Management practices, physically effective fiber, and ether extract are related to bulk tank milk de novo fatty acid concentration on Holstein dairy farms

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ABSTRACT

The objective of this study was to evaluate the relationship of management practices and dietary factors with de novo fatty acid concentration in bulk tank milk from commercial dairy farms milking Holstein cows. Farms were selected based on de novo fatty acid concentration during the 6 mo before the farm visit and were categorized as high de novo (HDN; 24.61 ± 0.75 g/100 g of fatty acids, mean $\pm$ standard deviation; $n = 19$) or low de novo (LDN; 23.10 ± 0.88 g/100 g of fatty acids; $n = 20$). Farms were visited once in February, March, or April 2015 and evaluated based on management and facility design known to affect cow behavior, physical and chemical characteristics of the diet, and ration formulation and forage analyses obtained from the farm's nutritionist. We observed no differences between HDN and LDN farms in farm size, time away from the pen for milking, days in milk, or body condition score. We detected no differences between HDN and LDN farms in milk fat or true protein yield; however, milk fat and protein content and de novo fatty acid yield per day were higher for HDN farms, as was gross income per unit of milk sold. High de novo farms tended to be more likely to deliver fresh feed twice versus once per day, have a freestall stocking density $\leq 110\%$, and provide ≥ 46 cm of feed bunk space per cow. We observed no detectable differences in forage quality or ration dry matter, crude protein, or starch content. However, ether extract was lower and physically effective neutral detergent fiber was higher for HDN farms. Feeding management, stocking density, dietary ether extract content, and the physical characteristics of the diet are related to de novo fatty acid, fat, and protein concentration in bulk tank milk from high-producing Holstein dairy farms.

Key words: de novo fatty acids, milk fat, milk protein

INTRODUCTION

De novo milk fatty acids (FA; C4 to C14) are those that are synthesized in the cow's mammary gland using acetate and butyrate—VFA that originate from the fermentation of fibrous feeds in the rumen (Palmquist et al., 1993). Mixed-origin milk fatty acids (C16) can be synthesized in the mammary cells from acetate and butyrate or can enter the mammary secretory cell preformed; at present, no method exists to determine these separately. Preformed FA ($\geq C18$) enter the mammary cell from the blood stream and originate from the cow's intestinal, liver, or adipose tissues.

A large and systematic change in the fatty acid composition of milk fat occurs with stage of lactation (Lynch et al., 1992); de novo FA start out as low a proportion of total FA in early lactation and increase to a relatively constant concentration when cows are in positive energy balance. In high-producing Holstein cows, de novo FA typically account for 18.2 to 28.4% of the total FA in milk fat (Jensen, 2002). Factors that affect ruminal conditions, such as nutrition and management, may influence milk fatty acid profiles and are the predominant factors that affect milk de novo fatty acid synthesis among cows of similar breed and stage of lactation (Palmquist et al., 1993; Bauman and Griinari, 2003).

High dietary carbohydrate fermentability and excessive ruminally available PUFA may depress de novo fatty acid synthesis (Harvatine and Bauman, 2011). Dietary fat supplementation may also influence milk fat composition by directly providing substrate for preformed milk FA (Stoffel et al., 2015), although the extent of fatty acid variation may be limited by the melting point of milk fat (Toral et al., 2013). Adequate physically effective NDF (**peNDF**; 20 to 23% of ration DM; Mertens, 2002) has been associated with an increase in ruminal pH (Allen, 1997; Kononoff and Heinrichs, 2003) and greater milk fat (Grant et al., 1990) and milk protein content (Caccamo et al., 2014). However, to our knowledge no studies have evaluated

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the relationship between the physical characteristics of the diet and bulk tank de novo FA concentration on commercial Holstein dairy farms.

In addition to the diet, feeding environment may also influence milk composition. For example, stocking density and facility design may influence feeding behaviors such as increasing feeding rate (Collings et al., 2011) or sorting against long particles (Sova et al., 2013), which increase a cow's risk for low ruminal pH (French and Kennelly, 1990). Management practices and dietary characteristics that result in low ruminal pH are associated with reduced milk fat content (Allen, 1997) via a shift to the alternate ruminal biohydrogenation pathway, leading to the formation of UFA isomers that downregulate the expression of genes related to de novo FA synthesis (Harvatine and Bauman, 2011).

