Effect of balancing low protein diets for methionine and lysine on performance of early lactation Holstein cows in hot environmental temperature

H. Abdi-Benemar[1], K. Rezayazdi[*2], M. Dehghan-Banadaky [2] and R. Kachuee[1]

[1]Department of Animal Science, College of agricultural sciences, University of Mohaghegh Ardabili, Iran.
[2]Department of Animal Science, Campus of agriculture and natural resources, University of Tehran, Karaj, Iran.
* Corresponding author, E-mail address: rezayazdi@ut.ac.ir

Abstract The aim of this study was to evaluate the effects of balancing low protein diets for methionine and lysine on performance of early lactation Holstein cows under hot environmental temperature. Twenty one multiparous Holstein cows in early lactation were allocated to three experimental rations including, 1) High protein ration with 17.5% CP, 2) Medium protein ration with 16% CP and 12 g/d rumen protected methionine (RP Met), 3) Low protein ration with 14.5% CP, 14 g/d RP Met and 5 g/d rumen protected lysine. There was no effect of ration on milk yield and milk fat percentage, but milk protein percent, N efficiency for milk production, milk urea nitrogen (MUN) and blood urea nitrogen (BUN) were affected significantly. N excreted in urine, N balance and creatinine concentration in urine decreased significantly by feeding low protein diets. Plasma concentrations of non-essential AA were not affected by treatments but, Methionine, Valine and Leucine concentrations were affected significantly. These results suggest that low protein diets with rumen protected amino acids can be an alternative for high protein diets for dairy cows under hot climates.
Keywords: low protein, methionine, lysine, heat stress, dairy cow

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Introduction

Dietary crude protein (CP) is necessary for microbial growth in the rumen and both feed and microbial proteins supply amino acid (AA) requirements of dairy cows (Blum et al., 1999). In animals exposed to high environmental temperature, dry matter intake (DMI) and subsequently, nutrients intake and milk yield decrease (Berman et al., 1985; Arieli et al., 2004). Increased nutrient density in the diet is a nutritional strategy to counteract the reduction in nutrient intake (West, 1998). In the case of protein nutrition, increased dietary protein concentration will be associated with an energetic cost (West, 2003). Excess N above requirement reduces metabolizable energy (ME) by 7.2 Kcal/g of N (Tyrrell et al., 1970). Otherwise, some researchers suggested that feeding low protein diets can be a useful feeding regimen for dairy cows under hot climate and reported similar performance between low and high protein diets (Higginbotham et al., 1989; Arieli et al., 2004). They attributed these results to alleviate the energy costs associated with N metabolism (Arieli et al., 2004).

Several studies have reported that decreasing CP content of dairy cow rations from 19.4 to 16.0 % had no effect on milk production, milk protein and fat concentrations and also, decreased urinary N loss and increased N utilization efficiency (Cunningham et al., 1996; Broderick, 2003; Davidson et al., 2003; Leonardi et al., 2003; Reynal and Broderick, 2005). However, decreasing CP content to levels lower than 16% decreased milk production and milk protein concentration (Piepenbrink et al., 1996; Olmos Colmenero and Broderick, 2006; Law et al., 2009). It has been shown that balancing diets for limiting amino acids improves the production response of dairy cows to low protein diets (Piepenbrink et al., 1996; Dinn et al., 1998; Leonardi et al., 2003; Broderick et al., 2008). Methionine (Met) and Lysine (Lys) are often the first two limiting amino acids (AA) for milk protein production by dairy cows (NRC, 2001). Schwab et al. (1992) stated that the best Lys to Met ratio in dairy cow rations for milk protein synthesis is 3 to 1. Also, Patton (2009) suggested that adding gram quantities of rumen protected methionine (RP Met) to fulfill the Met requirement, irrespective of metabolizable protein (MP) percentage, was the proper method to describe requirements for practical applications.

Using these experiences concerning feeding low protein diets in thermoneutral conditions for high temperature conditions may be useful. Also, effects of balancing

low protein diets with rumen protected amino acids (RP AA) and considering of Lys to Met ratio and adding gram quantities of RP AA to fulfill the AA requirement merits further study. Then, this experiment was designed to investigate the effect of balancing low protein diets with RP Met and Lys on performance of early lactation dairy cows under hot environmental temperature.

