Journal of Animal Science, 2020, Vol. 98, No. 8, 1–8

doi:10.1093/jas/skaa226
Advance Access publication July 18, 2020
Received: 18 May 2020 and **Accepted:** 13 July 2020
Animal Behavior and Cognition

ANIMAL BEHAVIOR AND COGNITION

Behavioral and cortisol responses to feeding frequency in pregnant sows under isocaloric intake

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Abstract

The study focused on behavioral and cortisol responses to feeding frequency in pregnant sows under isocaloric intake. Twenty-four sows [(Landrace × Yorkshire); BW 216.70 ± 3.98 kg; parity 3.04 ± 0.53] were balanced for parity and randomly assigned to 1 of 3 feeding frequency regimes. Sows were fed corn–soybean meal-based diet 1× [0730 (Control), T1], 2× [half ration at 0730 and 1530 hours, T2], or 3× [one-third portion at 0730, 1130, and 1530 hours, T3] from days 30 to 60 of gestation. Sows received 7055 kcal ME/d during gestation from 2.21 kg of diet formulated to contain SID Lys/ME of 1.71 g/Mcal. Saliva samples were collected every 2 hr from 0630 to 1830 hours on day 52 and assayed for cortisol using ELISA procedure. Behavior data were collected for 7 d from day 53 of gestation by affixing a remote insights ear tag to each sow. Each sow had 120,960 data points categorized into: “Active”, “Feed,” or “Dormant”. Because of housing constraint, all sows were housed in individual stalls in the same room presenting a potential limitation of the study. The data were analyzed using PROC MIXED and GLIMMIX procedures of SAS 9.4 for cortisol and behavior count data, respectively. Sow was the experimental unit. The area under the curve (AUC) is quantitative evaluation of response as threshold varies over all possible values. The T2 sows had reduced 12-hr cortisol AUC compared with control sows ($P = 0.024$) and T3 sows ($P = 0.004$), respectively. The T2 sows had lower 3 hr ($P = 0.039$) and 5 hr ($P = 0.015$) postfeeding cortisol AUC compared with control sows. Feed anticipatory activity (FAA), 24-hr total activity, and feeding activities (eating and/or sham chewing) were reduced for T2 sows relative to the control and T3 sows ($P < 0.01$). Consequently, T2 sows had lower 24-hr total activity ($P < 0.001$) and feeding activities ($P < 0.001$) AUC compared with both the control and T3 sows, respectively. The T3 sows had greater FAA ($P < 0.001$) and 24-hr total activity AUC ($P = 0.010$) compared with control sows. Our data although inconclusive due to small sample size, twice daily feeding appears to be the threshold that reduces sows’ total activity AUC, feeding activity AUC, and activation of hypothalamic–pituitary–adrenal axis, reduced hunger, and exhibit potential to improve sow welfare in relation to once and thrice daily feeding regimes under isocaloric intake per kilogram live metabolic weight.

Key words: behavior, feeding frequency, isocaloric intake, pregnant sows, saliva cortisol

Abbreviations

AUC	area under the curve
HPA	hypothalamic–pituitary–adrenal
FAA	feed anticipatory activity
RISMS	Remote Insight's Sow Management Solution
NTS	nucleus of the solitary tract

Introduction

Welfare of farm animals is important to producers, consumers, and society at large (Cornish et al., 2016). Modern hyperprolific sows are restrictively fed for efficient reproduction and to improve sow longevity. Restrictively, fed gestating sows can experience stress and impact on their behavior (Bernardino et al., 2016). 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 cortisol such that food restriction or starvation increases the mean glucocorticoids levels in humans and rat (Garcia-Belenguer et al., 1993; Kenny et al., 2014). Feed restriction in pregnant gilts elicited higher salivary cortisol levels than control gilts which had higher feed levels (Amdi et al., 2013). Increasing feeding frequency enables the performance of natural behavior to improve welfare compared with sows fed less often (Verdon et al., 2018). Our understanding of how feeding frequency under isocaloric intake in pregnant sows affects cortisol and sow behavior remains unknown.

