



# Effect of dietary protein level and corn processing on behavior activity of high producing dairy cows

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**ABSTRACT.** The objective of this experiment was to evaluate the effects of corn processing and protein level on the feeding, lying, and post milking standing (PMS) behavior in high producing cows. Eight Holstein cows were randomly assigned to diets containing either finely ground (FGC) or steam flaked (SFC) corn based on either low (LP) or high (HP) protein content. Cows receiving LP had lower milk yield than cows receiving HP with similar DMI. Moreover, FGC-fed cows had higher DMI than SFC-fed cows with similar milk yields. Eating and rumination time tended to be lower and chewing time was lower in HP-fed cows than LP-fed cows. Cows fed SFC tended to have higher laying rumination interval and lower lying rumination bouts than cows fed FGC. Total and average PMS were lower in cows fed HP than LP. Cows fed LP had higher chewing activity in the daytime than cows fed HP. Our results suggested that the protein level and corn processing affect the standing and lying behavior of high producing dairy cows, although, this effect is marginal. Results also indicated that probably any change in the diet that increases the rumination and eating times could also improve the PMS.

**Keywords:** Behavior pattern; corn flaking; dietary protein level; lying behavior.

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## Introduction

Due to the intense selection for higher milk yield, and better management and nutrition, milk yield has been considerably increased during the past decades. High producing dairy cows require more time for feeding (Dado & Allen, 1994) and their higher feeding time can affect their daily time budget. Daily time budget of a lactating cow includes lying, ruminating, eating, milking, socializing in alleys, drinking, and standing in the stalls (Grant & Albright, 2001). Natural pattern of eating and lying may affect milk yield and composition (Cooper, Arney, & Phillips, 2007). Given its importance in dairy cows, there was more interest in behavior activity in the studies during the past decades.

The behavior pattern of dairy cows are affected by nutrition and feeding management. Different factors such as grouping (Grant & Albright, 2001), stocking density (Fregonesi, Tucker, & Weary, 2007), milk production level (Norrington, Valros, & Munksgaard, 2012), parity (Steensels et al., 2012), days in milk (Deming, Bergeron, Leslie, & DeVries, 2013b), forage particle size (Grant, Colenbrander, & Albright, 1990), acidosis (DeVries, Beauchemin, Dohme, & Schwartzkopf-Genswein, 2009), and heat stress (Cook, Mentink, Bennett, & Burgi, 2007), influence the behavior pattern in cattle. Overstocking (Fregonesi et al., 2007) and heat stress (Cook et al., 2007) reduce the lying time and increase the standing time in dairy cows. The cows with acidosis spent less time ruminating (491 vs. 555 min. d<sup>-1</sup>) than cows without acidosis (DeVries et al., 2009). Grant et al. (1990) reported that decreasing the particle size of forage reduced the time spent on ruminating but had no effect on eating time.

The post milk standing (PMS) is another behavior activity that has been extensively investigated in recent studies (Deming, Bergeron, Leslie, & DeVries, 2013a; DeVries, Dufour, & Scholl, 2010; Watters et al., 2013; Watters, Barkema, Leslie, Von Keyserlingk, & DeVries, 2014). The amount of time cows spends standing after milking can influence the incidence of mastitis. Applying management practices to promote longer standing time after milking can also reduce the risk of mastitis. Previous studies reported varying PMS time in different housing and management conditions (Deming, Bergeron, Leslie, & DeVries, 2013a; DeVries et al., 2010; Watters et al., 2013; Watters et al., 2014).

As previously reported, milk production and DMI level can affect cow behavior. Factors affecting the feed intake or milk yield can also influence the behavior pattern in dairy cows. Among factors influencing the feed intake, milk production and, in turn, behavior pattern of cows, are changes in the level of dietary nutrient and grain processing. The dietary protein level is one of the most important factors that can affect the feed intake and milk yield. In literature, changes in the protein levels have had varying results on the performance of dairy cows. In some studies, by higher dietary level of protein milk production and feed intake were not affected (Mutsvangwa, Davies, McKinnon, & Christensen, 2016) or increased (Broderick, 2003). The most important source of energy for dairy cows is cereals. Among different grains, corn grain is a major energy source in the ruminant livestock industry and most of its energy comes from starch (about 75%; Theurer, Huber, Delgado-Elorduy, & Wanderley, 1999). Corn processing and, in particular, steam flaking is a widely-used method to formulate the dairy cow's diets and improves the performance of dairy cows (Theurer et al., 1999). Cooke, Bernard, and West (2008) reported that feeding steam flaked corn (SFC) instead of finely ground corn (FGC) decreased DMI, while Zhong, Li, Gao, Tan, and Ren (2008) reported a higher DMI in cows fed SFC compared to those fed FGC. Replacement of SFC with FGC has reportedly either increased (Cooke et al., 2008) or had no effect (Zhong et al., 2008) on the milk production.