Previous research has evaluated the relationship between management practices and bulk tank milk de novo FA content for lower-producing, smaller dairy farms that contain a variety of breeds (Woolpert et al., 2016), and it has predicted FA composition using data that describe the source and proportion of forages in the diet (Coppa et al., 2013). However, we need to better understand the relationships among cow management practices, dietary fat content, peNDF, and de novo FA concentration for high-producing Holstein dairy farms. The objectives of this study were to evaluate management practices, dietary chemical composition and physical characteristics, and lactation performance for high-producing Holstein dairy farms with high or low de novo FA concentrations in bulk tank milk. We hypothesized that Holstein dairy farms that optimized cow comfort and fed adequate peNDF would have higher de novo FA concentrations and higher milk fat and protein content in bulk tank milk.

MATERIALS AND METHODS

On-Farm Data Collection

Farms were enrolled based on their willingness to participate in the study and the concentration of de novo FA in bulk tank milk samples taken 2 to 20 times per month for each farm over the 6 mo before the study began. Farms were categorized as high de novo (HDN) or low de novo (LDN) based on their average de novo FA concentration (g/100 g of FA) for the 6 mo before the farm visit (September 2014 to February 2015; Table 1). To be eligible to participate in the study, farms needed to be a member of the St. Albans Cooperative Creamery (St. Albans, VT), average at least 25 kg of milk per cow per day, have 90% or more Holstein cattle, milk at least 50 cows, and calve year-round. Each farm was visited once between February 25 and April 24, 2015.

Management practices, BCS, and herd demographics were recorded by trained research personnel from Miner Institute (Chazy, NY), working with a farm owner or manager. The primary management factors assessed were stall stocking density, feed bunk space per cow, feeding frequency, feed push-up frequency, time away from pen for milking, and milking frequency.

Diets were sampled for chemical composition analysis as described by Woolpert et al. (2016). Previous research had found no detectable differences in the chemical composition of corn silage or hay crop silage for HDN versus LDN herds (Woolpert et al., 2016), so analysis of these components was omitted from the study. However, the most recent hay crop silage and corn silage analyses were obtained from the farm's nutritional consultant to assess the content of DM, CP,

Table 1. Milk composition data (used to select farms to participate in the study) representing monthly mean milk composition for high de novo (HDN; n = 19) and low de novo (LDN; n = 20) farms from September 2014 to February 2015

Item	HDN			LDN		
	Mean $\pm$ SD	Minimum	Maximum	Mean $\pm$ SD	Minimum	Maximum
Fat, %	3.96 $\pm$ 0.15	3.72	4.19	3.75 $\pm$ 0.19	3.43	4.15
True protein, %	3.19 $\pm$ 0.09	2.99	3.32	3.10 $\pm$ 0.07	2.98	3.20
De novo fatty acids (FA) ¹						
g/100 g of milk	0.92 $\pm$ 0.05	0.79	1.00	0.81 $\pm$ 0.06	0.72	0.92
g/100 g of FA	24.61 $\pm$ 0.75	22.57	26.15	23.10 $\pm$ 0.88	21.22	24.45
Mixed FA ²						
g/100 g of milk	1.53 $\pm$ 0.09	1.37	1.68	1.41 $\pm$ 0.11	1.19	1.65
g/100 g of FA	41.15 $\pm$ 1.04	39.28	43.35	39.94 $\pm$ 1.42	36.91	42.29
Preformed FA ³						
g/100 g of milk	1.27 $\pm$ 0.05	1.16	1.34	1.30 $\pm$ 0.05	1.20	1.41
g/100 g of FA	34.42 $\pm$ 1.35	31.99	37.31	36.96 $\pm$ 1.86	34.02	40.74

¹C4 to C14.

²C16, C16:1, and C17.

³Greater than or equal to C18.

organic-matter-corrected NDF with α -amylase and sodium sulfite (**aNDFom**), ether extract, starch (for corn silage only), and ash as a percentage of DM. Forage analyses were provided by participating nutritionists from 33 farms ($n = 15$ HDN; $n = 18$ LDN).

Total mixed ration samples were collected from 5 to 10 locations along the length of the feed bunk immediately after feed delivery. The TMR samples were mixed manually and subsampled using the quartering technique: mixed samples were divided into 4 equal subsamples. Two subsamples allocated diagonally were remixed and sent to a commercial laboratory for analysis (Dairy One, Ithaca, NY) as described by Woolpert et al. (2016). The TMR samples were analyzed using near infrared spectroscopy with a local algorithm that has been shown to have good to moderate utility to predict CP, aNDFom, CP, starch, and fat content ($R^2 > 0.81$; Berghazi et al., 2000; Mentink et al., 2006).

