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8 **Abstract**

9 The objective of this study was to investigate the influence of feeding frequency on a sow's reproductive
10 performance and stress response during gestation. A total of twenty multiparous sows (Yorkshire ×
11 Landrace, Darby Genetics, Republic of Korea) were used in a completely randomized design based on
12 their parity, body weight (BW), and backfat thickness (BFT), and the sows were allotted to two different
13 feeding systems: 1) once daily feeding (OF) and 2) twice daily feeding (TF) in corn-soybean meal based
14 diets. The gestation diet was formulated to contain 3,265 kcal of ME / kg, 12.90 % of CP, and 0.75 %
15 of total lysine. The lactation diet was formulated to contain 3,265 kcal of ME / kg, 16.80 % of CP, and
16 1.08 % of total lysine and provided ad libitum during lactation. In gestation, sow BFT and BF changes
17 were not affected by feeding frequency, but higher BW and BW gain from day 35 to 90 and day 35 to
18 110 were observed in OF sow ($p < 0.10$). In lactation, feeding frequency did not influence on BW, BW
19 gain, BFT, BF changes, average daily feed intake, and wean-to-estrus interval. Also, there were no
20 differences in litter size, litter weight and piglet weight in lactating sows. OF sows had higher ($p < 0.05$;
21 $p < 0.10$) protein, solid-not-fat, and total solid concentrations in colostrum compared to TF sows, while
22 OF sows had a lower ($p < 0.05$) lactose concentration in colostrum compared to TF sows. Sows in OF
23 showed significantly lower average daily water consumption (ADWC) from day 35 to 110 of gestation
24 ($p < 0.05$). While there were no significant differences in stereotypic behaviors and salivary cortisol
25 levels during gestation between treatments, the OF sows showed less time spending on the activity at
26 day 105 ($p < 0.05$). In conclusion, reduced feeding frequency increased BW gain during gestation,
27 decreased activation time, and changed the colostrum composition. This information may contribute to
28 the understanding of the physiological and behavioral change of gestating sows by manipulating feeding
29 frequency.

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31

32 **Keywords**

33 Behavior, Cortisol, Feeding frequency, Gestation, Sow

34

35 **Introduction**

36 The provision of proper management and nutrition for gestating sows is essential to ensure
37 successful reproductive performance and fetus health. Sows can experience chronic stress because of
38 physiological changes during placental and fetal development and mammary gland development and
39 changes in maternal body tissue reserves [1, 2]. In addition, sows are bred to produce piglets in limited
40 environments such as stalls, which can manage individual sows and avoid social stress, thereby
41 preventing aggression toward other sows [3]. However, this gestational stall hinders the free movement
42 and social interaction of sows, inducing poor welfare and mental conditions. Furthermore, pregnant
43 sows are fed a restricted amount of feed to control their body condition [4], which is lower than that of
44 self-feeding sows in nature. These limited environments for cage and feed intake may increase stress
45 levels and stereotypical behavior [5], thereby inducing poor reproductive performance in sows.

46 Controversial results have been found regarding the determination of the feeding frequency of
47 gestating sows. Several studies have shown that once-daily feeding (OF) in gestating sows reduces their
48 stereotypical behaviors with low stress levels compared with sows provided more than twice-daily
49 feeding (TF) during gestation, or neither feeding system affects their behavior [6, 7]. In addition, OF in
50 pregnant sows may improve sow behaviors compared with TF in pregnant sows. In contrast, Farmer et
51 al. [9] reported that reduced daily feeding frequency did not affect stress-hormone levels. Moreover,
52 multiple feeding regimens can lead to the spread of the nutrient load, resulting in improved nutrient
53 utilization [8].

54 Therefore, the objective of this study was to investigate whether gestational feeding frequency,
55 particularly when comparing OF with TF, affected the reproductive performances and stress responses
56 of pregnant sows. We hypothesized that feeding the same amount of energy per day with different
57 feeding frequencies would not affect reproductive performance, thus reducing stress responses and
58 stereotypical behaviors in pregnant sows.

