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Effect of protein level and grain source on milk production, nutrient digestibility and ruminal fermentation in primiparous Holstein cows

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Abstract

This study investigated whether the interaction of protein level and grain type can affect milk production, nutrient digestibility and rumen fermentation in primiparous Holstein cows. Four dietary treatments were used: high-protein with barley as the only grain source, HP-B; (2) high-protein with an equal mix of barley and maize, HP-BM; (3) low-protein with barley as the only grain source, LP-B and (4) low-protein with equal proportions of barley and maize, LP-BM. High-protein diets showed no improvement in milk or protein yield compared with low-protein, but barley and maize mix diets increased energy-corrected milk yield and fat yield compared with barley-only diets. The highest total apparent digestibility of dry matter, organic matter and neutral detergent fibre was observed for LP-BM whereas HP-BM showed the greatest crude protein digestibility. Treatment had no effect on total volatile fatty acid concentrations, molar proportion of acetate and propionate and acetate to propionate ratio. The lowest ruminal pH was observed for LP-B. High-protein diets resulted in greater concentrations of ammonia nitrogen (N), urinary N, blood and milk urea nitrogen compared with low-protein diets, whereas low-protein diets showed better nitrogen utilization efficiency. This study showed that primiparous lactating cows do not benefit from high-protein diets with different fermentation rates of grain sources, but barley and maize diets may improve milk production performance, ruminal fermentation and pH under the present dietary conditions. The current results on milk production performance should be interpreted with caution due to the small number of cows used (eight in each treatment).

Introduction

Dietary protein is the most important nutrient for increasing the milk nitrogen (N) yield in dairy cows (Huhtanen and Hristov, 2009). However, over-feeding of protein increases the cost of production and excretion of N to the environment (NRC, 2001; Lee *et al.*, 2012*a*). For instance, it has been reported that increasing dietary crude protein (CP) content from 148 to 167 (Lee *et al.*, 2012*b*) and 162 to 201 g/kg dry matter (DM) (Borucki Castro *et al.*, 2008) increased the excretion of urinary N to almost two-fold. Former studies showed that a dietary CP concentration greater than 167 g/kg DM had no effect on productive performances of lactating dairy cows (Cunningham *et al.*, 1996; Broderick, 2003; Socha *et al.*, 2008). However, it has recently been reported that milk production peaked at CP concentration of 173 (Law *et al.*, 2009) or 176 g/kg DM (Katongole and Yan, 2020).

Ruminal fermentation and microbial protein synthesis (MPS) occur in an integrated way in the rumen. Consequently, some dietary factors such as amount and types of carbohydrates have substantial impacts on protein requirement of ruminants, as carbohydrate fermentation rate can alter the efficiency of rumen N utilization (Broderick, 2003). Rapidly *v.* moderately fermentable grains (e.g. barley *v.* maize) can alter rumen degradation patterns and the requirement of dietary chemical composition for an optimal rumen fermentation of dairy cows (Beauchemin and Rode, 1997). Thus, the concentration of CP in the diet could differ due to differences in starch degradability or the rate of availability of energy for the rumen microbiome, a context that has not been considered well for investigating the optimal dietary CP in dairy cows. As a result, the key should be applying efficient nutritional strategies to induce a favourable synchronization of energy and protein for an optimal MPS and rumen characteristics and subsequently minimizing N excretion in urine and maximizing the animal productivity (Kaswari *et al.*, 2007).

Although the theory supports the effect of synchronized energy and protein for optimal MPS and rumen characteristics, practical results have been conflicting (Kolver *et al.*, 1998; Shabi *et al.*, 1998; Kaswari *et al.*, 2007). Differences in experimental methods and animals used have made it difficult to determine the effect of energy and protein synchronization from several other factors (Cabrita *et al.*, 2006). For instance, changes in feed ingredients or dry matter intake (DMI) alter the supplied amount of organic matter (OM), N and their fermentation rate in the rumen, which affect the efficiency of synchronized energy and N in the rumen (Yang *et al.*, 2001). Moreover, these studies used different ruminant species in various physiological stages, where the conversion efficiency of dietary N into animal product N is greatly diverse. Thus, it is difficult to generalize the effect of synchronization of energy and N on animal performance across all ruminants with different physiological needs. Primiparous cows have not been widely evaluated in terms of energy-N synchronization. It has been recommended to contain greater concentrations of CP in the diets for primiparous cows than for their multiparous counterparts, as they are in growing stage, their mammary gland is developing and they have lower DMI compared with multiparous cows (NRC, 2001). Consequently, they might benefit more from the amount and type of dietary protein and energy sources, when nutrients partition into the two main processes (i.e. growth and milk synthesis). Therefore, more studies are required to identify the effects of protein and energy availability interaction on ruminal fermentation characteristics and animal performance in a particular physiological condition.