Recent technological advances such as Remote Insight's Sow Management Solution (RISMS) is designed to allow continuous monitoring of pig activity using wireless ear tag. The ear tag data are processed using machine learning models trained to identify when an animal is performing feeding-related behaviors, active and dormant. The hypothesis tested in this experiment was that, given the same amount of energy per $BW^{0.75}$ d^{-1}, feeding multiple times would reduce the activation of HPA axis and improve sow welfare compare with feeding once per day. Therefore, objective of this study was to determine the effects of feeding frequency on cortisol response, feed anticipatory activity (FAA), indication of hunger, feeding and total activities of pregnant sows under isocaloric intake per kilogram live metabolic weight.

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.

Sows were kept and fed individually in stalls measuring (2.1 m × 0.59 m × 0.97 m) with fully slatted floors under temperature-controlled environment (21 to 23 °C) on a 9-hr light and 15-hr dark schedule, with light on at 0730 hours and turned off at 1630 hours. 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) after which sows were balanced for BW and BF. Throughout the experiment sows had unlimited access to water through nipple drinkers fitted to each stall. Feed offered to sows was restricted to 2.0 kg once daily from weaning through breeding to day 30, which is standard operating procedure of the sow unit for sows and gilt during that phase.

Experimental design, dietary treatments, and feed line calibration

Sows were balanced for parity and randomly allocated to 1 of 3 treatments with 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 was previously reported (Manu et al., 2019).

Experimental treatments were imposed from days 30 to 60 of gestation with 21 d adaptation. Body weights on day 30 was used to adjust the amount of feed fed. 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 × $BW^{0.75}$ kcal ME/d; NRC, 2012). To provide the daily energy intake, sows received on average 2.21 kg feed which supplied 7,055 kcal ME/d 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 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 hours (T1)), twice daily [half ration at 0730 and 1530 hours (T2)], or thrice daily [a third portion at 0730, 1130, and 1530 hours (T3)].

Measures recorded

Cortisol measurements

Multiparous and nulliparous pregnant sows of [Topigs Norsvin (Landrance × Large White (*Sus scrofa*)); total N = 24; 8 sows per treatment; parity 3.04 ± 0.53; and BW 216.70 ± 3.98 kg] were sampled. Saliva cortisol concentrations were measured on day 52 of gestation by collecting saliva samples 7 times during the day. Saliva samples were collected 1 hr before and after each feeding time and 3 hr after the last feeding occasion (i.e., 0630, 0830, 1030, 1230, 1430, 1630, and 1830 hours) using neutral synthetic swab Salivette (Sarstedt, Aktiengesellshaft 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 (Greenwood et al., 2016). Saliva samples were collected on ice and centrifuged 2 hr later at 2,500 × *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.

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 (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 interassay variations, samples from all treatments and same time points were analyzed within the same assay.

Collection of sow behavior data

A subset of multiparous and nulliparous pregnant sows [Topigs Norsvin (Landrance x Large White); total $N = 18$; 6 sows per treatment; initial average BW 222.89 ± 3.82 kg and average parity 3.61 ± 0.65] from the sows that were sampled for the physiological measure were studied for sow behavior from day 53 of gestation for 7 d without human interference. Data were collected by affixing a remote insights ear tag to each pregnant sow using ear tag applicator on day 48 of gestation. The ear tag sent 3 axis accelerometer data in *x*, *y*, and *z* plane collected at 2 Hz to a cloud database. The raw accelerometer data were then passed through a machine learning model which classified the activity of the sow every 5 s into 1 of 3 categories: "Active", "Feed," or "Dormant" (Table 1). This resulted in 120,960 data points per sow over the 7-d study period after 21 d adaptation to the feeding regime. The data were aggregated and reported every 15 min for 24-hr. The results presented are average daily "Feed" and/or "Active" classifications per sow. The study had 1 limitation due to housing constraint. Animals on different feeding frequency regimes could not be housed in different rooms within the barn. However, to reduce this expected impact on our results, an adjustment period of 21 d preceded any data collected. Additionally, experimental units were evenly distributed between locations, making sure experimental sows are not next to each other.

Determination of sow activity before feeding (FAA)

FAA in all sows was recorded as sow feeding activity which occurred 60 min before feeding (Table 1). Total daily FAA was the average of all feeding activities which occurred 60 min before feeding (de Godoy et al., 2015). The results presented are average daily FAA counts per sow. Average performance of feeding-related behaviors outside of the feed anticipatory times was determined to be indicator of hunger.