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60

61 **Materials and Methods**

62 A total of twenty gestating sows (Yorkshire × Landrace) with average body weight (BW) of 201.8 ±
63 12.54 kg and a parity of 2.8 ± 0.41 (parity 2 = 4 and parity 3 = 16) were allotted to one of two feeding
64 treatments by parity, BW, and BFT in completely randomized design (CRD) after confirming
65 pregnancy at day 35.8 ± 1.11 of gestation by ultrasound scanner (Dongjin BLS, Korea). The treatments
66 consisted of: 1) once daily feeding of 2.4 kg/d, or 2) twice daily feeding of 1.2 kg of a gestation diet
67 (Sows of 2nd parity fed 2.2 kg/d). All sows received the same lactation diet ad libitum after parturition
68 till weaning. A gestation diet based on corn-soybean meal contained 3,265 kcal of ME/kg, 12.90 % of
69 crude protein (CP), and 0.75 % of total lysine, respectively. A lactation diet was formulated to contain
70 3,265 kcal of ME/kg, 16.80 % of CP and 1.08 % of total lysine, respectively. All the diets met or exceed
71 the nutrient requirement of sows [10].

72 After confirming pregnancy at 35 days of gestation, sows were moved to gestation barn from breeding
73 barn. Diet was provided at 08:00 AM for the sows fed once daily and at 08:00 and 16:00 for the sows
74 fed twice daily, respectively. All sows were accommodated in individual gestation stalls (2.40 × 0.64
75 m) where the indoor temperature was regulated by automatic ventilation system (average 19 ± 2 °C).
76 At day 110 of gestation, sows were moved from gestation barn to farrowing crates (2.20 × 0.65 m)
77 with partition walls (2.50 × 1.80 m) after washing and disinfecting their body. During lactation, the
78 room temperature of farrowing barn was kept automatically at 25 ± 3 °C by heating lamps and
79 ventilation fans. After weaning, sows were moved to breeding barn again for the next conception.

80 Saliva samples were taken from 5 sows of each treatment at day 35, 70, 105 of gestation using a cotton
81 roll (Salivette®, Sarstedt AG & CO., Numbrecht, Germany) to analyze salivary cortisol concentration.
82 The saturated cottons with saliva were collected from their oral cavity immediately before and 3h after
83 feed delivery (8:00 and 11:00). Samples were frozen at -20°C, then cortisol concentration were
84 determined by an enzyme immunoassay with salivary cortisol kit (Salimetrics, State College, PA, USA).
85 Water consumption was measured from 8 sows of each treatment at day 35, 70 and 105 of gestation by
86 water meter (Sewha Precision Co., Ltd., Republic of Korea). Average water flow rate was adjusted to

87 range from 1.5 to 2 L/min. The water spills would be minimized because drinking of sows happened
88 directly from the nipple or from the feed bowl beneath the nipple. Therefore, although water
89 consumption represented the total quantity of water intake and spillage by sow, it also considered to be
90 equal to water intake.

91 Sow behaviors were recorded from 4 sows of each treatment during daytime (06:00-18:00) by CCTV
92 (Samsung Techwin Co., Ltd, Republic of Korea) at the same day with saliva collection. Recorded videos
93 were analyzed by direct view, and then the behaviors classified as stereotypic behavior (bar biting, sham
94 chewing and nosing the floor or feeder), activity (standing and moving without stereotypes, feeding and
95 drinking behaviors) and inactivity (lying and sitting), respectively [11,12,13]. One trained observer,
96 blind to the treatments, did count these behaviors. The percentage of stereotypic behavior in sows was
97 calculated as the proportion of abnormal behavior observed out of all behaviors exhibited during the
98 observation period.

99 The body weight (BW) and backfat thickness (BFT) of sows from all treatments were taken at day 35,
100 90, and 110 of gestation, 12 h and 21 d postpartum. BFT was measured at the P2 position (last rib, 65
101 mm from the center line of the back) on both sides of back bone using a lean-meter (Renco Corp.,
102 Minneapolis, MN, USA). Values from the two measurements were averaged to record a single BFT
103 measurement. During lactation, sow feed intake was measured at day 7, 14, and 21 of lactation.

104 A 5 mL of blood samples were collected from the anterior vena cava of piglet at 12 h and 21 d
105 postpartum. All samples were enclosed into serum-separating tube and centrifuged at 3,000 rpm and 4 °C
106 for 15 mins after clotting at room temperature for 30 mins. The upper liquid (serum) of the blood was
107 separated to a microtube (Axygen, Union City, CA, USA) and stored at -20 °C until later analysis.