As noted, several experiments examined the effect of dietary protein (Broderick, 2003; Mutsvangwa et al., 2016) or corn processing (Cooke et al., 2008; Zhong et al., 2008) on dairy cow's performance. In our knowledge, the effect of dietary protein level and corn processing and their interaction in 24-hour behavior pattern of high producing dairy cows has not been studied. Hence, the specific objective of this experiment was to determine the effects of, and interactions between, corn processing and dietary protein level on the feeding, lying and post milking standing behaviors in mid-lactation Holstein cows.

## Material and methods

### Experimental design, cow's management, and treatments

The experiment was conducted at Dairy Facilities of the Lavark Research Station (Isfahan University of Technology, Isfahan, Iran), from November 2015 to January 2016. Management and experimental protocols were performed in accordance with protocol no. 19293, approved by the Iranian Council of Animal Care (1995). Ambient temperature (Tdb, °C) and relative humidity (RH, %) were recorded using a temperature and humidity data-logger (ST-172; Fotronic Co., Melrose, MA) every 15 min. to determine the temperature-humidity index (THI):  $THI = (1.8 \times Tdb + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26.8)]$  (Dikmen & Hansen, 2009). The average THI, Tdb and RH for entire experiment period were 50, 8.2 °C and 51% respectively.

Eight multiparous Holstein cows were used in a replicated 4 × 4 Latin square design with parity (2 vs 3) being different between squares. Within each square, cows were randomly assigned to a sequence of four diets. Each period was 21 d including 14 d for diet adaptation and 7 d for sampling. Cows averaged 105 ± 9 DIM, 47.2 ± 3 kg milk d<sup>-1</sup>, and 614 ± 43 kg BW (mean ± SEM) at the beginning of the experiment. Throughout the experiment, each cow remained in a box stall (4 × 4 m) in a roofed barn with open sides. Each box stall was bedded with clean wood shavings that were refreshed twice daily to minimize mastitis. Each box had a concrete feed bunk and an automatic water trough. Animals did not access to forage outside of the barn.

A 2 × 2 factorial arrangement of treatments was used to provide two levels of protein (HP or LP) and two processing methods of corn (FGC or SFC). Consequently, diets were: steam flaked corn with high protein content (SFHP), steam flaked corn with low protein content (SFLP), finely ground corn with high protein content (FGHP), and finely ground corn with low protein content (FGLP, Table 1). High-protein and low-protein diets had 16.3% and 14.8% of protein on DM basis, respectively. Diets contained 40% corn as either finely ground or steam flaked. Forage comprised corn silage and chopped alfalfa hay. Forage to concentrate ratio was 40:60 on a DM basis. Feed was provided daily at 0930 and 1730 h and orts were weighed daily before feeding. Feed offered was adjusted daily to supply about 10% in excess of feed consumed.

The cows were milked three times a day at 0900, 1700, and 0100h in a herringbone milking parlor. Milk yields were recorded in the first 5 d of sampling period. Yields were averaged to determine mean milk yield production for each period. Milk was sampled during the first 5 d of each sampling period and analysed for SCC (Combifoss 5000, Foss Electric Hillerød, Denmark). Somatic cell scores were estimated as the natural logarithmic transformation of the SCC (cells mL<sup>-1</sup>) using the formula;  $SCS = 3 + (\ln [SCC/100,000]/0.693)$ , where SCC was expressed as cells mL<sup>-1</sup> (Shook, 1993).

