Short communication: Short-term changes in stocking density did not alter meal characteristics of lactating Holstein dairy cattle

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ABSTRACT

The study objectives were to determine the effect of short-term increases in stocking density and milking on meal duration, meal frequency, and time between meals and to determine the bioequivalence of different meal criteria in a competitive environment. Forty-eight Holstein dairy cows were allotted to 1 of 4 groups ($n = 12$ per group). Stocking density treatments of 100 (one cow per freestall and headlock), 113, 131, and 142% were assigned to groups using a 4×4 Latin square with treatments imposed for 14-d periods. On d 11 of each period, feeding time was recorded for 24 h using 10-min scan samples from direct observation. Meals were defined as repeated observations of eating with a maximum of 20, 30, or 40 min of not eating between observations constituting the same meal. A new meal was established when a cow was observed feeding and then not feeding for greater than 2 (20 min), 3 (30 min), or 4 (40 min) observations. To evaluate diurnal effects, the 24-h period of data was divided into 8-h intervals (based on milking time); morning (0400–1200 h), afternoon (1200–2000 h), and night (2000–0400 h). Feed delivery occurred daily at 0430 h, with feed pushed up throughout the day. A mixed linear model was used to determine the effect of stocking density and time of day on meals per day, meals per hour, meal duration, time between meals, and meal duration 2 h before and after milking. Regardless of stocking density, meal duration, meal frequency, meals per hour, and time between meals did not differ. Regardless of stocking density, mean meal duration was longer during the morning and afternoon compared with night. Meal duration was also greater after milking compared with before milking, regardless of stocking density. These results suggest meal length decreased throughout the day, relative to feed delivery, with periodic increases in length due to return from milking. Meals per hour, meal duration before and after milking, and meal fre-

quency established bioequivalence for the 20-, 30-, and 40-min meal criteria. Bioequivalence was not met for meal duration when the meal criterion was increased from 20 to 40 min. Short-term increases in stocking density of 14-d duration did not affect the feeding pattern of lactating dairy cows, indicating that mid-lactation dairy cows can compensate for reduced feed bunk access during short-term overstocking. When calculating feeding behaviors, including meal frequency and time between meals, using a meal criterion of 20, 30, or 40 min resulted in similar outcomes when using 10-min scan samples. Future studies should investigate changes in other behaviors, such as resting, which may be altered to compensate for reduced access to the feed bunk.

Key words: dairy cattle, stocking density, feeding behavior, meal criterion

Short Communication

Cows typically eat approximately 5 to 8 meals throughout the day (Tolkamp et al., 2000; DeVries et al., 2003b), which consist of short intervals of feeding and nonfeeding activity within a meal and longer intervals between meals (Tolkamp et al., 1998). Feeder design, social hierarchy, and stocking density (STDN) may all affect the frequency and duration of visits to the feeder (Tolkamp et al., 2000). Use of meals to characterize feeding behavior reduces the variability associated with feeder visits (Tolkamp et al., 2000). Therefore, using a meal criterion, or a minimum time interval between visits constituting a new meal (Tolkamp et al., 1998), is necessary when assessing feeding behavior. Specifically, this technique can be used when assessing meal frequency and length instead of individual feed bunk visits.

Overstocking is a common practice in the United States (USDA, 2010). Current guidelines recommend providing lactating cows a minimum of 0.6 m of feed bunk space or one cow per headlock (USDA, 2010). However, based on the Dairy 2007 survey, 57.1% of producers provided less than 0.6 m of feed bunk space, whereas 45.9% of producers provided 1 headlock to 1.1

Received March 20, 2015.

Accepted January 29, 2016.

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cows or more (USDA, 2010). Cows compensate for reduced time at the feeder by eating more frequent meals and increasing consumption rate (Olofsson, 1999) to maintain DMI (Krawczel et al., 2012b). Faster, less frequent meals, or “slug feeding,” may reduce ruminal pH levels and cause cows to develop subacute ruminal acidosis, which is also associated with laminitis (Nocek, 1997), making feeding behavior and meal characteristics an important consideration when managing dairy cows.