108 Colostrum and milk samples were taken from functional mammary glands of each sow of treatments at
109 24 h and 21 d postpartum, respectively. After collection, samples were stored in a freezer at -20 °C until
110 further analysis. Proximate analysis of colostrum and milk was conducted using Milkoscan FT120
111 (FOSS A/S, Hillerød, Denmark). The immunoglobulin G (IgG) and A (IgA) concentration of sow milk
112 and piglet serum were also determined by ELISA assay based on the manufacturer's instructions (Pig
113 IgG and IgA ELISA Quantitation Kit; Bethyl, Texas, USA).

114 The experimental data were analyzed using GLM procedure of SAS (SAS Institute, 2004). All data
115 were checked for normal distribution applying the Shapiro–Wilk test within the UNIVARIATE
116 procedure and by visual inspection of the plotted residuals. The repeated measures model for sow
117 performance, litter performance and other collected data included fixed effects of feeding frequency,
118 parity, and feeding frequency x parity, whereas sows were considered a random effect. Least squares
119 means of fixed effects with their corresponding SE were calculated using the LSMEANS statement of
120 SAS. The estimation method was based on residual maximum likelihood (REML). Data are presented
121 as means \pm SEM. Difference between least squares means was requested using PDIF of SAS and
122 significant differences were declared at $P \leq 0.05$ while a trend was considered between $0.05 < P \leq 0.10$.
123 The Tukey–Kramer’s adjustment method for multiple comparisons was used for means separation.

125 **Results & Discussion**

126 The effects of feeding frequency on sow performance and average daily water consumption
127 (ADWC) during gestation are listed in Table 1. No differences were found in BFT and backfat (BF)
128 changes during any gestation period. However, body weight BW gain during the mid-gestation period
129 (d 35–90) and overall period (d 35–110) was higher in once-daily feeding (OF) sows than in twice-daily
130 feeding (TF) sows ($p < 0.10$). These results are contrary to those of Holt et al. [7], who reported that
131 sow BW and BFT were significantly higher in the TF treatment group, regardless of gestation and
132 lactation. The differences between the present study and the work reported by Holt et al. [7] may be
133 related to the behavioral patterns of sows. In the present study, OF sows showed lower physical activity
134 than did TF sows. However, Holt et al. [7] found that sows fed OF spent more time standing, feeding,
135 and engaging in stereotypical behaviors than sows fed TF. Physical activity plays an important role in
136 regulating BW. Regular physical activity can help increase energy expenditure, prevent weight gain,
137 and promote weight loss. This is because physical activity burns calories, which can help offset the
138 calories consumed through food [15]. Noblet et al. [14] demonstrated that compared with the lying
139 posture, the standing posture in gestating sows increased heat production by 180 kcal per 100 min during
140 gestation, indicating that the high activity of gestating sows caused an increase in body heat, thereby

141 increasing energy utilization [15]. It seems likely that the feeding frequency determined in the present
142 study (one or two times per day) did not affect physiological changes in sows. However, reduced
143 activity in OF sows increased BW gain during mid-gestation. The lack of differences in BW was not
144 surprising because sows in their respective treatments were fed the same total quantity of feed each day.

145 There was a lower ADWC during the entire period of gestation ($p < 0.05$) in OF sows than in TF
146 sows. The higher ADWC in TF sows is probably related to feeding frequency and active behaviors [16].
147 Terlouw et al. [17] categorized excessive water consumption by sows as a form of stereotypical
148 behavior that cannot be controlled by normal physiological mechanisms. This abnormal behavior is
149 mostly because of some degree of frustration or stress [18]. However, this does not apply to the present
150 findings because the water consumption of sows in the present study was within the normal range (11–
151 15 L/day), according to the report by Brumm [19]. We hypothesized that multiple feeding frequencies
152 would lead to increased sow activation time, resulting in increased feeding motivation, which has been
153 implicated in the development of stereotypes [20]. Similar results were reported by Schneider et al. [21],
154 who compared feeding frequency (2 vs. 6 times/day) of group-housed gestating sows and indicated that
155 multiple-time feeding tended to increase active behaviors, specifically increasing the time spent sitting
156 and feeding, which was also found in the present study (Figure 1). These results suggested that a larger
157 meal with reduced feeding frequency could increase feed satiety and water consumption in pregnant
158 sows.