**Table 1.** Ingredients and chemical composition of experimental diets on DM basis.

Item	SFC <sup>1</sup>		FGC	
	HP	LP	HP	LP
Alfalfa hay	15.44	15.45	15.44	15.45
Corn silage	23.17	23.17	23.17	23.17
Ground corn grain	0	0	39.38	39.40
Flake corn grain	39.38	39.40	0	0
Soybean meal	11.66	8.11	11.66	8.11
Fish meal	3.01	2.32	3.01	2.32
Bypass soybean meal	2.05	4.44	2.05	4.44
Urea	0.31	0.08	0.31	0.08
Beet pulp	1.12	3.17	1.12	3.17
Calcium salts of fatty acids <sup>2</sup>	1.74	1.74	1.74	1.74
Sodium bicarbonate	0.69	0.69	0.69	0.69
Magnesium oxide	0.31	0.31	0.31	0.31
Dicalcium phosphate	0.12	0.12	0.12	0.12
White salt	0.08	0.08	0.08	0.08
Mineral and vitamin Premix <sup>3</sup>	0.92	0.92	0.92	0.92
Chemical composition				
DM, % of diet	55.52	55.54	56.32	56.01
NE <sub>L</sub> <sup>4</sup> , Mcal kg <sup>-1</sup> DM	1.76	1.76	1.62	1.62
OM	92.40	92.49	92.53	92.86
CP	16.12	14.75	16.28	14.88
NDF	31.78	31.76	30.86	30.94
ADF	14.42	14.33	13.70	13.89
Ether extract	4.65	4.64	4.69	4.66
NFC <sup>5</sup>	39.84	41.34	40.68	42.37

<sup>1</sup>SFC = steam flake corn; FGC = finely ground corn; HP = high protein; LP = low protein. <sup>2</sup>Nutracor, Wawasan, Malaysia. Composition: Ash 13%, Moisture 3%, Calcium 9%, Crude fat 85% (C12:0, 0.2%; C14:0, 1.2%; C16:0, 47%; C18:0, 5%; C18:1, 38%; C18:2, 8%). <sup>3</sup>Composition: 195 g kg<sup>-1</sup> of Ca, 21 g kg<sup>-1</sup> of Mg, 2.2 g kg<sup>-1</sup> of Mn, 0.3 g kg<sup>-1</sup> of Zn, 0.3 g kg<sup>-1</sup> of Cu, 0.12 g kg<sup>-1</sup> of I, 0.1 g kg<sup>-1</sup> of Co, 600,000 IU kg<sup>-1</sup> of vitamin A, 200,000 IU kg<sup>-1</sup> of vitamin D, and 0.2 g kg<sup>-1</sup> of vitamin E, 0.025 g kg<sup>-1</sup> of Se. <sup>4</sup>Calculated from NRC (2001). <sup>5</sup>Nonfibrous carbohydrates [NFC = OM - (NDF + CP + EE)] where EE = ether extract.

### Sampling, measurements, and analyses

Amounts of fresh TMR and refusal were recorded and sampled daily for each individual cow. The samples were refrigerated until the end of the collection period. At the end of collection period, these individual samples were combined, sub-sampled and then stored at -20°C for later analysis. Feed and orts samples were dried at 60°C in a forced-air oven for 48h and grinded using a Wiley mill with a 1-mm screen (Wiley mill, Arthur H. Thomas) and analyzed for NDF (using heat-resistant alpha-amylase and sodium sulfite) and ADF, according to Van Soest, Robertson, and Lewis (1991) with the Ankom Fiber Analyzer system (Ankom Technology, Macedon, NY), DM (method 925.40), ash (method 942.05), CP (method 2001.11), and ether extract (method 920.39) according to Association of Official Analytical Chemists (AOAC, 2002). Organic matter was calculated as OM = (100 - % ash).

### Chewing behavior

Eating, ruminating and lying activities were monitored visually for a 24h period on d 19 of each period. Chewing and lying activities were monitored every 5 min., and each activity (i.e., eating, ruminating, resting) was assumed to persist for the entire 5-min. (Grant et al., 1990). The cows were considered to be idle (no chewing activity) and standing when they were out of the barn. Thus, standing time represents the total standing time in the barn plus the total time spent outside and eating and ruminating were only recorded while the cows were in their stalls. Total chewing time was determined as the sum of total eating and ruminating times. A period of chewing was defined as at least 5 min. of chewing activity followed by at least 5 min. without chewing activity.

### Experimental design and analysis

Data were subjected to the MIXED MODEL procedure of Statistical Analysis System (SAS, 2002). The model included period, treatment, square, and the relevant interactions as fixed effects, and cow as a random effect.

The REML method was used to estimate least squares means, and the Kenward-Roger method was used to calculate denominator degrees of freedom. Normality of distribution and homogeneity of variance for residuals were tested using PROC UNIVARIATE (SAS, 2002). Effects of the factors were declared significant at  $p \leq 0.05$  unless otherwise noted and trends were discussed at  $p \leq 0.10$ . Means were compared using the Tukey multiple comparison test.