Material and Methods

Animal and Diets

Twenty one multiparous Holstein cows in early lactation (DIM =55 ± 10; BW = 583.6 ± 54 Kg; Milk production = 39 ± 2 Kg) were assigned to one of the three rations in a randomized complete design with a 35-days period. Cows were cared for according to the guidelines of the Iranian Council of Animal Care (1995). The experiment was performed in summer (from 15 June to 21 July) in the animal research station of Tehran University (Karaj, Iran). Ambient temperature and relative humidity records were taken from Iran Meteorological Service (Meteorological Organization of Iran) and summarized in Table 1. Rations were: 1) High protein diet with 17.5% CP, 2) Medium protein diet with 16% CP with 12 g/d rumen protected Met, 3) Low protein diet with 14.5% CP with 14 g/d rumen protected Met and 5 g/d rumen protected Lys. We did not include any control low protein diet without AA because the improvement in milk yield and milk protein percent and yield due to AA supplementation of low protein diets reported by earlier studies (Leonardi et al., 2003; Broderick et al., 2008) and adding a control diet has decreased the number of animals in each treatments. Supplementing of RP AA to diets was done based on AA deficiency of diets after decreasing CP level. An AA deficiency coding was used for describing AA deficiency of diets before adding RP AA as described by Patton (2010). Table 2 includes ingredients of experimental diets. Diets were formulated by the Mepron dairy ration evaluator 3.5 (Amino Cow Software).

Table 1. Ambient temperature and humidity.

Temperature, °C	
Average of max. Diurnal temp.	36.9
Average of min. Diurnal temp.	21.8
Average of Daily temp.	33.5
Days with max. temp. > 26 °C, %	100
Days with max. temp. > 32 °C, %	97.3
Days with max. temp. > 35 °C, %	91.9
Relative Humidity, (%)	
Average of max. Diurnal Humidity	53
Average of min. Diurnal Humidity	13
Average of Daily Humidity	33
THI[1]	
Average of max. Diurnal THI	77.3
Average of min. Diurnal THI	69.5
Average of Daily THI	71.2

[1]THI: temperature-humidity index

Table 2. Composition of the diets.

	1[1]	2	3
Ingredient, % of DM			
Alfalfa Hay	18.37	18.37	18.37
Corn Silage	20.41	20.41	20.41
Ground corn grain	11.84	11.84	11.84
Ground barley grain	12.57	12.57	12.57
Whole cottonseed	7.88	7.88	7.88
Corn gluten meal	2.94	0	0
Soybean meal	10.90	10.90	6.18
Wheat bran	0.37	2.18	6.18
Canola meal	3.84	3.84	3.84
Fat powder, prilled	0.61	1.21	1.82
Beet pulp	8.16	8.16	8.16
Limestone	0.77	0.81	0.85
White salt	0.20	0.20	0.20
Sodium bicarbonate	0.82	0.82	0.82
Di-calcium phosphate	0.16	0.12	0.12
Mineral and vitamin premix[2]	0.40	0.40	0.40
Methioplus[3]	0	0.11	0.12
Lys-50[4]	0	0	0.08

[1]1)17.5% CP Without RPAA, 2) 16% CP With RPMet, 3) 14.5% CP With RPMet and RPLys.
[2]Contained 195 g/Kg calcium, 21 g/Kg magnesium, 1000 mg/Kg cobalt, 300 mg/Kg copper, 120 mg/Kg iodine, 3000 mg/Kg iron, 2200 mg/Kg manganese, 3000 mg/Kg zinc, 1.1 mg/Kg selenium, 600000 IU/Kg vitamin A, 200000 IU/Kg vitamin D, 2000 mg/Kg vitamin E, 2500 mg/Kg antioxidant.
[3]measured 45.33% available Met by procedure of Berthiaume et al., 2000.
[4]measured 34.31% available Lys by procedure of Berthiaume et al., 2000.

The high protein level was chosen in order to fulfill the RDP, RUP, Lys and Met requirements (Table 3). The medium protein level was formulated with a lower CP content by removing the RUP supplement form the diet (corn gluten meal) and with RP Met for fulfilling Met requirements. The low protein diet was formulated by lowering CP content to a level lower than medium protein level by removing some of a more expensive protein supplement in the dairy cow rations (soybean meal). We used rumen protected fat powder for keeping energy density of diets in an equal level (1.59 Mcal/Kg) and wheat bran to balance diets for an equal predicted DMI level.