159 The BW, BW gain, BFT, BF change, and ADFI of sows during lactation and wean-to-estrus
160 interval were not affected by feeding frequency during gestation (Table 2). Similarly, Manu et al. [22]
161 reported that sows fed once, twice, or three meals per day during gestation did not show changes in BW,
162 BW gain, BFT, or BF change during lactation. Therefore, feeding frequency during gestation may not
163 affect sow performance during lactation.

164 An effect of feeding frequency was observed on colostrum composition, with OF sows having a
165 lower lactose concentration and higher protein, solid-not-fat, and total solid concentrations in the
166 colostrum (Table 3). However, no differences were observed in litter size, litter weight, and piglet
167 weight between lactating sows (Table 4). Water intake during gestation may affect the nutritional

168 content of the colostrum. TF sows showed higher ADWC than did OF sows, which, in turn, resulted in
169 the dilution of the colostrum and decreased nutrient concentrations. This can happen if sows have access
170 to unlimited water during gestation and lactation. Holt et al. [7] indicated that the litter performance of
171 lactating sows, including litter size and weight, was not affected by feeding frequency during gestation.
172 We hypothesized that appetite hormones, such as leptin, ghrelin, and glucagon-like peptide-1, play an
173 important role in the long-term regulation of feed intake and BW, thus achieving energy homeostasis
174 and resulting in fetal development. In human studies, alterations in maternal-placental-fetal leptin
175 exchange may modify fetal development and increase the risk of intrauterine growth retardation [23].
176 A similar result was found in a rodent study, which showed that high maternal leptin levels in obesity
177 might adversely affect fetal growth and development [24]. However, in the present study, feeding
178 frequency may not have affected the appetite hormone later, resulting in no effect on the litter
179 performance of lactating sows.

180 The effect of feeding frequency on the behavior of gestating sows during the daytime (06:00–
181 18:00) is shown in Figure 1. No significant differences between different feeding frequencies in
182 stereotypical behaviors were observed; however, OF sows showed lower activities at day 105 ($p < 0.05$)
183 of gestation than did TF sows. The occurrence of stereotypical behaviors can be found when the gut fill
184 and nutrient requirements in gestating sows cannot be satisfied owing to restricted feeding [25, 26].
185 Terlouw et al. [17] reported that stereotypical behaviors during gestation were stimulated by feed intake
186 and peaked after meals. Robert et al. [6] observed that gilts fed twice during the day performed more
187 activities and showed stereotypical behaviors before and after meals because they were not completely
188 satiated by induced feeding, and feeding a single daily meal resulted in the reduced anticipation of a
189 subsequent afternoon meal. Holt et al. [7] also found that sows fed a once-daily meal showed reduced
190 feeding and standing time, as well as decreased stereotypical behaviors throughout the day, with an
191 exception of mealtime during which they exhibited increased activity. In growing-finishing pigs with
192 restricted feeding conditions, Hessel et al. [27] reported that pigs with greater feeding frequency showed
193 more aggressive actions, less lying posture, longer belly-nosing time, and greater skin lesion scores than
194 shown by those with lower feeding frequency (3 times daily vs. 9 times daily). In the present study,

195 sows did not show significant differences in stereotypical behaviors between treatments; however, OF
196 sows tended to show decreased activity and increased inactivity during pregnancy, partially supporting
197 previous study results [7, 22].

198 Salivary cortisol levels were not associated with feeding frequency, either before or after meals
199 during gestation (Figure 2). Farmer et al. [9] demonstrated that compared with TF, OF increased the
200 cortisol level of sows after a morning meal, which indicated a greater stimulation of feed. In contrast,
201 Holt et al. [7] reported that the salivary cortisol concentrations of sows were mostly unaffected by
202 feeding frequency, and a declining trend of the hormone was observed as the pregnancy progressed,
203 consistent with the results of the present study.

204

205 **Conclusion**

206 Sows in OF under stall housing condition did not have negative impact on reproductive performance in
207 gestating sow litter size and weight. In addition, sows in OF induced decreasing active behavior and
208 water consumption in comparison to sows in TF. These results suggest that the OF is practical
209 alternative management for the pork producers, by enhancing labor efficiency in combination with
210 considering the welfare of gestating sows.