## Results

### Chewing activity

Milk yield was similar across corn processing, but cows fed FGC had greater DMI than cows fed SFC (Table 2). Eating, rumination and chewing times were not significantly different in cows fed SFC or FGC, and greater DMI in FGC-fed cows had no effect on chewing time. 'There were no treatment interactions' with respect to chewing activity. Cows fed HP had similar DMI and greater milk yield than cows fed LP. Eating ( $p = 0.06$ , 349.9 vs 355.9 min.  $d^{-1}$ ) and rumination ( $p = 0.08$ , 475.6 vs 497.5 min.  $d^{-1}$ ) time tended to be lower and chewing time was lower ( $p < 0.01$ , 862 vs 817.5 min.  $d^{-1}$ ) in cows fed HP diets than cows fed LP diets (Table 2).

**Table 2.** Least squares means of chewing activity of high producing dairy cows ( $n = 8$ ) fed diets containing either high (HP) or low (LP) level of protein supplemented with either finely ground (FGC) or steam-flaked corn (SFC).

Item	SFC		FGC		SEM	Contrast <sup>1</sup>		
	HP	LP	HP	LP		P	CP	P×CP
Dry matter intake, kg $d^{-1}$	26.69	26.04	28.03	28.00	0.57	<0.01	0.26	0.30
Milk yield, kg $d^{-1}$	46.93	45.41	47.17	45.14	1.51	0.96	<0.01	0.65
Eating								
Min. $d^{-1}$	345.62	366.25	338.13	361.67	11.17	0.59	0.06	0.89
Bout $d^{-1}$	14.00	14.12	14.62	13.65	0.85	0.92	0.62	0.52
Min. bout <sup>-1</sup>	25.42	26.12	23.67	27.74	1.79	0.97	0.19	0.35
Interval, min.	83.43	79.96	80.01	85.69	5.15	0.81	0.82	0.35
Rumination								
Min. $d^{-1}$	476.25	496.25	475.00	498.80	15.54	0.95	0.08	0.87
Bout $d^{-1}$	15.62	15.00	16.00	14.73	0.91	0.93	0.17	0.64
Min. bout <sup>-1</sup>	31.01	34.08	30.48	35.79	2.45	0.68	<0.01	0.43
Interval, min.	59.56	60.13	58.65	64.43	3.47	0.61	0.34	0.43
Total chewing activity								
Min. $d^{-1}$	821.88	862.50	813.13	861.53	19.29	0.75	<0.01	0.79
Bout $d^{-1}$	29.50	29.50	30.75	28.24	1.32	0.99	0.31	0.31
Min. bout <sup>-1</sup>	28.31	29.62	26.81	31.48	1.70	0.89	0.03	0.21
Interval, min.	24.36	22.56	23.57	23.58	1.25	0.93	0.48	0.48

<sup>1</sup>Contrasts for P (corn processing effect), CP (protein level effect) and interaction (P×CP).

### Lying and standing rumination

Protein content and corn processing had no effect on standing and lying rumination (Table 3). Cows fed SFC tended to have higher laying rumination interval (77.6 vs 70.1;  $p = 0.07$ ) and lower lying rumination bouts (13.2 vs 14.4;  $p = 0.03$ ) compared with cows fed FGC. In all treatments, cows approximately spent 17% (1.3 h  $d^{-1}$ ) of the total rumination while standing and spent 83% (6.8 h  $d^{-1}$ ) of rumination while lying.

### Lying and standing pattern

The treatments and their interaction had no effect on total lying and standing times (Table 4). Across all treatments, cows spent 12.9 h  $d^{-1}$  on lying (separated into 10.7 bouts  $d^{-1}$  with an average duration of 76.2 min. bout<sup>-1</sup>) and 11.1 h  $d^{-1}$  on standing (separated into 11.6 bouts  $d^{-1}$  with an average duration of 60.9 min. bout<sup>-1</sup>).

### Post milking standing

The effect of treatments on PMS is shown in the Table 5. Cows fed HP diets had lower total PMS (176 vs 197 min.  $d^{-1}$ ;  $p = 0.01$ ) and average PMS (58.6 vs 65.8 min.  $d^{-1}$ ;  $p = 0.01$ ) compared with cows fed LP diets. The LP-fed cows had higher PMS at 0900h (76 vs 67 min.;  $p = 0.04$ ) than HP-fed cows. The LP-fed cows had higher

eating and chewing times than cows fed HP diets (Table 2), and probably these cows spent more time for feeding after milking.