Methioplus (55% Met, Soda Nutrition Company, Italy) and Lys-50 (50% Lys, Soda Nutrition Company, Italy) were used as RP Met and RP Lys supplements, respectively. The addition of RP Met or RP Lys to the diets was based on Met and Lys requirements as gram per

Table 3. Chemical composition of the diets.

Chemical composition	1[1]	2	3
NE_l (Mcal/Kg of DM)[2]	1.59	1.59	1.59
CP (%DM)	17.5	16.0	14.5
RDP (%DM)[2]	11.3	11.0	10.2
RUP (%DM)[2]	6.2	5.0	4.3
EE (%DM)	4.5	5.1	5.8
NDF(%DM)	34.2	34.2	33.8
ADF (%DM)	24.0	24.1	24.4
NFC (%DM)[2]	35.9	36.6	37.7
Ash (%DM)	7.9	8.1	8.2
Ca (%DM)	0.76	0.77	0.78
P (%DM)	0.4	0.4	0.4
Lys (% of MP)[2]	7.13	7.59	7.78
Met (% of MP)[2]	2.15	2.52	2.62
Lys;Met ratio	3.32	3	3
AA deficiency code[4]	0	1	2

[1]1)17.5% CP Without RPAA, 2) 16% CP With RPMet, 3) 14.5% CP With RPMet and RPLys.
[2]Estimated using the Amino Cow, Mepron Dairy Ration Evaluator 3.5.
[3]NFC = 100 – (%NDF + %CP + %EE + %Ash)
[4]AA deficiency code = 0 for no predicted AA deficiency, 1 for predicted Met deficiency, 2 for predicted Met + Lys deficiency, and 3 for predicted Met + Lys + 1 other AA deficiency (Patton, 2010).

day for fulfilling their requirements (Patton, 2010).

Cows were housed in tie-stalls and milked at 02:00; 10:00 and 17:00 h. Animals were fed their diet as a total mixed ration (TMR) offered twice daily at 08:00 and 14:00 h for *ad libitum* consumption to allow for approximately 5% refusal. The determined RP AA products were top-dressed once a day at the morning feeding. Cows were routinely cooled by forced ventilation and two showering cycle lasting for 20 minutes before and after noon. Forced ventilation was provided by two fans in the shaded section of barn.

In Situ Measurements

For estimating ruminal resistance, intestinal digestibility and availability of AA in two RP AA products (Table 4), a three step technique was used as described by Berthiaume et al. 2000. This experiment was performed by two ruminal and intestinal cannulated cows and repeated two times. in each time, a total of 13 small polyester bags (3.5 × 5.5 cm; pore size, 52 ± 10 µm) were filled with 1.5 g of RP Met or Lys and put into a large mesh bag and suspended in the rumen for 4.5 h (Berthiaume et al., 2000). After removal from rumen, four bags were washed by hand under cold tap water until no color was visible. The remaining 9 bags were immediately transferred into a pepsin-HCl solution (pH = 2) for 2.5 h at 39°C to mimic abomasal digestion. After recovery, another 4 bags were washed as described earlier. Thereafter, the 5 remaining bags were inserted into the small intestine through the duodenal cannula at a rate of one bag in each 30 minutes (De Boer et al., 1987). Bags were recovered from feces within 24 h, thoroughly washed and analyzed for DM and Met or Lys content. Bags and their contents were dried in 40°C for 72 hours because of the lower melting point of the lipid protection layer on the RP AA.

Crude protein degradability of concentrate ingredients was determined prior to the beginning of the experiment using 6 cannulated nonlactating cows (Table 5). Duplicate Dacron polyester bags containing 5 g (DM basis) of material (9 × 15 cm, 52 ± 5 µm pore size) were incubated in each cow for 0, 2, 6, 12, 24 and 48 h to determine *in situ* DM and CP disappearance. After incubation, bags were immediately rinsed in cold water

Table 4. Mean disappearance of AA from two ruminally protected AA in different parts of the gastrointestinal tract.