The remaining 2 subsamples were analyzed separately for particle size distribution on an as-fed basis using the Penn State Particle Separator as described by Heinrichs and Kononoff (2002) and modified to include a 4-mm sieve as reported by Kmicikewycz et al. (2015). Residues on the 3 sieves and the pan were weighed, and the 2 replicates were averaged. The peNDF was calculated by multiplying the proportion of TMR retained on and above the 4-mm sieve by the NDF content of the ration (Kmicikewycz et al., 2015). A weighted average of peNDF based on the number of cows consuming each diet was calculated for each farm. Outliers ($n = 3$) were identified and removed from the data set when Cook's D exceeded 0.13.

Ingredients for concentrate feeds included in all diets fed to lactating cows on each farm were requested from the feed companies. Diets were provided by participating nutritionists from 33 farms ($n = 15$ HDN; $n = 18$ LDN). Feed products were categorized based on previous associations with milk fat content or composition as described by Woolpert et al. (2016).

Milk Yield, Composition, and FA Analysis

Milk yield was determined using the number of cows milking during the farm visit and the average bulk tank milked shipped during the month of the farm visit. Milk composition data were averaged for the month of the farm visit (February, March, or April 2015) and analyzed as reported by Woolpert et al. (2016). In short, milk fat, true protein, and anhydrous lactose percentages were determined using a Fourier transform mid-infrared spectrophotometer (Lactoscope FTA; Delta Instruments, Drachten, the Netherlands) at the St. Albans Cooperative Creamery payment testing laboratory (St. Albans, VT). Calibration for measure-

ment of fat, true protein, anhydrous lactose, and FA parameters was done using modified milk calibration samples (Kaylegian et al., 2006) produced monthly. All laboratory mean reference chemistry values were produced by a network of 10 to 12 laboratories for the main components (Wojciechowski et al., 2016), and the reference values for FA composition of the same set of samples were determined as previously described (Wojciechowski and Barbano, 2016).

Milk FA analysis was conducted at the St. Albans Cooperative Creamery payment testing laboratory at the same time as the component milk testing, as described by Woolpert et al. (2016), using partial least squares chemometric prediction models based on the mid-infrared spectra described by Wojciechowski and Barbano (2016).

Statistical Analysis

Statistical analyses were conducted using SAS (version 9.2; SAS Institute Inc., Cary, NC). Data were summarized by HDN and LDN group and checked for normality using the UNIVARIATE procedure. Data were considered to be normally distributed when the Shapiro-Wilks W was >0.85 . Differences in management, milk composition, and diet for HDN versus LDN farms were determined using the GLIMMIX procedure. De novo group was the fixed effect and farm was the random effect using the following equation,

$$Y_{ij} = \mu + \alpha_i + R_j + E_{ij},$$

where Y_{ij} is the dependent variable, μ is the overall mean, α_i is the fixed effect of de novo group, R_j is the random effect of farm, and E_{ij} is the residual error.

All milk composition and diet composition variables that were normally distributed were tested using a Gaussian distribution with an identity link function. Management data that were not normally distributed were analyzed using an alternative distribution, and the model was checked for overdispersion using the chi-squared/degrees of freedom ratio. Models with a ratio close to 1.0 were considered a good model fit (Gbur et al., 2012). The management variables, distributions, and link functions used in the analysis are listed in Table 2. The inclusion of supplements or additives in the concentrate mix was analyzed using a binary distribution and expressed as the percentage of farms in each category, the odds ratio, and the 95% confidence limit (HDN group was the referent).

A Poisson distribution with a log link function was used to test the number of cows milking on each farm and the number of times feed was pushed up at the feed bunk. A negative binomial distribution with a log

Table 2. Distributions and link functions used to test variables between high de novo and low de novo herds

Variable	Distribution	Link function
Cows milking, no.	Poisson	Log
Time away from pen, h	Gaussian	Identity
Feeding frequency		
Freestall	Binary	Logit
Tiestall	Gaussian	Identity
Push-up frequency		
Freestall	Poisson	Log
Tiestall	Poisson	Log
DIM	Gaussian	Identity
Feed bunk space, cm/cow	Negative binomial	Log
Stocking density, cows/stall	Gaussian	Identity
BCS	Gaussian	Identity
Feed delivery frequency	Binary	Logit
Milking frequency	Binary	Logit

link function was used to test linear feed bunk space available per cow. Feed delivery frequency (1 or 2 times per d), milking frequency (2 or 3 times per d), and the inclusion of supplements or additives in the concentrate mix were analyzed using a binary distribution and expressed as the percentage of farms in each category, the odds ratio, and the 95% confidence limit (HDN group was the referent; Table 2).