Dairy cows follow a diurnal feeding pattern with feed bunk attendance highest during the day and early evening and lowest late at night and during early morning hours (DeVries et al., 2003a). However, feeding motivation is typically highest after fresh feed delivery and the return from the milking parlor, with feed push up adding a little variation to the pattern (DeVries et al., 2003a). Considering that milking times can increase occupancy at the feed bunk and STDN can increase competition at the feed bunk, incremental increases in STDN may have negative effects on the feeding behavior of dairy cattle, especially around the time of milking.

The prevalence of overstocking the feed bunk creates a need to understand how it is affecting feeding behavior of dairy cows, particularly when cows are highly motivated to feed. Further, a competitive environment at feeding may affect meal length calculations. Therefore, the objectives of this study were to (1) determine the effect of short-term increases in STDN, and milking on meal duration and frequency of lactating Holstein dairy cattle, (2) determine the effect of time of day on feeding behavior, and (3) determine what meal criterion is most appropriate to use when cows are feeding in a competitive environment using bioequivalence.

This data set was part of a larger study (Krawczel et al., 2012a). Twelve focal cows from 4 pens ($n = 48$) were selected from a larger group of 44 primiparous and 92 multiparous lactating Holstein dairy cows. Cows were housed in a naturally ventilated, 4-row free stall barn at the William H. Miner Agricultural Research Institute (Chazy, NY), and feed delivery occurred once daily at 0430 h, with feed pushed up throughout the day. Full details of cow selection, housing, and management were described by Krawczel et al. (2012a).

Cows were assigned to 1 of 4 treatment groups (100, 113, 131, and 142% STDN; $n = 34$ cows per group) using a 4×4 Latin square design. Treatment periods lasted for 14 d (Krawczel et al., 2012a). Previous research has shown that 1 d is sufficient to generate treatment differences in feeding behavior of dairy cattle (Grant et al., 1990). Further, direct behavioral observations occurred on d 11 due to no conflicts with other

sampling activities or herd health checks occurring to allow for 24 h of uninterrupted behavioral monitoring. Feeding behavior of focal cows (feeding, ruminating, drinking, or no activity) was recorded by 4 observers at 10-min intervals (Mitlöhner et al., 2001; Endres et al., 2005). Cows were considered feeding if their head was through the headlock at the time of observation.

The 10-min intervals used to monitor feeding behavior were used to develop meal criteria, or minimum time interval between visits constituting a new meal (Tolkamp et al., 1998). Many observational studies take advantage of a 10-min scan sampling technique, which was similar to continuous scan sampling for feeding behavior (Mitlöhner et al., 2001), making meal criteria of multiples of 10 necessary to use in the present study. Meal criteria of 20, 30, and 40 min were selected based on previous research by Tolkamp et al. (1998) who estimated a meal criterion of 44.7 min in a noncompetitive environment using preassigned automated feeders, and DeVries et al. (2003b) who estimated a pooled meal criterion of 27.7 min in a competitive environment using a post-and-rail feeding design. Using a range of 20- to 40-min meal criteria allows for comparison of feeding behaviors when cows are in a competitive environment but using a headlock design, which reduces the number of displacements and competition compared with the post-and-rail design (Endres et al., 2005).

Meal duration, meal frequency, and time between meals were calculated using the 3 meal criteria of 20, 30, and 40 min. Meal duration was defined as repeated observations of feeding with a maximum of 20, 30, or 40 min of not feeding between observations constituting the same meal. The total number of observations was multiplied by 10 min to determine meal feeding time. For example, when using a 20-min meal criterion, if a cow was feeding for one observation and then noted as not feeding for greater than 2 observations, the next feeding observation would establish a new meal. Time between meals was calculated by adding the total number of not-eating events after meals had been calculated using the relevant meal criterion, and then multiplying the total by 10 min. Meal frequency was the total number of meals calculated during the 24-h period. Meals per hour was calculated by totaling the number of meals within each hour block.