Precision and validation of the machine learning model

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 ~94% confidence. Briefly, to validate the machine learning model, video of sow behavior was labeled for the distinct behavior categories (dormant, active, and feeding) and corresponding ear tag accelerometer data were used to train and test the machine learning model to identify when those behaviors occurred.

Chemical analysis and feed composition

The dry matter, crude protein, crude ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed by the methods previously described (Manu et al., 2019). Basically, the diet was corn- and soybean-based (4431 kcal/ g GE, 15.7% crude protein, 13.3% NDF, 4.80% ADF).

Calculation of behavior and cortisol area under the curve (AUC)

The activity and feeding-related behavior AUC (count × hr), and cortisol AUC (ng × hr/mL) were calculated for 24 and 12 hr respectively, using trapezoidal summation rule: ∑{[(Ct + Ct + 1) × 0.5] × Δ*I*}; where C_t is either the activity and feeding-related behavior 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 Δ*I* in hours between data points, and ∑ 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 were 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 transformed count behavior, FFA, and AUC data were analyzed by fitting a logistic model using the GLIMMIX procedure. Back-transformed geometric means (with 95% confidence interval) were reported. Cortisol data collected repeatedly were analyzed as repeated measures ANOVA using the PROC MIXED procedure of SAS (SAS Inst. Inc.). The model included fixed effects of treatment, time, and treatment × 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 the Kenward–Roger's method (Kenward and Roger, 1997). Differences in basal cortisol concentration at 0630 and 1830 hours were compared using a one-sided paired test with PROC *T*-test in SAS. Cortisol AUC, basal, 1-, 3-, and 5-hr postfeeding 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 $P \le 0.05$ and $0.05 < P \le 0.10$ for all statistical tests, respectively.

Result

Cortisol response to feeding frequency under isocaloric intake in pregnant sows

The 12-hr salivary cortisol concentrations in pregnant sows are presented in Table 2. Treatment by time interaction was not

Table 1. Ethogram of pregnant sows' behavioral activity

Type of behavior	Description of behavior
1. Total activity	The number of 5 speriods that the model detected sow movement; average value per 15 min for 24 hr
2. Total feed activity	The number of 5 s periods that the model detected feeding behavior (eating and/or sham chewing); average value per 15 min for 24 hr.
3. Total FAA	The average of all feeding related behavior 1-hr before feeding (de Godoy et al., 2015); average per 15 min.
4. Indication of hunger	Feeding-related behavior outside of the feeding anticipatory times; average per 15 min.

To record feeding activity, the ear tag accelerometer captures the movement of the head. The sow's head has a distinct gyration when chewing. The model currently cannot distinguish between sham-chewing and the chewing of feed. "Active" behavior or activity is movement excluding "Feed" behavior and small motions such as dream tremors and very brief movements of the head (e.g., to shake a fly off).

Table 2. Effect of feeding frequency in pregnant sows under isocaloric intake on cortisol concentration and AUC (least squares means)

	Time of day							
Item	0630 hours	0830 hours	1030 hours	1230 hours	1430 hours	1630 hours	1830 hours	Total AUC, ng h/mL[4]
T1[1]	0.663	0.698	0.457	0.319	0.372	0.345	0.484	326.02[b]
T2[2]	0.523	0.412	0.313	0.191	0.335	0.264	0.458	246.52[a]
T3[3]	0.743	0.502	0.560	0.346	0.430	0.388	0.593	348.42[b]
SEM	0.07	0.08	0.07	0.07	0.07	0.08	0.08	23.52

[1]Sows received their daily gestation ration once at 0730 a.m.
[2]Sows daily gestation ration was split into 2 and each portion offered at 0730 a.m. and 1530 hours.
[3]Sows daily gestation ration was split into 3 and each portion offered at 0730 a.m., 1130 a.m. and 1530 hours.
[4]Total AUC was calculated using the trapezoidal summation method.
[a,b]Least squares means in each column of feeding frequency followed by different superscripts significantly differ ($P < 0.05$) (Tukey–Kramer adjusted).

significant ($P = 0.754$). Peak cortisol concentrations of 0.66, 0.52, and 0.74 ng/mL occurred at baseline (0630 hours) for treatments 1, 2, and 3, respectively, but there was no difference between treatments ($P \geq 0.10$). Cortisol levels were affected by time with concentration at 0630 hours being greater than 1030, 1230, 1430, and 1630 hours ($P < 0.05$). The T2 sows had lower 12-h cortisol AUC compared with T1 ($P = 0.024$) and T3 sows ($P = 0.004$). The 12-h cortisol AUC did not differ between T1 and the T3 sows ($P = 0.622$).