Additional analyses were conducted for dietary and management factors by converting interval data into nominal data surrounding a common or industry recommended cut-point (Table 3). The data were then analyzed using the GLIMMIX procedure using a binary distribution and a logit link function and were expressed as the percentage of farms in each category, the odds ratio, and the 95% confidence limit (HDN group was the referent). Differences were declared significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Farm Characterization and Milk Composition

A total of 40 farms were visited ($n = 20$ HDN; $n = 20$ LDN). One HDN farm was removed from the data set due to missing milk composition data, because their milk was shipped to a different processing plant during the month of the farm visit. Therefore, 39 farms ($n = 19$ HDN; $n = 20$ LDN) were included in the final analysis.

Milk composition data from the 6 mo before the farm visit are presented in Table 1. Milk FA composition and bulk tank fat and protein tests normally vary across time within a farm. The data in Table 1 represent the average for the HDN and LDN herds across time before the study and were used for general selection and grouping of farms from the St. Albans Coopera-

Table 3. Cut-point and literature used to categorize management characteristics for analysis using odds ratios

Variable	Cut-point	Reference	Comments
Stocking density, cows/stall	≤ 1.10 cows/stall	von Keyserlingk et al., 2012	The average freestall stocking density for the northeastern United States is approximately 1.10 cows/stall
Feed bunk space, cm/cow	≥ 46 cm/cow	Krawczel et al., 2012	Treatments of 46 cm/cow or less reduced lying behavior
Feed push-up frequency	≥ 5 times per d	Miller-Cushon and DeVries, 2015	This study found no effect of feed push-up on behavior or performance. Their frequent push-up treatment was 5 times per d. However, this is the only known study in the published literature to compare a reasonable feed push-up frequency for commercial farms
Freestall feeding frequency	1 vs. 2 times per d	DeVries and von Keyserlingk, 2005	Two times vs. 1 time per d reduced sorting and increased feeding time. All freestall farms on the current study fed 1 or 2 times per d
Tiestall feeding frequency	≤ 3 times per d	Gibson, 1984	Tiestall-housed cows with low milk fat increased milk fat when fed 3 vs. 1 time per d
Time away from pen for milking	≤ 3 h per d	Charlton et al., 2014	Lying time affected when cows were away from the pen for 3.7 or more h per d
Milking frequency	2 vs. 3 times per d	Allen et al., 1986	All farms milked 2 or 3 times per d. Milking 3 vs. 2 times per day is associated with increased milk production

tive (St. Albans, VT) to include in the study. During the months of the study, the milk FA composition may have changed slightly compared with the average from the months before the study. When interpreting milk FA data to improve milk fat and protein production, changes in the relative proportion and output (grams per cow per day) of the groups of FA within a farm across time are used to diagnose the need for management or feeding changes. The real power in the data for routine on-farm management would be to review the changes in values for a farm across time, relating them to milk protein and fat content in the bulk tank milk and to feeding and management changes that caused increases or decreases.

Descriptive statistics, including the frequency of management practices, feeding strategies, and facility designs for HDN and LDN farms are presented in Table 4. We found a similar number of freestall facilities for HDN and LDN herds (16 and 14, respectively) that practiced TMR feeding (18 and 15, respectively), but fewer tiestall facilities (3 and 6, respectively) that practiced component feeding (1 and 5, respectively) for HDN versus LDN herds. The HDN and LDN herds had similar heat abatement (mostly fans), and a variety of freestall feeding systems were used, including post and rail, headlocks, H-bunks, or a combination of these, as well as 3 and 2 rows of stalls per pen.