To evaluate diurnal effects, the 24 h of data were divided into 3 time blocks related to milking times: morning (0400 to 1150 h), afternoon (1200 to 1950 h), and night (2000 to 0350 h). To determine the effect of milking on meal duration, data were summarized into 2-h blocks before and after each milking. Milking times ranged from 0430 to 0640 h (**Milk1**), 1230 to 1430 h (**Milk2**), and 2030 to 2330 h (**Milk3**), with the first

cow leaving at the first time and the last cow returning at the latter time.

The observational unit of this study was the cow and the experimental unit was the pen. Separate models were developed for each of the meal criteria, 20, 30, and 40 min, analyzing the variables of interest. The MIXED procedure of SAS (SAS 9.3, SAS Institute Inc., Cary, NC) was used to evaluate the effect of STDN on meals per day with pen $\times$ period $\times$ STDN as a random variable. Meals per hour, meal duration, time between meals, and meal duration before and after milking were summarized by pen $\times$ period $\times$ STDN. The MIXED procedure of SAS was used to determine the effect of STDN, time block, and the interaction of STDN and time block on meals per hour, meal duration, and time between meals, with pen $\times$ period $\times$ STDN and pen $\times$ period $\times$ STDN $\times$ time block as the random variables and observations repeated by time, with pen $\times$ period $\times$ STDN as the subject and an autoregressive covariate structure. Finally, the MIXED procedure of SAS was used to determine the effect of STDN, time before and after milking, and the interaction between STDN and time before and after milking on feeding duration with pen $\times$ period $\times$ STDN as a random variable. Normality of data was confirmed using the univariate procedure of SAS.

Meal criteria were evaluated for bioequivalence, the concept that 2 treatments are biologically equivalent (Tempelman, 2004), of meal duration, meal frequency, meals per hour, and meal duration before and after milking using the method described by Tempelman (2004). Bioequivalence was established if the 95% confidence interval was completely within the predetermined bioequivalence interval at an α of 0.05. Bioequivalence intervals for meal duration and daily meal frequency were established from Tolcamp et al. (2000) and set at ± 8.1 min/bout and ± 0.8 meals/d, respectively. No information has been reported on meals per hour under conditions similar to our study. Therefore, to create an equivalence interval, the interval for daily meal frequency was divided by 24 to estimate that for meals per hour.

Regardless of meal criterion, STDN and the interaction between STDN and time block did not affect meal duration (Table 1; $P > 0.10$), time between meals ($P > 0.10$), or meals per hour ($P > 0.10$). Similarly, STDN did not affect meal frequency ($P > 0.10$), regardless of meal criterion. Meal duration before or after milking was unaffected by STDN and the interaction between STDN and return from milking, regardless of meal criterion ($P > 0.10$). Diurnal changes were evident in meal duration ($P < 0.01$; Table 2), with cows eating for the longest duration during the morning and afternoon and shortest duration during the night. Cows consumed

Table 1. Least squares means ($\pm$ SE) of feeding behavior of focal cows from each pen in response to each stocking density treatment using a 20-, 30-, or 40-min meal criterion