**Table 3.** Least squares means of standing and lying rumination of high producing dairy cows (n = 8) fed diets containing either high (HP) or low (LP) level of protein supplemented with either finely ground (FGC) or steam-flaked corn (SFC).

Item	SFC		FGC		SEM	Contrast <sup>1</sup>		
	HP	LP	HP	LP		P	CP	P×CP
Standing rumination								
Min. d <sup>-1</sup>	89.37	95.00	70.00	69.06	24.01	0.16	0.88	0.83
Bout d <sup>-1</sup>	5.37	4.62	5.25	4.97	0.86	0.87	0.49	0.74
Min. bout <sup>-1</sup>	15.03	16.92	12.84	14.85	2.80	0.23	0.27	0.97
Interval	251.88	327.43	237.43	250.06	46.32	0.25	0.27	0.42
Lying rumination								
Min. d <sup>-1</sup>	386.87	401.25	405.00	428.77	26.62	0.18	0.26	0.78
Bout d <sup>-1</sup>	13.50	12.87	14.12	14.74	0.93	0.03	0.99	0.26
Min. bout <sup>-1</sup>	28.75	31.73	29.38	30.70	2.23	0.88	0.13	0.55
Interval	76.19	79.08	72.44	67.70	6.34	0.07	0.81	0.35

<sup>1</sup>Contrasts for P (corn processing effect), CP (protein level effect) and interaction (P×CP).

**Table 4.** Least squares means of total standing and lying of high producing dairy cows (n = 8) fed diets containing either high (HP) or low (LP) level of protein supplemented with either finely ground (FGC) or steam-flaked corn (SFC).

Item	SFC		FGC		SEM	Contrast <sup>1</sup>		
	HP	LP	HP	LP		P	CP	P×CP
Total standing								
Min. d <sup>-1</sup>	676.25	680.62	663.75	643.18	32.3	0.29	0.73	0.59
Bout d <sup>-1</sup>	11.62	11.12	11.87	11.95	0.83	0.33	0.70	0.59
Min. bout <sup>-1</sup>	59.56	69.09	58.66	56.12	7.70	0.21	0.52	0.27
Interval	73.53	79.60	75.48	75.55	5.52	0.83	0.54	0.55
Total lying								
Min. d <sup>-1</sup>	763.75	759.38	776.25	796.82	32.31	0.29	0.73	0.59
Bout d <sup>-1</sup>	10.75	10.12	10.87	10.93	0.82	0.41	0.62	0.54
Min. bout <sup>-1</sup>	74.23	79.61	75.49	75.47	5.63	0.78	0.60	0.60
Interval	59.62	69.15	58.65	56.12	7.79	0.21	0.52	0.28

<sup>1</sup>Contrasts for P (corn processing effect), CP (protein level effect) and interaction (P×CP).

**Table 5.** Least squares means of post milk standing (PMS) of high producing dairy cows (n = 8) fed diets containing either high (HP) or low (LP) level of protein supplemented with either finely ground (FGC) or steam-flaked corn (SFC).

Item	SFC		FGC		SEM	Contrast <sup>1</sup>		
	HP	LP	HP	LP		P	CP	P×CP
Total PMS (min. d <sup>-1</sup> )	176.88	201.25	175.00	193.57	10.90	0.54	0.01	0.71
Average PMS (min. d <sup>-1</sup> )	58.96	67.08	58.33	64.52	3.63	0.54	0.01	0.71
PMS at 0900h (min.)	66.25	78.12	68.12	73.19	5.85	0.70	0.04	0.39
PMS at 1700h (min.)	66.25	65.62	61.25	66.89	4.52	0.66	0.56	0.47
PMS at 0100h (min.)	44.37	57.50	45.62	53.85	6.21	0.85	0.10	0.70
SCS <sup>2</sup>	4.62	4.67	4.47	4.30	0.23	0.22	0.76	0.61

<sup>1</sup>Contrasts for P (corn processing effect), CP (protein level effect) and interaction (P×CP). <sup>2</sup>SCS = Somatic cell scores.