No differences in milk, fat, and true protein yields were detected between HDN and LDN farms, but milk fat and true protein content were higher ($P < 0.01$) on HDN farms (Table 5). This positive relationship between de novo FA and milk fat and true protein

percentage agreed with the results of Barbano et al. (2014), who evaluated bulk tank milk composition on over 400 commercial dairy farms. De novo FA expressed as g/100 g of FA and as g/100 g of milk were higher ($P < 0.01$) on HDN farms, and preformed FA expressed as g/100 g of FA and as g/100 g of milk were lower ($P < 0.01$ and $P = 0.02$, respectively) on HDN farms. These results were consistent with previous research indicating that HDN farms had higher milk fat and true protein content in bulk tank milk (Woolpert et al., 2016). De novo FA yield, expressed as g/d, was higher ($P < 0.01$) for HDN farms, but we detected no difference detected in milk yield ($P = 0.91$), suggesting that cows on HDN farms synthesized more de novo FA. However, milk weights per cow were not measured directly; instead, they were estimated indirectly based on the number of cows milking on the day of the farm visit and the average bulk tank milk shipped per day during the month of the farm visit. Thus, the uncertainty in the milk weight data is higher than that for the milk composition data. Further research is needed under conditions in which milk weight per cow per day can be accurately measured, along with milk composition, to determine whether greater de novo FA synthesis is always associated with greater milk fat and true protein yields.

The difference in income per cow between the HDN and LDN herds would depend on the actual milk price at a given point in time. The average fat and protein price for Federal Milk Order No. 1 for February through April 2015 (US Department of Agriculture, 2015) was \$4.19 and \$5.74 per kg, respectively. Therefore, at 30 kg of milk per cow per day, the average HDN farm earned a gross of \$5.00 and \$5.49 per cow for fat and protein, respectively. The average LDN farm at 30 kg of milk per cow per day earned a gross of \$4.75 and \$5.30 per cow for fat and protein, respectively. These differences for fat and true protein between HDN and LDN herds at 30 kg of milk would result in gross income differences of \$9,125 for fat and \$6,935 for true protein per 100 milking cows per year.

An individual cow's FA profile varies throughout her lactation (Lynch et al., 1992; Palmquist et al., 1993). However, no differences were observed in the number of cows milking or the average DIM between HDN and LDN farms (Table 6; $P = 0.74$ and 0.61 , respectively). Therefore, the observed differences in de novo synthesis in this study were unlikely to be caused by differences in stage of lactation between HDN and LDN farms.

Herd Management Practices

Feed bunk space (measured in linear centimeters per cow) tended to be higher on HDN farms (Table 6; P

Table 4. Description of management practices and facility design (number of farms) between high de novo (HDN) and low de novo (LDN) commercial dairy farms

Item	HDN	LDN
Farms enrolled	20	20
Farms in final data set	19	20
Freestall facility	16	14
2 Rows per pen	5	1
3 Rows per pen	11	13
Tiestall facility	3	6
Certified organic	0	1
Component feeding	1	5
TMR feeding	18	15
Uses test-day program ¹	8	11
Computer management	12	14
Fans over stalls	14	12
Fans over feed bunk	5	5
Freestall feeding system		
Post and rail	7	5
H-bunk	6	1
Headlocks	1	3
Combination	2	5

¹Dairy Herd Improvement Association (Vermont DHIA, White River Junction, VT; Dairy One, Ithaca, NY).

Table 5. Least squares means of milk composition factors for high de novo (HDN; n = 19) and low de novo (LDN; n = 20) farms for the month of the farm visit

Item	HDN	LDN	SEM	P-value
Milk yield, kg/d	31.9	32.1	0.9	0.91
Fat, %	3.98	3.78	0.04	<0.01
Fat, kg/d	1.27	1.21	0.03	0.25
De novo fatty acids (FA) ¹				
g/100 g of milk	0.99	0.86	0.01	<0.01
g/100 g of FA	25.99	23.78	0.22	<0.01
g/d	315.6	276.2	9.5	<0.01
Mixed FA ²				
g/100 g of milk	1.48	1.35	0.02	<0.01
g/100 g of FA	38.86	37.36	0.37	<0.01
g/d	472.0	434.2	15.2	0.08
Preformed FA ³				
g/100 g of milk	1.32	1.38	0.02	0.02
g/100 g of FA	34.60	38.21	0.50	<0.01
g/d	419.0	439.3	10.4	0.17
True protein, %	3.19	3.08	0.02	<0.01
True protein, kg/d	1.02	0.99	0.03	0.44
MUN, mg/dL	12.1	12.9	0.5	0.25
Anhydrous lactose, %	4.65	4.66	0.02	0.66
Anhydrous lactose, kg/d	1.46	1.51	0.05	0.51

¹C4 to C14.²C16, C16:1, and C17.³Greater than or equal to C18.