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213 **Acknowledgments**

214 Not applicable.

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224 **References**

- 225 1. Kensinger RS, Collier RJ, Bazer FW, Ducsay CA, Becker HN. Nucleic acid, metabolic and
226 histological changes in gilt mammary tissue during pregnancy and lactogenesis. *J Anim Sci.*
227 1982;54:1297-308. <https://doi.org/10.2527/jas1982.5461297x>.
- 228 2. Dourmad JY, Etienne M, Prunier A, Noblet J. The effect of energy and protein intake of sows on
229 their longevity. *Livest Prod Sci.* 1994;40:87-97. [https://doi.org/10.1016/0301-6226\(94\)90039-6](https://doi.org/10.1016/0301-6226(94)90039-6)
- 230 3. McGlone JJ, Vines B, Rudine AC, Dubois P. The physical size of gestating sows. *J Anim Sci.*
231 2004;82:2421-27. <https://doi.org/10.2527/2004.8282421x>.
- 232 4. Weldon WC, Lewis AJ, Louis GF, Kovar JL, Giesemann MA, Miller PS. Postpartum hypophagia
233 in primiparous sows: I. Effects of gestation feeding level on feed intake, feeding behavior, and
234 plasma metabolite concentrations during lactation. *J Anim Sci.* 1994;72:387-94.
235 <https://doi.org/10.2527/1994.722387x>
- 236 5. Tatemoto P, Bernardino T, Morrone B, Queiroz MR, Zanella AJ. Stereotypic Behavior in Sows Is
237 Related to Emotionality Changes in the Offspring. *Frontiers in Veterinary Science.* 2020;7.
238 <https://doi.org/10.3389/fvets.2020.00079>
- 239 6. Robert S, Bergeron R, Farmer C, Meunier-Salaun MC. Does the number of daily meals affect
240 feeding motivation and behaviour of gilts fed high-fi bre diets? *Appl Anim Behav Sci.* 2002;76:105-
241 17. [https://doi.org/10.1016/S0168-1591\(02\)00003-5](https://doi.org/10.1016/S0168-1591(02)00003-5)
- 242 7. Holt JP, Johnston LJ, Baidoo SK, Shurson GC. Effects of a high-fiber diet and frequency of feeding
243 on behavior, reproductive performance, and nutrient digestibility in gestating sows. *J Anim Sci.*
244 2006;84:946-55. <https://doi.org/10.2527/2006.844946x>.
- 245 8. Jenkins DJA, Wolever TMS, Vuksan V. Nibbling versus gorging: metabolic advantages of increased
246 meal frequency. *N Engl J Med.* 1989;321:929-34. <https://doi.org/10.1056/NEJM198910053211403>
- 247 9. Farmer C, Meunier-Salaun MC, Bergeron R, Robert S. Hormonal response of pregnant gilts fed a
248 high-fiber or a concentrate diet once or twice daily. *Can J Anim Sci.* 2002;82:159-64.
249 <https://doi.org/10.4141/A01-039>
- 250 10. NRC. Nutrient Requirements of Swine. 11th rev. ed. Natl. Academy Press, Washington, DC.
251 Pedersen BK. Water intake and pig performance, Proceedings of Teagasc Pig Conference,
252 1994;p.50-4.
- 253 11. Fraser D. The effect of straw on the behavior of sows in tether stalls. *Anim Prod.* 1975;21:59-68.
254 <https://doi.org/10.1017/S0003356100030415>