### Diurnal variations in behavior activity

The corn processing and treatment interactions had no significant effect on behavior activity during the day (0600 to 1800h) and night (1800 to 0600h) (Table 6). Cows fed LP diets had higher chewing activity (434.9 vs 405.9 min.; p = 0.05) and tended to have higher rumination activity (202.9 vs 187.5 min.; p = 0.08) during the daytime than cows fed HP diets. Cows fed LP diets had higher chewing activity (Table 2; 862 vs 817 min. d<sup>-1</sup>; p < 0.01) than cows fed HP diets, and this increase in chewing activity happened in daytime and cows had similar chewing activity at nighttime. Chewing activity was not different during the daytime (420.4 min.) and nighttime (420.0 min.) in all treatments. The eating (224.8 vs 128.7 min.) and standing (387.3 vs 277.0 min.) activities were higher during the daytime than nighttime, and rumination (195.2 vs 291.2 min.) and lying (331.4 vs 440.4 min.) activities were lower in day than night.

**Table 6.** Least squares means of diurnal variations in behavior activity of high producing dairy cows (n = 8) fed diets containing either high (HP) or low (LP) level of protein supplemented with either finely ground (FGC) or steam-flaked corn (SFC).

Item	SFC		FGC		SEM	Contrast <sup>1</sup>		
	HP	LP	HP	LP		P	CP	P×CP
Day								
Chewing	408.75	432.50	403.13	437.34	14.25	0.97	0.05	0.72
Eating	220.63	231.25	216.25	231.30	11.64	0.85	0.29	0.85
Lying	324.38	328.75	333.75	338.59	20.35	0.57	0.78	0.98
Standing	395.63	385.63	386.25	381.78	21.05	0.71	0.68	0.87
Rumination	188.12	201.25	186.87	204.51	9.37	0.90	0.08	0.79
Rumination lying	146.88	159.38	160.63	177.13	13.71	0.07	0.10	0.81
Rumination standing	43.12	35.00	26.87	26.92	10.61	0.13	0.60	0.60
Night								
Chewing	412.50	435.62	410.62	421.52	14.41	0.49	0.15	0.59
Eating	125.00	135.00	121.88	132.86	7.00	0.68	0.11	0.93
Lying	442.50	431.25	443.13	444.91	17.72	0.63	0.75	0.66
Standing	277.50	288.75	276.88	264.98	17.66	0.39	0.98	0.41
Rumination	287.50	300.62	288.75	287.73	12.16	0.52	0.51	0.44
Rumination lying	240.62	238.75	245.00	251.80	15.15	0.48	0.84	0.72
Rumination standing	46.25	60.00	43.12	42.68	16.12	0.38	0.56	0.54

<sup>1</sup>Contrasts for P (corn processing effect), CP (protein level effect) and interaction (P×CP).

## Discussion

Increased chewing time in cows fed LP diets (Table 2) may have been related to increased level of beet pulp in LP diets, which was used instead of soybean meal. Because beet pulp is a source of nonforage fiber and had greater NDF than soybean meal, maybe it caused greater chewing activity than soybean meal. In contrast, Bahrami-Yekdangi et al. (2014) reported that different levels of dietary protein had no effect on chewing time. High-yielding dairy cows typically spend about 4 to 6 h d<sup>-1</sup> eating and 7 to 10 h d<sup>-1</sup> ruminating (Dado & Allen, 1994). In the current study and across treatments, the eating (5.9 h d<sup>-1</sup>) and rumination (8.1 h d<sup>-1</sup>) times are within the range of guideline.

In general, cows spend most of rumination time in lying than standing (Steensels et al., 2012). In the studies conducted by Grant et al. (1990) and Norring et al. (2012), milk production was 28 and 38 kg d<sup>-1</sup>, and the cows ruminated 477 and 427 min. d<sup>-1</sup>, respectively. In our experiment using high producing dairy cows (45 kg d<sup>-1</sup>), the rumination time was 476 min. d<sup>-1</sup>, which is similar to previous studies, indicating that the production level did not affect the amount of rumination. However, cows in the studies of Grant et al. (1990) and Norring et al. (2012) spent 61% and 66% of rumination while lying respectively, which was lower than our experiment. These results suggest that when milk production and DMI are increased, the cows spend more time ruminating while lying, and they probably spend most of their standing time on eating. In contrast, Norring et al. (2012) reported that higher-yielding cows spent more time ruminating while standing and less time lying than lower-yielding cows.