Thus, it was hypothesized that feeding ground barley grain instead of combined ground barley and maize grains with high concentration of CP in diets may lead to a decrease in urinary N excretion, an increase in N utilization efficiency and an improvement of productive performance as a result of improved MPS. This study was, therefore, designed to evaluate the interaction of two dietary protein concentrations (high *v.* low) and two grain sources with different degradability (barley or barley + maize) on rumen fermentation characteristics, N efficiency and milk production in primiparous Holstein cows.

Materials and methods

Cows, treatments and management

This experiment was conducted at the Dairy Facilities of the University of Zanjan (Zanjan, Iran). The Animal Care Committee of University of Zanjan (ID 1353) approved all procedures involving animal care and management.

Eight primiparous Holstein cows (560 ± 65 kg of body weight; 50 ± 15 days in milk; DIM) were used in a replicated 4 × 4 Latin square design with four 21-day periods. Cows were allocated within the square based on DIM (≤50 and >50 days). Each experimental period had 14 days of adaptation followed by 7 days of sampling and data collection. Cows were housed in individual free boxes (4 × 4 m) bedded with wheat straw and were allowed to 1-h daily exercise. Cows were fed with one of the four dietary treatments (Table 1) consisted of: (1) high-protein concentration (195 g/kg of DM) with barley as the only grain source, HP-B; (2) high-protein concentration (193 g/kg of DM) with an equal mix of barley and maize grains, HP-BM; (3) low-protein concentration (159 g/kg of DM) with barley as the only grain source, LP-B and (4) low-protein concentration (156 g/kg of DM) with an equal mix of barley and maize grains, LP-BM. Maize (*Zea mays* L. ssp.) and barley (*Hordeum* spp.) grains contained 700 and 580 g/kg of starch; 85 and 110 g/kg of CP; 110 and 200 g/kg of neutral detergent fibre (NDF); 32 and 70 g/kg of acid detergent fibre; 40 and 22 g/kg of ether extract, respectively (all in DM basis). Whole maize and barley grain densities were 540 and 500 g/l, respectively. Both grains were ground by using a commercial hammer mill (sieve size of 2 mm) most commonly used in dairy farms in Iran. In each period, diets were provided as total mixed rations (TMRs) and were formulated using the Cornell-Penn-Miner Dairy model (CPM-Dairy 3.0). Cows were fed *ad-libitum* twice daily at 09.00 and 16.00 h, and fresh water was available at all times.

Table 1. Ingredients and chemical composition of the experimental diets

	Dietary treatments			
	Barley		Barley + maize	
Items	HP	LP	HP	LP
Ingredients (g/kg DM)				
Alfalfa hay	200	200	200	200
Maize silage	200	200	200	200
Ground barley	282	362	123	168
Ground maize	–	–	123	168
Wheat bran	69.5	107	85.4	107
Fish meal	32.2	15.0	32.1	10.6
Soybean meal	139	55.8	136	64.2
Cottonseed meal	53.4	36.5	77.2	58.8
Calcium carbonate	9.40	9.40	9.40	9.40
Vitamin-mineral mix[a]	2.2	2.2	2.20	2.20
Salt	2.70	2.70	2.70	2.70
Sodium bicarbonate	9.50	9.50	9.50	9.50
Chemical composition				
NEL[b], MJ/kg DM	7.21	6.98	7.35	7.23
CP, g/kg DM	195	159	193	156
RDP, g/kg DM	122	104	120	102
RUP, g/kg DM	72	54	73	54
Ether extract, g/kg DM	57.0	43.0	63.0	50.0
NDF, g/kg DM	329	335	323	319
peNDF, g/kg DM	195	190	211	204
NFC, g/kg DM	367	410	370	424
Starch, g/kg DM	232	279	243	304

HP, high protein; LP, low protein; DM, dry matter; NEL, net energy for lactation; CP, crude protein; RDP, rumen degradable protein; RUP, rumen undegradable protein; NDF, neutral detergent fibre; peNDF, physically effective NDF; NFC, non-fibre carbohydrate.
[a]Supplemental minerals and vitamins per kg contained 196 g calcium, 96 g phosphorus, 19 g magnesium, 46 g sodium, 3000 mg iron, 300 mg of copper, 2000 mg manganese, 100 mg cobalt, 3000 mg zinc, 100 mg iodine, 1 mg selenium, 400 mg antioxidants, 500 000 IU vitamin A, 100 000 IU vitamin D3 and 100 mg vitamin E.
[b]Calculated from CPM Dairy (V. 3.0).