- 255 12. Jensen P. An ethogram of social interaction patterns in group-housed dry sows. *Appl Anim Ethol.*
256 1980;6:341-50. [https://doi.org/10.1016/0304-3762\(80\)90134-0](https://doi.org/10.1016/0304-3762(80)90134-0)
- 257 13. Arellano PE, Pijoan C, Jacobson LD, Algers B. Stereotyped behaviour, social interactions and
258 suckling pattern of pigs housed in groups or in single crates. *Appl Anim Behav Sci.* 1992;35:157-
259 66. [https://doi.org/10.1016/0168-1591\(92\)90006-W](https://doi.org/10.1016/0168-1591(92)90006-W)
- 260 14. Noblet J, Dourmad JY, Etienne M. Energy utilization in pregnant and lactating sows: Modeling of
261 energy requirements. *J Anim Sci.* 1990;68:562-72. <https://doi.org/10.2527/1990.682562x>
- 262 15. Cronin GM, van Tartwijk JMFM, van der Hel W, Verstegen MWA. The influence of degree of
263 adaptation to tether-housing by sows in relation to behavior and energy metabolism. *Anim Prod.*
264 1986;42:257-68. <https://doi.org/10.1017/S0003356100017979>
- 265 16. Robert S, Matte JJ, Farmer C, Girard CL, Martineau GP. High-fibre diets for sows: Effects on
266 stereotypies and adjunctive drinking. *Appl Anim Behav Sci.* 1993;37:297-309.
267 [https://doi.org/10.1016/0168-1591\(93\)90119-A](https://doi.org/10.1016/0168-1591(93)90119-A)
- 268 17. Terlouw EMC, Lawerence AB, Illius AW. Influences of feeding level and physical restriction on
269 development of stereotypies in sows. *Anim Behav.* 1991;42:981-91. [https://doi.org/10.1016/S0003-3472\(05\)80151-4](https://doi.org/10.1016/S0003-3472(05)80151-4)
- 271 18. Fraser D, Patience JF, Phillips PA, McLeese JM. Water for piglets and lactating sows: quantity,
272 quality and quandaries. In: Haresign W, Cole DJA. (Editors). *Recent Advances in Animal Nutrition.*
273 Butterworths; 1990. London, p. 137-160.
- 274 19. Brumm M. Patterns of Drinking Water Use in Pork Production Facilities. *Nebraska Swine Report.*
275 2006. P.10-3.
- 276 20. Pedersen BK. Water intake and pig performance, *Proceedings of Teagasc Pig Conference,*
277 1994;p.50-4.
- 278 21. Schneider J, Tokach M, Dritz S, Nelssen J, DeRouchey J, Goodband R. Effects of feeding schedule
279 on body condition, aggressiveness, and reproductive failure in group-housed sows. *J Anim Sci*
280 2007;85:3462-69. <https://doi.org/10.2527/jas.2007-0345>
- 281 22. Manu H, Lee S, Ren P, Pangeni D, Yang X, Baidoo SK. Effect of feeding frequency and sow parity
282 based on isocaloric intake during gestation on sow performance. *J Anim Sci.* 2019;97:2154–2164.
283 <https://doi.org/10.1093/jas/skz099>
- 284 23. Briffa JF, McAinch AJ, Romano T, Wlodek ME, Hryciw, DH. Leptin in pregnancy and development:
285 a contributor to adulthood disease? *Am J Physiol Endocrinol Metab.* 2015;308:335-50.
286 <https://doi.org/10.1152/ajpendo.00312.2014>

- 287 24. Valteau JC, Sullivan EL. The Impact of Leptin on Perinatal Development and Psychopathology. *J*
288 *Chem Neuroanat.* 2014;61-62, 221-32. <https://doi.org/10.1016/j.jchemneu.2014.05.001>
- 289 25. Lawrence AB, Terlouw EMC. A review of behavioral factors involved in the development and
290 continued performance of stereotypic behavior in pigs. *J Anim Sci.* 1993;71:2815-25.
291 <https://doi.org/10.2527/1993.71102815x>.
- 292 26. Whittaker X, Spooler HAM, Edwards SA, Lawrence AB, Corning S. The influence of dietary fiber
293 and the provision of straw on the development of stereotypic behaviour in food restricted pregnant
294 sows. *Appl Anim Behav Sci.* 1998;61:89-102. [https://doi.org/10.1016/S0168-1591\(98\)00183-X](https://doi.org/10.1016/S0168-1591(98)00183-X)
- 295 27. Hessel EF, Wülbers-Mindermann M, Berg C, Van den Weghe HFA, Algers B. Influence of increased
296 feeding frequency on behavior and integument lesions in growing-finishing restricted-fed pigs. *J*
297 *Anim Sci.* 2006;84:1526-34. <https://doi.org/10.2527/2006.8461526x>

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300 **Tables and Figures**301 **Table 1.** The effect of feeding frequency on body weight and backfat thickness in gestating sows