= 0.06). When categorized as above or below 46 cm of space per cow, HDN herds tended ($P = 0.06$) to be 10 times more likely to provide at least 46 cm of feed bunk space (Table 7). Krawczel et al. (2012) found no effect of feed bunk space on milk fat percentage or milk composition, but that was a short-term study (14 d periods), unlike the present study, in which bulk tank milk would reflect longer-term responses to stocking density. Additionally, mean feed bunk space for LDN farms for the current study was 39.8 cm per cow, which was more overcrowded than the highest feed bunk stocking density treatment in the Krawczel et al. (2012) study. Other research has reported that a decrease of 10 cm/

cow in feed bunk space is associated with a reduction of 0.06 percentage points in milk fat content (Sova et al., 2013). Commonly observed changes in feeding behavior with decreased feed bunk space (i.e., greater rate of eating, fewer and larger meals) predispose the cow to lower ruminal pH that may alter FA biohydrogenation pathways and result in depressed de novo FA synthesis (Grant and Albright, 2001; Harvatine and Bauman, 2011; Campbell et al., 2015).

We detected no difference in overall stall stocking density (Table 6; $P = 0.41$), but HDN farms tended to be 4.7 times more likely to have a stocking density ≤ 1.10 cows per stall (Table 7; $P = 0.08$). Most freestall

Table 6. Least squares means of management data for high de novo (HDN; n = 19) and low de novo (LDN; n = 20) farms

Item	HDN	LDN	SEM	P-value
Cows milking, no.	190	211	46	0.74
Time away from pen, h ¹	3.5	3.3	0.4	0.71
Tiestall feeding frequency	3.3	4.1	1.1	0.57
Push-up frequency				
Tiestall	4.9	6.1	1.4	0.88
Freestall ²	1.6	1.8	0.9	0.32
DIM	170	176	8	0.61
Feed bunk space, ³ cm/cow	50.0	39.8	3.7	0.06
Stocking density, ³ cows/stall	1.11	1.16	0.04	0.41
BCS	3.05	3.03	0.02	0.65

¹Includes only freestall farms with a designated holding area (n = 16 HDN; n = 14 LDN).²Freestall bunks included post and rail, headlocks, and H-bunks. For freestall push-up frequency, farms with H-bunks (n = 6 HDN; n = 1 LDN) were not included in the analysis, because feed cannot be pushed up in an H-bunk.³Freestall only (n = 16 HDN; n = 14 LDN).

Table 7. Effects of milking and freestall feeding frequency on bulk tank milk fatty acid composition among high de novo (HDN) or low de novo (LDN) farms

Item	HDN, %	LDN, %	OR ¹ (95% CI)	P-value
Milking frequency ²	63.19	50.00	1.71 (0.44–6.70)	0.59
Time away from pen ≤ 3 h/d ³	78.72	75.15	1.22 (0.16–9.12)	0.84
Freestall feeding frequency ⁴	68.84	30.68	4.99 (0.89–27.99)	0.07
Freestall push-up frequency ≥ 5 ⁵	29.82	77.10	0.12 (0.02–1.02)	0.05
Stall stocking density ≤ 1.10 cows/stall ⁶	65.29	21.35	4.74 (0.83–27.21)	0.08
Feed bunk space ≥ 46 cm per cow ⁷	43.71	7.12	10.13 (0.91–112.41)	0.06
Tiestall feeding frequency ⁸	67.38	42.51	2.79 (0.06–134.62)	0.56

¹OR = odds ratio. The HDN group was set as the referent.

²Percent of farms that milked twice per day.

³Percent of freestall farms that removed the cows for 3 or more hours per day for milking. Farms that allowed access to the freestall pen during milking were excluded from the analysis.

⁴Percent of farms that fed twice per day.

⁵Percent of freestall farms that pushed up the feed 5 or more times per day. Farms with H-bunk managers were excluded from the analysis.

⁶Percent of freestall farms with freestall stocking density at 1.10 cows per stall or less.

⁷Percent of freestall farms with less than 46 cm of feed bunk space per cow.