Item	Variable	Stocking density treatment ¹				df	F-value	P-value
		100%	113%	131%	142%			
20-min meal criterion ²	Meal duration, ³ min	33.9 $\pm$ 2.9	36.8 $\pm$ 2.9	37.4 $\pm$ 2.9	38.5 $\pm$ 2.9	12	0.44	0.73
	Meals per hour, no./h	0.29 $\pm$ 0.02	0.27 $\pm$ 0.02	0.28 $\pm$ 0.02	0.27 $\pm$ 0.02	12	0.34	0.80
	Time between meals, min	160.1 $\pm$ 6.6	176.9 $\pm$ 6.6	169.9 $\pm$ 6.5	178.9 $\pm$ 6.6	12	1.66	0.23
	Meal frequency, no./d	7.0 $\pm$ 0.2	6.6 $\pm$ 0.2	7.0 $\pm$ 0.2	6.7 $\pm$ 0.3	12	0.77	0.53
30-min meal criterion ²	Meal duration, min	36.3 $\pm$ 3.2	39.7 $\pm$ 3.2	39.7 $\pm$ 3.2	40.3 $\pm$ 3.2	12	0.33	0.81
	Meals per hour, no./h	0.29 $\pm$ 0.02	0.27 $\pm$ 0.02	0.28 $\pm$ 0.02	0.27 $\pm$ 0.02	12	0.32	0.81
	Time between meals, min	120.9 $\pm$ 6.7	131.6 $\pm$ 6.6	117.0 $\pm$ 6.2	120.7 $\pm$ 6.4	12	0.95	0.45
	Meal frequency, no./d	6.9 $\pm$ 0.2	6.4 $\pm$ 0.2	6.8 $\pm$ 0.2	6.6 $\pm$ 0.2	12	0.85	0.49
40-min meal criterion ²	Meal duration, min	40.1 $\pm$ 3.1	43.0 $\pm$ 3.2	44.8 $\pm$ 3.1	44.9 $\pm$ 3.2	12	0.50	0.69
	Meals per hour, no./h	0.27 $\pm$ 0.02	0.26 $\pm$ 0.02	0.26 $\pm$ 0.02	0.26 $\pm$ 0.02	12	0.28	0.84
	Time between meals, min	121.2 $\pm$ 6.7	131.2 $\pm$ 6.6	116.4 $\pm$ 6.2	121.2 $\pm$ 6.5	12	0.92	0.46
	Meal frequency, no./d	6.6 $\pm$ 0.2	6.3 $\pm$ 0.2	6.4 $\pm$ 0.2	6.3 $\pm$ 0.2	12	0.75	0.54

¹Stocking densities were 1.00, 1.13, 1.31, and 1.42 cows per stall and headlock.

²Meal criterion is the minimum time interval between visits constituting a meal.

³Meals were defined as repeated observations of eating with a maximum of 20, 30, or 40 min of not eating between observations.

more meals per hour during the afternoon than during the morning and night ($P < 0.01$; Table 2). Time between meals was greatest in the morning and shortest at night ($P < 0.01$; Table 2). Meal duration varied in relation to return from milking ($P < 0.01$; Table 3) with cows eating for longer duration after Milk1 and for the shortest duration before Milk1.

Meals per hour, meal duration before and after milking, and meal frequency calculated using the 20-, 30-, or 40-min meal criterion were bioequivalent ($P > 0.05$; Table 4). Meal durations calculated with meal criterion of 20 or 40 min were not similar ($P < 0.05$).

Results for feeding behavior, including meal duration, meal frequency, meals per hour, and time between meals were unaffected by short-term changes in STDN. However, diurnal alterations in feeding behavior existed, particularly around return from milking. Also, this is the first study aimed to determine whether meal criterion in a competitive feeding environment had an effect on different feeding behaviors and the bioequivalence of those meal criteria.

The present study found no effect of short-term changes in STDN on meal characteristics. This contradicts previous work by Olofsson (1999) who determined that increasing STDN increased meal frequency while reducing feeding duration. However, a meal criterion of 5 min was used to calculate meals, compared with the 20-, 30-, or 40-min meal criteria in the current study, which may be a reason for the differences in meal frequency and length. Additionally, in the present study, a maximum STDN of 142% may have avoided differences in feeding times. However, because the STDN imposed closely relates to the 142% overstocking rate practiced in regions of North America (von Keyserlingk et al., 2012), it may be more advantageous to investigate the long-term effect of STDN reflective on those experi-

enced by lactating dairy cows in commercial settings rather than higher STDN.

Longer meals occurred during the morning and afternoon compared with night, regardless of STDN. This is consistent with the existing literature (DeVries et al., 2003a) where feed bunk attendance was greatest during the early morning and daytime hours. However, this may also be largely driven by milking times and fresh feed delivery (DeVries et al., 2003a). In the current study, cows had the longest meal duration during the 2 h after 0400 h milking, which followed feed delivery. DeVries and von Keyserlingk (2005) determined that delivery of fresh feed increased feeding time more than returning from milking, whereas feed push-ups did little to alter the diurnal feeding behavior. Meal duration tapered to near zero during the 2 h before the 0400 h milking. Cows milked 3 times per d and fed once per day were least active between 0000 and 0400 h (Krawczel et al., 2012a), whereas cows milked and fed twice per day were least active between 2000 and 0400 h (Endres et al., 2005). This shadows the pattern cows follow in relation to feeding and milking times (DeVries et al., 2003a) and when cows may be more motivated to rest than eat.