Sample collection and chemical analysis

Samples of TMRs and orts were collected during days 15–21 of each period and stored at −20°C until analysis. Samples were composited by period, dried at 55°C for 48 h in an oven and

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Table 2. Coefficients of total tract apparent digestibility of dietary components in dairy cows fed with four diets with different protein concentrations and grain sources

	Treatments				S.E.M.	P value		
	Barley		Barley + maize					
Items	HP	LP	HP	LP		Protein	Grain	*P* × *G*
DM	0.65	0.65	0.66	0.70	0.011	0.03	0.003	0.01
OM	0.67	0.65	0.67	0.71	0.010	0.24	<0.001	<0.001
NDF	0.60	0.57	0.62	0.65	0.007	0.78	<0.001	<0.001
CP	0.71	0.59	0.69	0.64	0.016	<0.001	0.17	0.01

HP, high protein; LP, low protein; *P* × *G*, protein and grain interaction; DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; CP, crude protein.

ground through a 1-mm screen using a Wiley mill (Wiley Company pulverizer Ogaw Seiki, Ltd., Tokyo, Japan). OM concentration was determined after subtraction of ash content that was determined after burning the samples at 600°C for 2 h. CP concentration was analysed using the Kjeldahl method (Tecator, Hoganas, Sweden). NDF content was determined with sodium sulphite and alpha-amylase according to Van Soest *et al.* (1991). The acid insoluble ash (AIA) concentration of the diets and faecal samples was determined according to Van Keulen and Young (1977). The concentration of AIA was used as an internal marker to calculate total tract apparent digestibility of dietary components. In this regard, faecal samples were collected at 4-h intervals for 24 h on day 15. On the last day of each period at 11.30 a.m., rumen fluid sample was collected using a stomach tube. The initial 100 ml of aspirated ruminal fluid was discarded to minimize saliva contamination. The pH of the rumen fluid was measured using a mobile pH meter (HI8314, Hanna Instruments, Italy). An 8-ml aliquot of strained ruminal fluid sample was mixed with 2 ml of 25% (wt/vol) metaphosphoric acid solution containing 2 g/l of 2-ethyl butyric acid (an internal standard, Sigma). The content was allowed to settle for 15 min. Rumen fluid samples were centrifuged at 3000 × ***g*** for 20 min at 4°C (Mikro 220R, Hettich, Germany) and the supernatant was kept at −20°C for subsequent volatile fatty acid (VFA) analysis. For VFA analysis, a 5-ml supernatant was transferred into a 15-ml test tube. The tube was centrifuged at 10 000 × ***g*** and 4°C for 15 min. A 1-μl aliquot of the upper phase was injected into a Varian 3400 gas chromatograph (Varian Inc., Walnut Creek, CA, USA) equipped with an injector at 170°C, a flame-ionization detector at 175°C and a packed column (2 m × 2 mm i.d. glass column containing 1–1965 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb W). Another 8-ml aliquot of rumen fluid was preserved with 2 ml of 1% (wt/vol) sulphuric acid and stored at −20°C for ammonia-N analyses. . Urine samples were collected before the morning milking in 50-ml tubes on day 21. Fifteen millilitres of samples was acidified immediately by diluting with 60 ml of 0.072 N H_2SO_4. The representative sample was stored at −20°C until analysis for total N by the Kjeldahl method. Daily milk yield was recorded for all cows for 84 days in the four experimental periods. All cows were milked three times daily at 06.00, 14.00 and 20.00 h. To determine milk composition, milk samples were collected for two consecutive days per week (on days 18 and 19 of each period) and then analysed for milk fat, protein and urea nitrogen concentrations (MUN; Milcoscan TM S 50, Denmark).

Statistical analysis

Data were analysed using the Proc MIXED in SAS. The REML method was set to estimate least squares means, and Kenward–Roger method was used to calculate denominator's degrees of freedom. The model for data analysing was as follows:

$$Y_{ijkl} = \mu + T_i + C_j + G_k + P_l + (G_k \times P_l) + e_{ijkl}$$

where Y_{ijkl} is the variable of interest, μ is the overall mean, T_j is the fixed effect of period, C_k is the random effect of cow, G_l is the fixed effect of the grain source, P_m is the fixed effect of protein level, $(G_k \times P_l)$ is the interaction of grain source and protein level and e_{ijkl} is the residual error.