302

Criteria	Treatment ¹		SEM ²	<i>p</i> -value
	OF	TF		
No. Sows	10	10	-	-
Body weight, kg				
d 35	202.0	201.7	2.96	0.948
d 90	228.4 ^c	222.2 ^d	2.93	0.068
d 110	243.1 ^c	237.3 ^d	3.19	0.076
Body weight gains, kg				
d 35-90	26.4 ^c	20.6 ^d	1.23	0.054
d 90-110	14.6	15.1	0.74	0.922
d 35-110	41.1 ^c	35.7 ^d	1.45	0.067
Back-fat thickness, mm				
d 35	19.0	19.0	0.99	1.000
d 90	20.2	20.7	0.92	0.747
d 110	21.1	22.0	0.91	0.562
Back-fat changes, mm				
d 35-90	1.2	1.7	0.48	0.747
d 90-110	0.9	1.3	0.43	0.797
d 35-110	2.1	3.0	0.60	0.562
ADWC³, L / day				
d 35-90	9.5 ^a	12.4 ^b	0.75	0.028
d 90-110	11.9 ^a	14.8 ^b	0.79	0.034
d 35-110	10.7 ^a	13.6 ^b	0.63	0.029

^{a,b}Means with different superscripts in the same row significantly differ ($P < 0.05$).

^{c,d}Means with different superscripts in the same row numerically differ ($P < 0.10$).

¹OF = once daily feeding; TF = twice daily feeding.

²Standard error of means.

³Average daily water consumption.

303 **Table 2.** The effect of feeding frequency during gestation on body weight, backfat thickness, average
 304 daily feed intake and weaning to estrus interval in lactating sows

305

Criteria	Treatment ¹		SEM ²	p-value
	OF	TF		
No. Sows	10	10	-	-
Body weight, kg				
12 h postpartum	220.2	215.3	2.66	0.191
d 21 of lactation	219.5	217.9	3.00	0.735
Body weight gain, kg				
d 0-21	-0.7	2.6	1.24	0.309
Back-fat thickness, mm				
12h postpartum	20.2	22.3	1.07	0.246
d 21	17.5	18.8	0.92	0.486
Back-fat changes, mm				
d 0-21	-2.7	-3.6	0.65	0.640
Average daily feed intake, kg/d				
d 0-7	5.98	5.81	0.120	0.588
d 8-14	6.76	6.88	0.157	0.706
d 15-21	7.01	6.75	0.175	0.413
Overall	6.58	6.48	0.098	0.556
Weaning to estrus interval, day				
	4.5	4.8	0.28	0.213

¹OF = once daily feeding; TF = twice daily feeding.

²Standard error of means.

306 **Table 3.** The effect of gestation feeding frequency on colostrum and milk composition of lactating sows

307

Criteria	Treatment ¹		SEM ²	p-value
	OF	TF		
Fat, %				
Colostrum	6.78	6.77	0.567	0.995
Milk (d 21)	7.17	6.76	0.289	0.642
Lactose, %				
Colostrum	4.02 ^a	4.42 ^b	0.168	0.049
Milk (d 21)	5.82	5.95	0.074	0.954
Protein, %				
Colostrum	8.96 ^a	6.94 ^b	0.936	0.041
Milk (d 21)	4.80	4.59	0.107	0.891
Solid-not-fat, %				
Colostrum	13.43 ^a	11.84 ^b	0.785	0.049
Milk (d 21)	10.83	10.76	0.084	0.974
Total solid, %				
Colostrum	21.71 ^c	20.22 ^d	0.915	0.081
Milk (d 21)	19.26	18.65	0.362	0.746

^{a,b}Means with different superscripts in the same row significantly differ (P<0.05).

^{c,d}Means with different superscripts in the same row numerically differ (P<0.10).

¹OF = once daily feeding; TF = twice daily feeding.

²Standard error of means.