Meals per hour, meal duration before and after milking, and meal frequency established bioequivalence for the 20-, 30-, and 40-min meal criteria. Bioequivalence was not met for meal duration when the meal criterion was increased from 20 to 40 min. However, bioequivalence was met when increased from 20 to 30 min or 30 to 40 min. This indicates that meal duration is more sensitive to changes in meal criterion than meals per hour, meal frequency, and meal duration before and after milking. As the current study determined no difference in meal behavior with increased competition, it is unlikely that the lack of bioequivalence in related

Table 2. Least squares means ($\pm$ SE) of feeding behavior of focal cows from each pen during different time blocks using a 20-, 30-, and 40-min meal criterion

Item	Variable	Time block ¹			df	F-value	P-value
		Morning	Afternoon	Night			
20-min meal criterion ²	Meal duration, ³ min	37.6 $\pm$ 1.9 ^a	40.3 $\pm$ 1.8 ^a	32.0 $\pm$ 1.8 ^b	24	10.49	<0.01
	Meals per hour, no./h	0.27 $\pm$ 0.01 ^b	0.32 $\pm$ 0.01 ^a	0.25 $\pm$ 0.01 ^b	24	6.35	<0.01
	Time between meals, min	210.1 $\pm$ 5.8 ^a	171.1 $\pm$ 5.4 ^b	142.2 $\pm$ 5.9 ^c	24	25.47	<0.01
30-min meal criterion ²	Meal duration, ³ min	40.1 $\pm$ 2.0 ^a	42.6 $\pm$ 1.9 ^a	34.4 $\pm$ 2.0 ^b	24	8.41	<0.01
	Meals per hour, no./h	0.26 $\pm$ 0.02 ^b	0.31 $\pm$ 0.01 ^a	0.25 $\pm$ 0.01 ^b	24	6.08	<0.01
	Time between meals, min	144.9 $\pm$ 5.7 ^a	125.0 $\pm$ 5.4 ^b	97.9 $\pm$ 5.6 ^c	24	17.28	<0.01
40-min meal criterion ²	Meal duration, ³ min	44.6 $\pm$ 2.2 ^a	47.4 $\pm$ 2.0 ^a	37.6 $\pm$ 2.2 ^b	24	8.45	<0.01
	Meals per hour, no./h	0.25 $\pm$ 0.01 ^b	0.30 $\pm$ 0.01 ^a	0.24 $\pm$ 0.01 ^b	24	5.28	0.01
	Time between meals, min	143.2 $\pm$ 5.8 ^a	125.2 $\pm$ 5.4 ^b	99.2 $\pm$ 5.7 ^c	24	15.02	<0.01

^{a-c}Means with different superscripts differ ($P < 0.01$).

¹Time blocks were morning (0400–1150 h), afternoon (1200–1950 h), and night (2000–0350 h), based on milking time.

²Meal criterion is the minimum time interval between visits constituting a meal.

³Meals were defined as repeated observations of eating with a maximum of 20, 30, or 40 min of not eating between observations.

Table 3. Least squares means ($\pm$ SE) of meal duration of focal cows before and after milking according to milking time using a meal criterion of 20, 30, or 40 min

Item	Meal duration (min)						df	F-value	P-value
	Milk1 ¹		Milk2 ¹		Milk3 ¹				
	Before ²	After ²	Before ²	After ²	Before ²	After ²			
20-min meal criterion ³	4.8 ± 1.8 ^c	34.0 ± 1.8 ^a	28.2 ± 1.8 ^b	26.2 ± 1.8 ^b	29.9 ± 1.8 ^{ab}	28.4 ± 1.8 ^b	60	38.31	<0.01
30-min meal criterion ³	4.8 ± 1.8 ^c	34.4 ± 1.8 ^a	28.5 ± 1.8 ^b	26.6 ± 1.8 ^b	31.0 ± 1.8 ^{ab}	29.9 ± 1.8 ^{ab}	60	38.08	<0.01
40-min meal criterion ³	5.0 ± 2.0 ^d	35.9 ± 2.0 ^a	29.2 ± 2.0 ^{bc}	27.1 ± 2.0 ^c	32.5 ± 2.0 ^{ab}	32.4 ± 2.0 ^{abc}	60	35.33	<0.01