Effect of feeding frequency on basal, pre-, and postfeeding cortisol concentrations and AUC

Least squares means of basal, pre-, and postfeeding cortisol concentrations and AUC with respect to feeding frequency are presented in Table 3. The T2 sows tended to have lower cortisol levels 1-hr postfeeding compared with the T1 ($P = 0.071$) but similar to T3 sows ($P = 0.644$). The T1 and T3 sows did not differ in cortisol levels 1-hr postfeeding ($P = 0.299$). Sows on twice daily feeding schedule (T2) had reduced cortisol concentration 3-hr after feeding relative to sows fed 3 times daily ($P = 0.034$) but similar to sows fed once daily ($P = 0.341$). Sows receiving their daily feed once (T1) and thrice (T3) daily did not differ in cortisol levels 3-hr postfeeding ($P = 0.440$). The 5-hr postfeeding cortisol concentrations were not affected by feeding frequency ($P > 0.10$). The T2 sows had reduced both the 3-hr ($P = 0.039$) and 5-hr ($P = 0.015$) cortisol AUC in comparison to control sows. Similarly, the T2 sows tended to have reduced 3-hr ($P = 0.072$) but reduced 5-hr ($P = 0.008$) cortisol AUC, respectively, relative to T3 sows. The 3-hr ($P = 0.908$) and 5-hr ($P = 0.986$) cortisol AUC were similar for T3 and T1 sows.

Behavioral activity of pregnant sows in response to feeding frequency under isocaloric intake

The behavioral activities with respect to feeding frequency are presented in Table 4. Total sow activity and total feeding activity were reduced for T2 sows compared with T1 and T3 sows ($P < 0.001$). The T3 sows had tendency to have greater total activity ($P = 0.095$) but greater feeding activities ($P = 0.025$) relative to T1 sows. The T2 sows had reduced FAA compared with T1 ($P = 0.0004$) and the T3 ($P = 0.0001$) sows. The T3 sows had greater FAA in comparison to T1 sows ($P = 0.003$). Sows on 3 times daily feeding regime (T3) had greater indication of hunger compared with sows fed at once daily ($P = 0.044$) and twice daily ($P < 0.0001$). Indication of hunger was greater in T1 sows relative to T2 sows ($P < 0.0001$). Sows on twice daily feeding regime had reduced total ($P < 0.0001$) and feeding ($P < 0.0001$) activity AUCs compared with control sows and sows fed thrice daily, respectively. The T3 sows had greatest total activity AUC ($P < 0.0001$) and feeding activity AUC ($P \leq 0.035$) compared with both T1 and T2 sows, respectively. Relative to T1 sows, the T2 sows had reduced total activity AUC ($P < 0.0001$) and feeding activity AUC ($P < 0.0001$). The T1 sows had 2 peaks of feeding activities at 0730 and 1530 hours whereas the T3 sows had peaks at all the feeding times (Figure 1). The T2 sows had 3 lowest feeding activity peaks throughout the day. Control sows had 2 peaks of feeding activities, whereas the T3 sows had elevated feeding activity at each feeding time (Figure 2).

Table 3. Basal, postfeeding cortisol concentrations, and AUC with reference to first partial or full feeding (least squares means)[1]

	Treatment				
Variable	T1[2]	T2[3]	T3[4]	SEM	P-value
Time 0 (baseline), ng/mL	0.66	0.53	0.76	0.11	0.276
1 hr after feeding, ng/mL	0.69	0.43	0.52	0.13	0.083
3 hr after feeding, ng/mL	0.45[ab]	0.31[a]	0.56[b]	0.06	0.043
5 hr after feeding, ng/mL	0.32	0.20	0.34	0.06	0.128
Total AUC[5], ng hr/mL	108.27[a]	72.73[b]	102.82[ab]	13.53	0.030
Total AUC[6], ng hr/mL	154.16[a]	103.07[b]	156.74[a]	15.61	0.005

[1]Total AUC was calculated using the trapezoidal summation method.
[2]Sows received their daily gestation ration once at 0730 hours.
[3]Sows daily gestation ration was split into 2 and each portion offered at 0730 and 1530 hours.
[4]Sows daily gestation ration was split into 3 and each portion offered at 0730, 1130, and 1530 hours.
[5]AUC from time 0 to 3 hr after first partial or full feeding.
[6]AUC from time 0 to 5 hr after first partial or full feeding.
[ab]Least squares means within a row uncommon superscript significantly differ ($P < 0.05$) (Tukey–Kramer adjusted).