⁸Percent of tiestall farms feeding 3 times per d or less.

facilities in the northeastern United States have more rows of freestalls than the corresponding amount of bunk space per cow, and have a stocking density of approximately 1.10 cows per stall (von Keyserlingk et al., 2012). The results of the current study were consistent with the observations of Woolpert et al. (2016), who found that higher stocking density was related to lower de novo FA content in bulk tank milk.

We detected no difference in tiestall feeding frequency ($P = 0.56$). However, HDN freestall farms tended to be 5 times more likely to feed cows twice versus once per day (Table 7; $P = 0.07$). Previous research to evaluate feed management and milk FA found no detectable difference in freestall feeding frequency, but an increase in tiestall feeding frequency for HDN compared with LDN herds (Woolpert et al., 2016). Sova et al. (2013) found that freestall-housed cows on commercial farms fed twice versus once per day decreased sorting against long particles, and increased DMI, milk yield, eating time, and rumination time. Rottman et al. (2014) reported an increase in milk fat content when cows were fed 4 times versus 1 time per day. The results of the present study suggest that the increase in milk fat content observed by Sova et al. (2013) and Rottman et al. (2014) may have been due to an increase in de novo FA synthesis.

We detected no differences in the overall mean of feed push-up frequency between HDN and LDN herds (Table 6; $P = 0.88$ and $P = 0.32$ for tiestall and freestall herds, respectively). However, HDN farms were less likely to push up the feed when the cut-point of pushing up the feed 5 or more times per day was used for analysis (Table 7; $P = 0.05$). Typically, pushing up the feed allows the cow greater access, and has been associated with increased milk yield and feed efficiency

(Armstrong et al., 2008). However, previous research has also indicated that fresh feed delivery motivates cows to eat more effectively than simply pushing up the feed (DeVries et al., 2003; DeVries and von Keyserlingk, 2005). The LDN freestall herds may have pushed up the feed more often because they fed the cows less frequently.

Very little research has evaluated the effects of feed push-up frequency on behavior or lactation performance. Armstrong et al. (2008) reported an increase in milk yield and feed efficiency when feed was pushed up every 30 min for the first 2 h after feeding, compared with 1 time per hour in the first 2 h after feeding. However, Miller-Cushon and DeVries (2015) reported no difference in behavior or lactation performance when comparing a push-up frequency of 3 versus 5 times per d. Future research is needed to better understand the effects of feed delivery and feed push-up frequency on rumen fermentation and milk fat composition.

Dietary Strategies

We detected no differences in dietary DM, CP, ADF, NDF, or starch, but ether extract in the TMR was lower ($P < 0.01$) on HDN farms (3.7 vs. 4.0% of DM for HDN and LDN farms, respectively; Table 8). Although we found no significant differences between HDN and LDN herds in the amount of dietary NDF or starch, differences in the fermentability of starch or NDF sources could have existed, but we did not measure this. Future research should assess potential differences in carbohydrate fermentability between diets fed to HDN and LDN herds.

Still, these results were consistent with previous research evaluating dietary chemical composition on

Table 8. Least squares means of dietary chemical composition for diets from high de novo (HDN) and low de novo (LDN) farms¹

Item	HDN	LDN	SEM	<i>P</i> -value
DM, %	41.7	43.7	1.3	0.23
CP, % of DM	15.6	16.2	0.4	0.29
aNDFom, ² % of DM	37.8	35.4	1.0	0.20
Ether extract, % of DM	3.7	4.0	0.1	<0.01
Starch, % of DM	22.6	24.3	1.2	0.29

¹Data were mathematically composited by the number of cows that consumed the diet, and analyzed using farm as the experimental unit.

²Organic-matter-corrected NDF with α -amylase and sodium sulfite.

Table 9. Least squares means of particle size distribution using the Penn State Particle Separator for diets from high de novo (HDN) and low de novo (LDN) farms

Item	HDN	LDN	SEM	<i>P</i> -value
peNDF, ¹ % of DM	26.8	21.4	1.1	<0.01
19-mm sieve, % as fed	16.5	11.6	2.7	0.18
8-mm sieve, % as fed	38.6	38.4	1.9	0.92
4-mm sieve, % as fed	16.4	11.8	1.4	0.02
Pan, % as fed	28.5	38.2	2.2	<0.01

¹peNDF = physically effective NDF.