The other possible interactions except for the effect of grain source × protein level as well as square and its interaction with period, grain source and protein level were tested earlier and they were excluded from final models due to non-significance. Normality of distribution and homogeneity of variance for residuals were tested and ensured using Proc Univariate. Post hoc comparisons with evaluate the differences among means was tested by Tukey test. The *P* values <0.05 were declared as significant.

Results

Diet characteristics

The nutrient composition of the ingredients and their inclusion rates in the diet are shown in Table 1. Across diets (on DM basis), the NDF content ranged from 319 to 335, and NFC from 367 to 424 g/kg. The net energy for lactation contents in HP-B, LP-B, HP-BM and LP-BM were 7.21, 6.98, 7.35 and 7.23 MJ/kg of DM, respectively. The NFC and starch contents of high-protein diets (g/kg of DM) were lower than those of low-protein diets. Therefore, low-protein diets provided more carbohydrate compared with high-protein diets.

Dry matter intake, body condition score and body weight, and nutrient digestibility

No significant differences in dry matter intake, body weight and body condition score were observed among dietary treatments (Table 3). Coefficients of total tract digestibility of dietary components are presented in Table 2. The interaction of grain source and protein level was significant for apparent digestibility of all

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Table 3. DMI, body condition score, blood urinary nitrogen, milk yield and composition of dairy cows fed with four diets with different protein concentrations and grain sources

	Treatments				S.E.M.		*P* value	
	Barley		Barley + maize					
Items	HP	LP	HP	LP		Protein	Grain	*P* × *G*
DMI (kg/day)	16.4	16.0	16.5	17.0	0.44	0.80	0.09	0.17
Body weight	548	548	546	554	14.4	0.80	0.77	0.44
Body condition score	3.31	3.28	3.31	3.37	0.10	0.88	0.64	0.80
Milk yield (kg/day)	29.4	29.4	31.1	30.1	1.02	0.50	0.10	0.50
ECM[a] (kg/day)	26.4	26.7	29.1	27.6	0.99	0.39	0.01	0.22
Milk fat yield (kg/day)	0.81	0.81	0.93	0.86	0.049	0.31	0.01	0.38
Milk fat (g/kg)	28.1	27.9	30.3	29.3	1.47	0.56	0.09	0.71
Milk protein yield (kg/day)	0.87	0.92	0.95	0.91	0.041	0.90	0.19	0.15
Milk protein (g/kg)	30.4	31.8	31.0	30.8	1.12	0.46	0.83	0.36
Milk lactose yield (kg/day)	1.31	1.32	1.41	1.29	0.069	0.30	0.43	0.18
Milk lactose (g/kg)	4.36	4.54	4.60	4.36	0.163	0.42	0.73	0.21
Milk urea nitrogen (mg/dl)	17.4	12.2	17.1	11.5	0.34	<0.001	0.05	0.38
BUN	24.4	15.3	23.8	15.0	0.40	<0.001	0.10	0.69

HP, high protein; LP, low protein; *P* × *G*, protein and grain interaction.
[a]Energy-corrected milk; calculated as (milk production × (0.383 × % fat + 0.242 × % protein + 0.7832)/3.1138) (Østergaard *et al.*, 2003).

dietary components (DM, OM, NDF and CP). Our results showed that LP-BM had the greatest coefficient of DM, OM and NDF digestibility compared with other treatments ($P < 0.05$). CP digestibility was greater for high-protein diets than that for low-protein diets ($P < 0.001$) and the greatest CP digestibility was observed for cows fed with HP-B diet.

Milk yield, milk composition and blood urea nitrogen (BUN)

Regardless of the level of CP, an equal mix of barley and maize grains resulted in higher milk fat yield (kg/day). The effect of the grain source was observed for ECM, as HP-BM cows had the greatest ECM compared with other dietary treatments ($P < 0.01$; Table 3). Concentration of MUN was influenced by level of protein, as cows fed with high-protein diets had greater MUN compared with cows fed with low-protein rations. Other measurements of milk yield and composition were not affected by protein concentration, grain type or their interaction (Table 3). Similar to MUN, concentration of BUN in cows fed with high-protein diets was greater ($P < 0.01$) than in cows fed with low-protein rations.