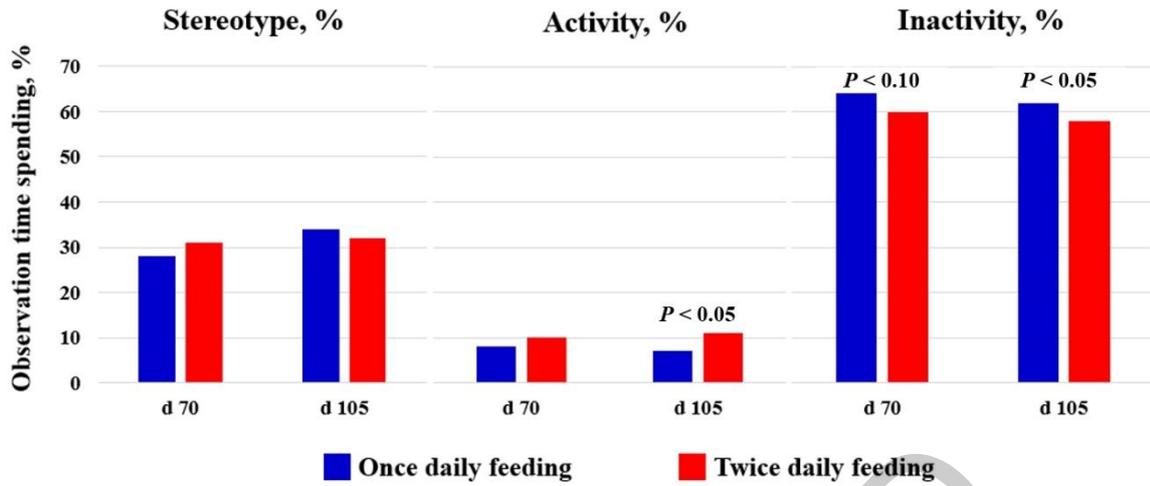
308 **Table 4.** The effect of gestation feeding frequency on litter size, litter weight and piglet weight in
 309 lactating sows

Criteria	Treatment ¹		SEM ²	p-value
	OF	TF		
No. Sows	10	10	-	
Litter size, no. of piglets				
Total born	12.7	11.9	0.76	0.343
Stillborn	1.3	1.2	0.40	0.910
Mummy	0.0	0.0	0.00	-
Born alive	11.4	10.6	0.53	0.295
After-cross-fostering	10.8	10.8	0.14	-
Death	0.3	0.4	0.13	0.726
Weaning pigs	10.5	10.4	0.17	0.758
Litter weight, kg				
At birth	19.82	17.34	1.054	0.152
After-cross-fostering	17.34	17.29	0.653	0.975
d 21	71.08	70.08	1.653	0.745
Litter daily weight gain (d 0-21)	2.56	2.51	1.442	0.715
Piglet weight, kg				
At birth	1.58	1.53	0.071	0.332
After-cross-fostering	1.60	1.61	0.063	0.971
d 21	6.77	6.75	0.130	0.966
Piglet daily weight gain (d 0-21)	0.25	0.25	0.100	0.938

¹OF = once daily feeding; TF = twice daily feeding.

²Standard error of means.

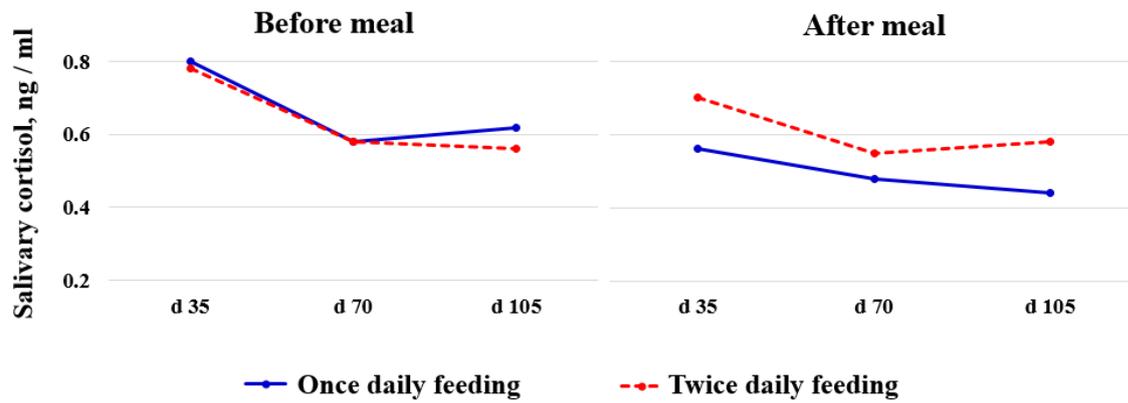
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312

313 **Figure 1.** The effect of feeding frequency on gestation sow activities (%) during 12 h observation from 06:00 to 18:00.

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315

316 **Figure 2.** The effect of feeding frequency on salivary cortisol concentrations before and after morning meal of gestating

317 sows (ng/ml)

318

319

320

ACCEPTED