^{a-d}Means with different superscripts within the same row differ ($P < 0.01$).
¹Milking times corresponded to approximately 0400 h (Milk1), approximately 1200 h (Milk2), and approximately 2000 h (Milk3).
²Before refers to the 2 h before milking and after refers to the 2 h after milking.
³Meal criterion is the minimum time interval between visits constituting a meal.

to increased aggression. However, sampling techniques, such as scan samples versus continuous monitoring, and housing environment, such as headlocks versus post-and-rail barriers, may play a role in meal behavior and, thus, meal criteria. Therefore, when conducting feeding behavior research using 10-min scan samples in a headlock system, using a shorter meal criterion of 20 or 30 min may result in similar estimations for meal variables.

Limitations exist in the current study, including behavioral sampling time period and the calculation of meals. The current study used 1 d to record behavioral observations within each period, as this was the only day allowing for 24 h of uninterrupted observation. Many factors can influence the day-to-day variation in dairy cow behavior, including social structure (Grant and Albright, 2001), estrus cycles (Halli et al., 2015, Pahl et al., 2015), and disease (Fogsgaard et al., 2015). To help mitigate these variations, the study allowed 9 d to acclimate to the pens and establish a hierarchy, although cow feeding behavior was only affected during the 1 h after the first fresh feed delivery after regrouping (von Keyserlingk et al., 2008). Individual cow data were removed if a cow displayed estrus behavior. Also, regular health exams were performed on cows. In addition, the use of 10-min scan samples to determine meals is not a validated technique. A high correlation exists between feeding data collected using continuous sampling and 10-min scan sampling in feedlot cattle (Mitlöhner et al., 2001). Further, previous research has used feed bunk visit data to mathematically calculate meals (Tolkamp et al., 2000; DeVries et al., 2003b), and although similar approaches were used, the results varied from 28- to 45-min meal criteria, demonstrating the variability and effect of the environment on the meal criteria. However, readers must use caution when considering the current technique because it is an invalidated approach, unlike those previously mentioned.

In conclusion, short-term increases in STDN did not influence the feeding pattern of lactating dairy cows. However, cows typically ate for longer bouts more frequently during the morning and afternoon hours, and meal duration was greatest after feed delivery and after milking, in agreement with previously reported results of diurnal feeding behavior in dairy cattle. When calculating feeding behaviors, including meal frequency and time between meals, using a meal criterion of 20, 30, or 40 min resulted in similar outcomes when using 10-min scan samples. Future studies should investigate changes in behaviors that may become altered when cows are overstocked for longer periods. If cows do not alter feeding behavior, other behaviors such as resting may be altered to compensate.

Table 4. The bioequivalence of selected response variables among meal criterion using 90% confidence intervals of the difference of LSM

Variable	Meal criterion ¹ compared			Equivalence interval
	20 vs. 30	20 vs. 40	30 vs. 40	
Meal duration, min	−5.08 to 1.35	−8.83 to −2.39 ^a	−6.97 to −0.52	±8.1 min
Meals per hour, no./h	−0.01 to 0.02	0.00 to 0.03	−0.01 to 0.03	±0.03 no./h
Meal duration before and after milking, min	−3.29 to 2.04	−4.41 to 0.92	−3.79 to 1.54	±8.1 min
Meal frequency, no./d	−0.27 to 0.56	0.01 to 0.83	−0.14 to 0.69	±0.8 no./d

^a90% confidence intervals without a superscript are bioequivalent ($P > 0.05$).

¹Meal criterion is the minimum time interval between visits constituting a meal.

ACKNOWLEDGMENTS

The authors thank the dairy barn and research staff at the William H. Miner Agricultural Research Institute (Chazy, NY) for their assistance with this project. This research was supported by USDA Hatch funds and the USDA-AFRI (CSREES Award No.: 2008-35204-04584).

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