Ruminal fermentation characteristics and nitrogen efficiency

The concentrations of total VFA (mmol/l) was greater for the diets containing mix of maize and barley than that for the diets containing only barley. However, the proportions of different VFAs (mol/100 mol) as well as acetate : propionate ratio were not different among the dietary treatments (Table 4). Ruminal pH ($P = 0.02$) and NH_3-N concentrations ($P = 0.004$) were affected by protein concentration and grain source interaction. Ruminal pH had lowest in the LP-B group, followed by HP-B and then in LP-BM and HP-BM groups. Ruminal NH_3-N concentrations were greatest in the HP-BM group, followed by the HP-B group and lowest in the LP-BM group, and it was greater in high-protein diets than in low-protein diets. Cows fed with high-protein diets had lower ($P < 0.001$) N utilization efficiency and higher urinary N concentration than cows fed with high-protein diets.

Discussion

Contrary to our assumption that primiparous cows may gain advantages from high-protein diets, feeding extra protein either with barley as the sole grain source or with an equal mix of barley and maize grains did not improve N utilization efficiency (milk protein/CP intake). Our results are consistent with other researchers who did not find advantages of feeding an increased dietary CP from 160 to 172 g/kg DM on N efficiency (milk protein/CP intake) or BCS of primiparous cows in early and mid-lactation (Mäntysaari *et al.*, 2004). However, these authors reported an increase in milk and milk protein yield by increasing dietary CP content (Mäntysaari *et al.*, 2004), which was not in agreement with our result. Although studies on primiparous cows are limited, some studies showed positive effects of feeding diets with CP >160 g/kg DM on milk production of multiparous Holstein lactating cows (Broderick, 2003; Reynal and Broderick, 2003). The higher milk yield by additional CP in these studies mostly has been related to an increased DMI due to greater rumen degradable protein (RDP) concentration. Therefore, diets with low levels of RDP may impair nutrient digestibility and thus do not support an efficient MPS, resulting in decreased DMI (NRC, 2001; Broderick, 2003; Law *et al.*, 2009). In the current study, although cows fed with low-protein diets tended to receive a lower amount of RDP (1.70 ± 0.07 *v.* 1.98 ± 0.03 kg/day for low- *v.* high-protein diets, respectively), DMI or milk yields were not affected by this difference. Similarly, the greatest NDF digestibility

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Table 4. Ruminal fermentation characteristics, urinary nitrogen and nitrogen utilization efficiency of dairy cows fed with four diets with different protein levels and grain sources

	Treatments					*P* value		
	Barley		Barley + maize					
Items	HP	LP	HP	LP	s.e.m.	Protein	Grain	*P* × *G*
Rumen pH	6.00	5.82	6.10	6.14	0.069	0.21	<0.001	0.02
Total VFA,[a] mmol/l	121	123	128	130	4.1	0.52	0.03	0.93
VFA, mol/100 mol								
Acetate	66.4	64.8	67.5	66.4	0.87	0.13	0.14	0.76
Propionate	18.4	17.7	17.9	18.2	0.30	0.48	0.99	0.15
Butyrate	11.4	13.7	11.2	11.5	1.21	0.29	0.32	0.41
Isobutyrate	0.89	0.98	0.80	1.04	0.151	0.30	0.93	0.63
Isovalerate	1.31	1.27	1.28	1.36	0.193	0.87	0.80	0.58
Valerate	1.56	1.47	1.28	1.50	0.152	0.66	0.40	0.28
Acetate to propionate ratio	3.61	3.65	3.76	3.65	0.087	0.63	0.24	0.25
NH_3-N, mmol/l	10.6	9.20	11.3	8.15	0.391	<0.001	0.56	0.004
Urinary nitrogen, mg/dl	45.0	37.7	45.7	36.1	1.08	<0.001	0.59	0.13
Nitrogen efficiency	26.7	34.7	27.5	31.5	1.94	<0.001	0.36	0.15

HP, high protein; LP, low protein; *P* × *G*, protein and grain interaction.
[a]Total volatile fatty acids.