Table 10. Grain ingredient inclusion from high de novo (HDN; n = 15) and low de novo (LDN; n = 18) farms

Item	HDN, %	LDN, %	OR ¹ (95% CI)	<i>P</i> -value
Tallow ²	24.91	35.22	0.61 (0.10–3.63)	0.57
Rumen inert fat	50.00	70.68	0.41 (0.08–2.23)	0.29
Monensin ³	75.09	58.87	2.11 (0.36–12.29)	0.39
Yeast	33.25	29.32	1.20 (0.21–6.80)	0.83
Animal-derived protein	33.23	23.42	1.63 (0.27–9.75)	0.58
Rumen-protected amino acid	41.61	23.43	2.33 (0.41–13.40)	0.33
Dried distillers grain	58.38	70.68	0.58 (0.11–3.16)	0.52

¹OR = odds ratio. The HDN group was set as the referent.

²Percentage of farms feeding product.

³Rumensin (Elanco Animal Health, Indianapolis, IN).

Table 11. Least squares means of corn silage analysis provided by farms' nutritionists for high de novo (HDN) and low de novo (LDN) farms

Item	n	HDN	LDN	SEM	<i>P</i> -value
Corn silage					
DM, %	23	29.5	32.3	1.3	0.11
CP, % of DM	22	8.5	8.0	0.2	0.10
aNDFom, ¹ % of DM	10	43.2	42.6	1.9	0.83
Ether extract, % of DM	20	3.1	3.1	0.1	0.88
Starch, % of DM	20	27.7	31.9	1.9	0.11
Ash, % of DM	20	3.7	3.6	0.2	0.57
Hay crop silage					
DM, %	24	33.3	42.8	2.8	0.02
CP, % of DM	23	17.3	16.5	0.7	0.37
aNDFom, % of DM	12	50.2	48.4	3.3	0.69
Ether extract, % of DM	21	4.1	3.7	0.2	0.12
Starch, % of DM	18	1.7	1.7	0.3	0.99
Ash, % of DM	21	10.5	9.6	0.7	0.32

¹Organic-matter-corrected NDF with α -amylase and sodium sulfite.

HDN and LDN farms (Woolpert et al., 2016). High amounts of dietary fat are a risk factor for milk fat depression, and diets with a FA content as low as 3% of DM can reduce milk fat content and de novo FA yield when free oils rich in linoleic acid are fed (Stoffel et al., 2015). Future research should consider FA profiles in addition to dietary ether extract content and rate of free fat release from the feed in the rumen to better understand the effect of the ration on lactation performance and milk fat composition.

Physically effective NDF was higher for TMR fed to cows on HDN farms (Table 9; $P < 0.01$). Caccamo et al. (2014) found no changes in milk fat or milk yield, but did find an increase in milk true protein content for commercial dairy farms that fed adequate peNDF. Mertens (2002) recommended a minimum peNDF of approximately 21% of DM to avoid milk fat depression; the peNDF for HDN herds in the present study was 26.8%—associated with the highest levels of milk de novo FA.

We detected no difference in the inclusion of feed products (Table 10; $P > 0.10$) or chemical composition of hay crop silage or corn silage (Table 11; $P > 0.10$) included in the lactating diets, except for DM percentage (33.3 and 42.8% for HDN and LDN farms, respectively). A study of grass silages from commercial dairy farms indicated that hay crop silage of 34% of DM is optimal for silage fermentation (O'Brien et al., 2007) and results in the highest quality of silage. The silage DM content for the HDN farms most closely resembled this optimal DM content.

CONCLUSIONS

We related management and dietary practices that affect cow behavior and rumen conditions to de novo FA concentrations in bulk tank milk on Holstein dairy farms. At a constant level of milk production, the gross income per unit of milk produced was higher on the high de novo farms because of higher milk fat and true protein concentrations. Management (i.e., frequent feed delivery and increased feed bunk space per cow) and dietary (i.e., adequate physically effective fiber and lower ether extract) factors that differed between HDN and LDN farms have been shown in earlier studies to affect ruminal function. Therefore, the de novo FA concentration of bulk tank milk may be an important tool for monitoring ruminal function on commercial dairy farms. Its routine use for on-farm management would involve tracking changes in the values for the same farm across time and relating the values milk protein and fat content in the bulk tank milk, as well as associated feeding and management changes. However, the present study was correlational in nature and does not

imply a causal relationship. Future research is needed to evaluate whether a change in one or more of the management or nutritional strategies identified in the current study causes an increase in de novo FA synthesis and milk fat and true protein content on commercial dairy farms.

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