Adequate daily lying time is necessary to ensure the optimal health and welfare in dairy cattle. Cow's requirement for lying behavior is demonstrated by an increase in this behavior immediately after cows have been deprived of the opportunity to lie down (Cooper et al., 2007). Deming, Bergeron, Leslie, and DeVries (2013a) reported that lying bout time are negatively associated with milk yield, and in this study, average time of lying bout (76.2 min.) is similar to Deming, Bergeron, Leslie, and DeVries (2013a) (78.1 min.). However, the average lying time observed in the present study was slightly higher than 11 h d<sup>-1</sup> reported by Ito, Weary, and Von Keyserlingk (2009), Watters et al. (2013) and Deming, Bergeron, Leslie, and DeVries (2013b) and similar to 13 h d<sup>-1</sup> reported by Drissler, Gaworski, Tucker, and Weary (2005), Fregonesi et al. (2007) and Krawczel et al. (2012). There is some variability between studies in terms of frequency of lying bouts per day (9 to 13 bouts d<sup>-1</sup>) and the time of lying bouts (72 to 88 min.) [11 bouts of 85 min. (Drissler et al., 2005); 9 bouts of 88 min. (Ito et al., 2009); 9 bouts of 78 min. (Deming, Bergeron, Leslie, & DeVries, 2013b); 9 bouts of 85 min. (Watters et al., 2013)].

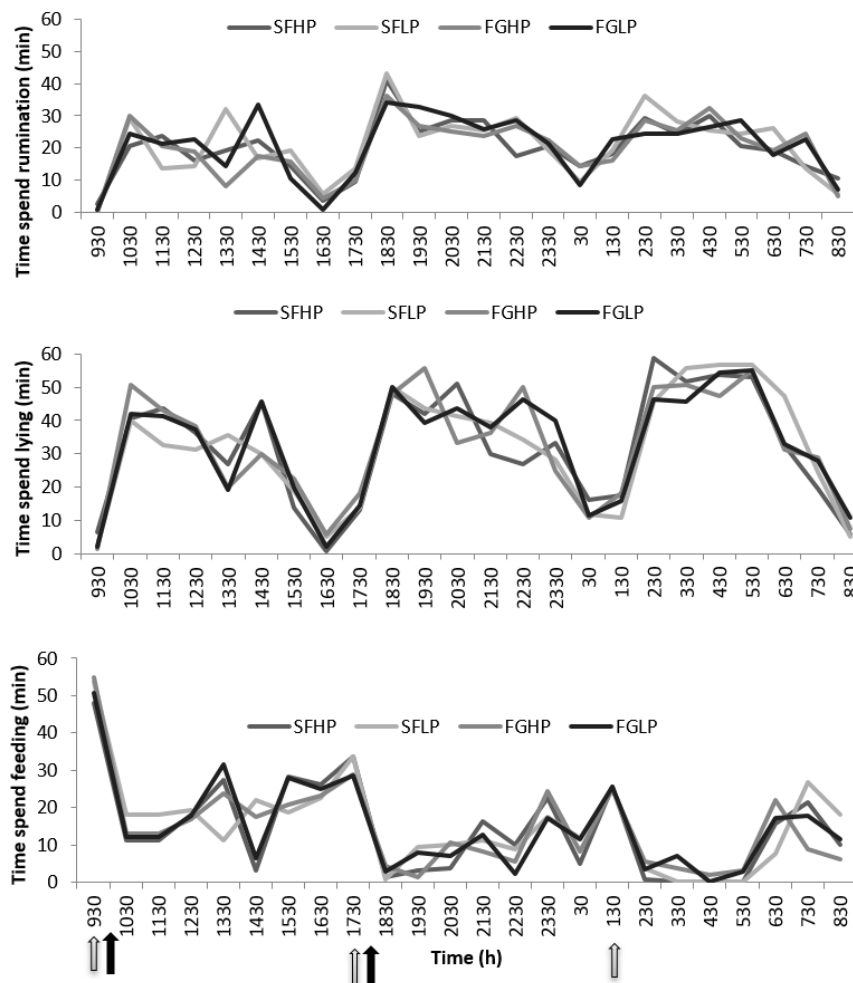
DeVries et al. (2010) reported that cows that lay down, on average, for the first time 40 to 60 min. after milking tended to have 1.4 times lower odds of a new intramammary infection caused by environmental bacteria compared to cows that lay down within 40 min. after milking. In the current trial and in agreement with DeVries et al. (2010), cows laid down between 40 to 80 min after milking and the somatic cell score were not different between treatments.