Discussion

The RISMS technology is designed to identify pig's activity 24 hr using wireless ear tag. The ear tag data were periodically sent to

Table 4. Pregnant sow's behavior counts per 15 min and AUC in response to feeding frequency under limit-fed condition (geometric mean [95% confidence interval])[1]

Variable	Treatment			P-value
	T1[2]	T2[3]	T3[4]	
Total activity, counts	274.1[a] (268.8 to 279.5)	134.3[b] (130.7 to 138.1)	295.7[a] (289.1 to 302.3)	<0.001
Total feeding activity, counts	153.7[b] (150.5 to 157.0)	54.9[a] (53.3 to 56.6)	173.0[c] (168.8 to 177.3)	<0.001
Total FAA[5], counts	95.1[b] (89.4 to 101.2)	38.3[a] (35.1 to 41.8)	167.1[c] (155.5 to 179.5)	<0.001
Indication of hunger[6], counts	156.9[b] (153.6 to 160.3)	56.8[a] (55.1 to 58.6)	174.0[c] (169.8 to 178.4)	<0.001
Total activity AUC[7], counts hr	340, 565[b] (334, 272 to 346, 977)	170, 687[a] (166, 265 to 175, 227)	374, 887[c] (366, 944 to 383, 001)	<0.001
Total feeding activity AUC[7], counts hr.	188, 452[b] (184, 587 to 192, 398)	70, 210[a] (68, 171 to 72, 310)	218, 072[c] (212, 912 to 223, 357)	<0.001

[1]Least squares means were calculated from transformed data and then back transformed for presentation of data.
[2]Sows received their daily gestation ration once at 0730 hours.
[3]Sows daily gestation ration was split into 2 and each portion offered at 0730 and 1530 hours.
[4] Sows daily gestation ration was split into 3 and each portion offered at 0730, 1130, and 1530 hours.
[5]Total FAA was recorded 1-hr preprandial for each feeding occasion.
[6]Feeding-related behavior outside of feeding anticipatory times.
[7]Total AUC was calculated using the trapezoidal summation method.
[a–c]Least squares means within a row with uncommon superscript significantly differ ($P < 0.05$) (Tukey–Kramer adjusted).

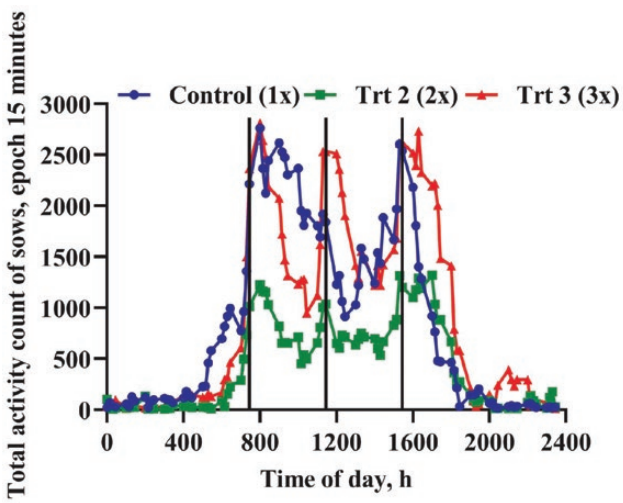

Figure 1. Temporal pattern of pregnant sow's total activity measured and averaged over a 24-h period over 7 d on different feeding frequencies under limit-fed conditions. The graph represents sows fed 1× daily at 0730 hours (blue curve, T1), 2× daily at 0730 and 1530 hours (green curve, T2), and 3× daily at 0730, 1130, and 1530 hours (red curve, T3). Vertical black lines indicate feeding time for each treatment.