observed for LP-BM may indicate that CP or RDP was sufficient in low-protein rations for proper fibre digestion (Lee *et al*., 2012*a*, *b*). Therefore, low-protein rations in this study were not likely deficient in RDP fraction. Similarly, it has been reported that inadequate rumen undegradable protein (RUP) also can cause a reduced metabolizable protein flowing to the duodenum (NRC, 2001; Broderick, 2003; Law *et al*., 2009). Therefore, deficient RUP can limit milk yield and milk protein production as well. In the current study, cows that received high-protein rations consumed 290 g/day more RUP (1.19 ± 0.00 *v.* 0.90 ± 0.03 kg/day for high- and low-protein rations, respectively). However, either milk protein or milk yield was not affected by this difference. One of the limitations of this study was using only eight cows for investigating the animal productive performances, however, similar to our results, milk protein content and yields were not affected by increasing dietary CP content in other studies (Wang *et al*., 2007; Cyriac *et al*., 2008; Law *et al*., 2009). Thus, diets with CP concentration at 160 g/kg DM with RUP of 54 g/kg DM could be optimum to meet dietary CP requirement of cow yielding around 30 kg/day of milk and feeding extra protein may not result in additional benefits for primiparous cows.

Feeding large amounts of highly fermentable carbohydrate may cause a low rumen pH (i.e. <5.8) (Bramley *et al*., 2008). In this situation, microbial maintenance requirements increase and MPS rate reduces remarkably (Russell and Wilson, 1996). In the current study, the lowest ruminal pH was observed in cows fed with the LP-B diet. This group of cows received the greatest barley amount compared with other treatments (362 *v.* 282, 123 and 168 g/kg DM for LP-B, HP-B, HP-BM and LP-BM, respectively). Although a reduction in rumen pH after feeding highly fermentable carbohydrate (e.g. barley) is expected (Obara *et al*., 1991), it has not been observed in all studies (Moran, 1986; Gozho and Mutsvangwa, 2008). Regardless of the grain type, the differences in the amount of starch consumed by cows should be considered as an important factor on ruminal pH changes, which is likely one of reasons showing no difference in the rumen pH by feeding different types of grains (Moran, 1986; Gozho and Mutsvangwa, 2008). Cows in the LP-B group had the lowest digestibility of NDF and CP. Given that the fermentation of dietary components is limited by the rumen pH below 6.0 (Mould *et al*., 1983; Cerrato-Sánchez *et al*., 2007), the reduced digestibility of NDF and CP for LP-B group may be related to low rumen pH this group. Due to the confounding effects of different feed ingredient levels in the diet (e.g. fish meal, cottonseed meal and soybean meal), the reasons of changes of nutrient digestibility among treatments are not clearly explainable. However, the high DM, OM and NDF digestibility observed in LP-BM might, in part, indicate that high levels of CP may impact negative effects on nutrient digestibility (Mould *et al*., 1983). A proper rumen pH (approximately >6.0 for modern dairy cows) allows most groups of rumen microbes to grow and ferment dietary components more efficiently, especially fibrous feeds by cellulolytic bacteria (Strobel and Russell, 1986). Similarly, providing a more synchronized rate of protein and energy release supplied by the combination of barley and maize may result in an improved ruminal digestibility of feed fractions (Firkins, 1996).

Many factors determine the efficient utilization of NH_3-N by ruminal microbes, but Theurer *et al*. (1999) noted that total starch intake should be considered as the main factor. The greater concentrations of NH_3-N observed for cows fed with high-protein rations indicate that extra N provided by these diets could not be efficiently used towards MPS. Our results are supported by others, who reported that synchronization between N and carbohydrate release in the rumen improved N utilization by the rumen microorganisms (Chamberlain and Choung, 1995; Seo *et al*., 2010). Consequently, the greater concentrations of CP increased

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the concentrations of urinary N, BUN and MUN in cows fed with the high-protein rations. Accumulated NH_3-N is absorbed across the ruminal wall and after conversion to urea causes an increase in BUN concentration. Because urea is a small molecule, it can diffuse freely from the blood stream into tissues such as udder. In agreement with our results, other researchers also reported that urinary N, MUN and BUN concentrations were associated with the dietary CP level (Broderick, 2003; Hynes *et al.*, 2016).

Conclusion

Considering the low number of cows for a productive performance study, the current study showed that feeding high levels of dietary protein either with the barley diet or with a combination of barley plus maize did not result in an improvement in milk production performance or ruminal fermentation characteristics in early lactating primiparous Holstein cows under our experimental conditions. However, this study showed that diets containing an equal mix of barley and maize instead of diets with barley only may increase ECM and fat yield, which may, in part, be attributed to better ruminal microbial fermentation and health as evident from greater VFA concentration and ruminal pH. However, confounding effects of some dietary ingredients which led to different RDP : RUP ratio in high- or low-protein diets, made it difficult to conclude that synchronization of energy and protein release caused these results.

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Conflict of interest. The authors declare no conflicts of interest.

Ethical standards. Not applicable.

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