Post milking standing is influenced by many factors such as the presence of fresh feed (DeVries et al., 2010), parity (Deming, Bergeron, Leslie, & DeVries, 2013a), and stocking density (Watters et al., 2014). To our knowledge, the effect of changes in diet nutrients on the PMS of dairy cows has not been examined. Previous studies have

reported varying PMS under different housing and management conditions [35 min. (Tyler et al., 1997); 55 min. (DeVries & Von Keyserlingk, 2005); 79 min. (DeVries et al., 2010); 75 min. (Deming, Bergeron, Leslie, & DeVries, 2013b)]. The variation in PMS indicates variable behavior among different housing and management systems. Availability of fresh feed following the return from milking has been used to encourage cows to remain standing (while feeding) than to lie down (Tyler et al., 1997; DeVries & von Keyserlingk, 2005). Peeler, Green, Fitzpatrick, Morgan, and Green (2000) reported lower clinical mastitis in farms that offered fresh feed after both morning and evening milking. In the current trial, fresh feed was not provided to cows after milking at midnight (0100h), and because of this cows had lower PMS time at 0100h (50 min.) than 0900 (71 min.) and 1700h (65 min.).

In agreement with our finding, DeVries et al. (2010) reported higher eating activity during the daytime and the early evening and lower eating activity during the late evening and early morning. In our study, rumination activity increased approximately by 20% during the nighttime interval of 1800 to 0600h compared with 0600 to 1800h. Similarly, Grant et al. (1990) observed that nighttime rumination activity increased by 29% compared with daytime activity.

As you can observe in Figure 1, time spent feeding peaked after offering feed in the morning (0930) and afternoon (1730) and return from milking (0130). These increases coincided with a decrease in ruminating and lying times. Feed delivery had more influence on feeding time than return from milking, and cows spent more time on feeding after morning and afternoon feed delivery in comparison with return from milking without feed delivery on midnight. As a result, higher proportions of feed are consumed immediately after the delivery of fresh feed. In agreement with our results, DeVries et al. (2010) reported that the diurnal feeding pattern of dairy cows was mostly influenced by the time of feed delivery, feed push-up and milking, and feed delivery is the primary factor effecting daily feeding activity pattern.



**Figure 1.** Diurnal pattern for rumination time, lying time, and feeding time for high producing dairy cows fed TMR twice daily (approximately 0930 and 1730h; indicated with arrows black arrows) and milked three times per day (approximately 0900, 1700 and 0100h; indicated with white arrows). SFHP = steam flaked corn with high level of protein, SFLP = steam flaked corn with a low level of protein, FGHP = finely ground corn with a high level of protein, FGLP = finely ground corn with a low level of protein.



In our study and in agreement with Schirmann, Chapinal, Weary, Heuwieser, and Von Keyserlingk (2012), and since cows are not able to feed and ruminate simultaneously, when they spend more time on feeding they will have shorter time for rumination. Cows spent more time to ruminate (1030, 0630 and 0230) after periods of high feeding time (0930, 1730 and 0130; Figure 1). In our study, cows spent more time ruminating about 1h after periods of high feed intake, however in Schirmann et al. (2012) this relationship peaks approximately at 4h after feeding. As previously reported, rumination and lying behavior are associated (Schirmann et al., 2012); in our study, the rumination was associated with lying down and cows preferred to ruminate when lying down. The pattern of lying behavior in this study was similar to that previously reported (Fregonesi et al., 2007), with the lowest lying time corresponding to peaks in feeding behavior. Mattachini, Riva, and Provolo (2011) observed the daily lying and standing behaviors of cows housed in a 2x/d day parlor milking free-stall barn, and found that peak periods of lying behavior occurred during the early morning and nighttime and that standing and lying behaviors were strongly influenced by management practices within 1-2h after milking. The time spent ruminating was highest at night and between feedings during the day.

## Conclusion

The results indicate that behavior pattern of high-producing dairy cows are affected by corn processing and dietary protein levels, although, this effect is marginal. Cows spent more time for lying and rumination at nighttime and for eating and standing at daytime. Moreover, any change in diet formulation or processing that can increase chewing time, probably increases the PMS length. These results indicate that changes in the management and housing system is more effective than diet changes on the behavior pattern in high-producing dairy cows. Availability of fresh feed after milking is an important factor to encourage cows to remain standing.

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