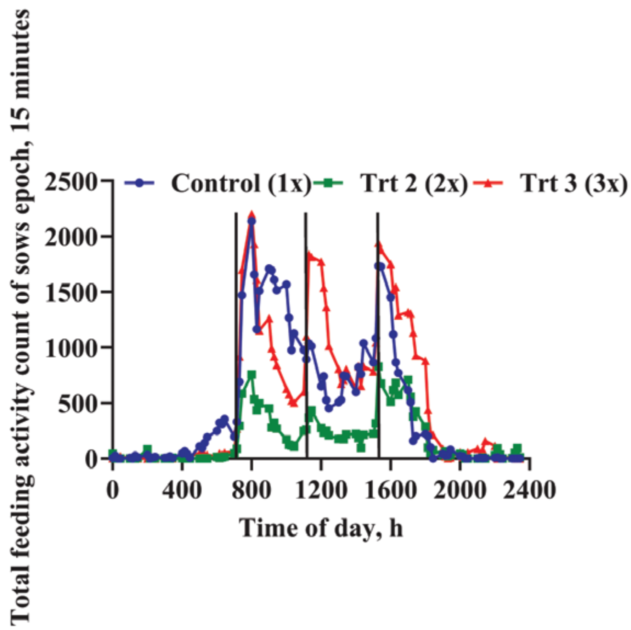

Figure 2. Temporal pattern of pregnant sow's feeding activity measured and averaged over a 24-h period over 7 d on different feeding frequencies under limit-fed conditions. The graph represents sows fed 1× daily at 0730 hours (blue curve, T1), 2× daily at 0730 and 1530 hours (green curve, T2), and 3× daily at 0730, 1130, and 1530 hours (red curve, T3). Vertical black lines indicate feeding time for each treatment.

a gateway in the barn that forwards it to Google's Cloud Platform where the movement data are processed using machine learning models trained to identify when an animal is performing feeding-related behaviors, active and dormant. Increasing feeding frequency enables the performance of natural behavior to improve welfare compared with sows fed less often (Verdon et al., 2018). However, this study had a limitation due to housing constraint. Animals on different feeding frequency regimes could not be housed in different rooms within the barn and the sound of feeding could have stimulated feeding behavior in the other sows. However, to reduce this expected impact on our results, we allowed 3 wk acclimation to the feeding regimes after which data were collected. Additionally, experimental units were evenly distributed between stalls, making sure experimental sows are not next to each other.

Cortisol is a steroid hormone secreted by the adrenal gland and has a circadian rhythmicity with greater concentration around 08:30 hr 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 hours was greater relative to 1830 hours time point 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 ~50% of their ad libitum feed intake (Meunier-Salaün et al., 2001). Splitting the limited feed further into 2 or 3 meals and fed multiple times within the day did not alter the basal 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 in a metabolic chamber had basal cortisol levels that did not change on varying meal frequency. Although 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 basal cortisol levels when all treatments groups had similar energy intake per kilogram live metabolic weight.

In studies where data are collected at various time points, the selection of a single primary endpoint may be arbitrary or not valid. In such situation, it may be beneficial to use a summary measure such as an AUC analysis. The calculations integrate the individual subject serial measurements over the entire study period and quantify the response as AUC (Ohkawara et al., 2013). 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 with sows on single feeding regime. Conversely, Holt et al. (2006) did not elicit a response of feeding frequency (2× daily at 0730 and 1430 hours) on salivary cortisol concentration relative to sows fed 1× daily. The differences in results are attributable to the extent of sampling, method of evaluation, and turnover rate of cortisol. In the current study, we sampled our sows every 2 hr from 0630 to 1830 hours and evaluated them using the AUC. Holt et al. (2006) evaluated their experimental animals using single time point measurements at 1300 hours. 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 (Stewart et al., 2017). Additionally, it is difficult to interpret saliva or plasma concentrations of any hormone. The concentration of cortisol at any time depends on secretion and clearance rates. Without knowing turnover rate, blood or saliva concentrations of cortisol are poor indicators of what is actually occurring (Friend, 1980).

Feeding frequency had significant effect on the feeding activity, which mirrored total sows' activity as observed in Figures 1 and 2. Sow group fed once daily at 0730 hours did not exhibit peak feeding or activity at 1130 hours. At 1130 hours (4 hr after sows had received their full meal at 0730 hours), it can be speculated that the sows had adequate gut fill or might not have attained postabsorptive state and therefore the desire to feed minimal. Sow group on once daily feeding regime had increased feeding activity at 0730 hours and 8 hr after feeding (1530 hours), whereas a different pattern of reduced sow feeding activity was observed in sows on twice daily feeding schedule. Overall, sows on twice daily feeding regime had reduced feeding and total activity compared with sows fed once and thrice daily. The 0730 and 1530 hours time points correspond to 24 and 8 hr after the morning feeding for sows fed once daily and 8 and 16 hr after the 1530 hours feeding for sows fed twice daily. Therefore, sows fed once daily were highly motivated to feed at 1530 hours and the next morning (0730 hours) than the sows fed twice daily since they experienced postabsorptive state at 0730 hours. On the contrary, Robert et al. (2002) reported that gilts fed 2 meals per day on concentrate diet had similar activity compared with gilts fed once daily. However, these researchers only evaluated sow feeding related behaviors in 5 min before the morning ration at 08:00 hours. Assessment of sow welfare 24 hr per day in the current study might have altered sow's feeding activity. Intuitively, increasing the feeding frequency for pregnant sows should improve satiation and their welfare because energy for stereotypic behaviors could decline and enhanced productivity. In support of this theory, twice daily feeding tended to improve gestating sow's back fat gains, reduced the number of stillborn, and increased the number of piglet wean relative to control sows fed once daily (Manu et al., 2019).

Sows on 3 times daily feeding regime had similar total and feeding activity relative to sows fed once daily which was replicated in pigs (Robert et al., 2002) but conflicts with earlier published report in cats (de Godoy et al., 2015). Robert et al. (2002) reported that the number of meals served daily had no major influence on feeding motivation of gilts fed concentrate diets in an operant test. Cats fed 4 times daily had increased (~18%) voluntary physical activity than similar group fed once 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 suggest that twice daily feeding appears to be the threshold of feeding frequency under limit fed conditions as it reduces total and feeding activities, indication of hunger as well as cortisol AUC in pregnant sows. However, cortisol AUC has not been related to feed restriction in sows (de Leeuw and Ekkel, 2004; Toscano et al., 2007) and hence warrant further investigation.

Sows on 3 times 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 frequent daily feeding regimen of 2 kg than pigs receiving 6 kg of feed once in 3 d. In line with our findings, sows fed thrice daily had increased total and feeding activity compared with sows fed twice 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 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 twice daily had the lowest peaks at all feeding time. Although the pattern of sows fed once and 3 times daily was similar, sow fed 3 times had 3 peaks while sows on once daily feeding regime had only 2 peaks. Therefore, sows on 3 times daily feeding regime had greater total activity AUC compared with the control sows and sows fed twice 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 once daily. When meal size is too small to induce satiety, nonfeeding activities would persist (Terlouw et al., 1993; Robert et al., 2002).

Behavioral activities preceding feed provision is termed "food anticipatory activity" (FAA) (Johnston, 2014). Sows on 3 times daily feeding regime had greatest FAA compared with sows fed at once and twice daily. This observation is in line with previous results in cats. Cats fed 4 times daily had increased FAA compared with cats fed once 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. 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 to induce meal termination. 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 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).

Conclusion

Our study indicates that twice daily feeding appears to be the threshold that reduces sows' total activity AUC, feeding activity AUC, indication of hunger, and activation of HPA axis and exhibits potential to improve sow welfare in relation to once and thrice daily feeding regimes under isocaloric intake per kilogram live metabolic weight. Sows on once and thrice daily feeding schedules had similar cortisol AUC, feeding, and total activities AUCs but sows fed thrice daily had greatest FAA and indication of hunger using conventional pregnant sow's diet.

Limitation of study

The study had 1 limitation due to housing constraint. Animals on different feeding frequency regimes could not be housed in different rooms within the barn. However, to reduce this expected impact on our results, we allowed 21 d acclimation to the feeding regimes. Also, experimental units were evenly distributed within the row, making sure experimental sows are not next to each other. This limitation may highlight the need for further research.

Acknowledgments

The authors would like to acknowledge the staff of Southern Research and Outreach Center (SROC)—Waseca, Sow Unit, University of Minnesota, for husbandry and assistance with experimental procedures. Research was supported by funds from United States Department of Agriculture (USDA)—National Institute of Food and Agriculture (NIFA—2010-04438).

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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