Item	Methioplus	Lys-50
Measured AA content (%DM)	53.0 ±2.7	47.0 ±1.9
Rumen[1]	31.5 ±1.4	39.5 ±1.2
Post rumen[2]	66.2 ±1.0	56.7 ±2.4
Total tract[3]	97.7 ±2.4	96.1 ±3.8
Ruminal resistance[4]	68.5 ±1.4	60.6 ±1.2
Postruminal Digestibility[5]	96.6 ±1.4	93.6 ±4.1
Available Met[6]	45.3 ±1.7	34.3 ±3.2

[1]Met disappearance after 4.5 h in rumen
[2]Met disappearance in HCL solution and Intestine
[3]Rumen + Postrumen
[4]100-Rumen
[5]Postrumen/(100-rumen)
[6]Postrumen*(100-rumen)

Table 5. Amino acid composition and ruminal crude protein degradability of some of concentrate ingredients.

	soybean meal	canola meal	corn gluten	corn grain	barley grain
Amino acid composition (%DM)					
Val	2.03	1.9	2.14	0.32	0.37
Ilu	2.04	1.49	2.06	0.19	0.28
Lue	3.55	2.86	7.92	0.62	0.49
Arg	3.83	2.86	1.05	0.44	0.51
Lys	2.54	1.35	0.82	0.27	0.32
Met	0.59	0.55	1.14	0.13	0.14
Thr	1.8	1.23	1.88	0.26	0.3
Phe	2.4	1.66	3.17	0.28	0.41
His	1.4	0.8	1.29	0.26	0.26
Ruminal crude protein degradability characteristics					
a[1] (%)	19.3	23.2	4.1	22.7	26.4
b[2] (%)	78.1	72.1	95.6	75.2	67.1
c[3] (%/h)	9.1	7.3	4.8	4.5	13.9

[1]proportion of water soluble N in the total N of a feed
[2]proportion of potentially degradable N other than water soluble N in the total N of a feed
[3]fractional rumen degradation rate per hour of the *b* fraction of feed N with time, t

and washed in a commercial washing machine for 2 cycles of 15 min. The 0 h bags were not incubated in cows but followed the same washing procedure. Bags were dried in a forced-air oven at 55 °C for 48 h to determine DM disappearance. Residues from each cow, for each time point, were composited and analyzed for CP. Animals were fed a TMR with forage to concentrate ratio of 60:40. The amount fed was based on NRC (2001) recommendations to feed 10 percent more than maintenance requirements. This information was used for formulation of the experimental diets.

Sampling and analysis

TMR samples were collected weekly, dried at 55°C for 48 h, ground through a Wiley mill (1-mm screen). Samples of faeces were collected from all cows for 5 days (d 25 to 30), and composited by cow, dried at 55 °C for 48 h and ground by Wiley mill (1-mm screen). Feed and feces were analyzed for DM, CP, EE, ash (AOAC, 1990), NDF and ADF (Van Soest et al., 1991). Nonfiber carbohydrate was calculated as 100 – (CP % + NDF % + ether extract % + ash %), (NRC, 2001). Acid Insoluble Ash (AIA) was used as internal marker to calculate apparent nutrient digestibility as described by Van Keulen and Young (1977).

Temperature-Humidity Index (THI) was calculated based on ambient temperature and relative humidity records gathered by Iran meteorological Organization as T (ºF) – (0.55 – 0.55 × H) × (T (ºF) – 58) (NOAA, 1976). Milk production was measured and recorded daily from second week. Milk samples were collected three times a week from the 3 milking at each day, put into ice bunk, immediately sent to lab and analyzed for milk fat, milk protein and milk urea nitrogen (MUN). On d 28, blood samples were taken by venipuncture of coccygeal vein 4 h after the morning feeding and used for analysis of metabolites. Another blood sampling was done 2, 6 and 10 h after the morning feeding, for determination of plasma free AA concentrations (Blum et al., 1999; Südekum et al., 2004). Blood samples were immediately centrifuged at 3000×g at 4°C for 15 min and frozen at -20°C until analysis.

For analysis of AA, samples of concentrates and samples from the *In situ* experiment were pretreated with performic acid. Hydrobromic was then added to destroy performic acid and then digested with 6 *N* HCl (AOAC, 1997). For determination of AA concentrations in plasma, samples were thawed at room temperature and three plasma samples of each cow composited by cow and deproteinized using sulfosalicylic acid. Amino acids were quantified by a High Performance Liquid Chromatography system (HPLC) that was set up for AA separation (Acme 9000, YOUNG LIN, Korea).

On d 28, urine spot sampling was done every 8 h during a 24 h period. Samples were composited by cow with equal volume and analyzed for creatinine concentration and urine output (L/d) was calculated as BW (kg) × creatinine excretion rate (mg/kg of BW/d) divided by creatinine concentration (mg/L). We used 29 mg/kg of BW/d creatinine for the creatinine excretion rate as described by Valadares et al. (1999).

Body weights were measured at the beginning and end of the experiment at 07:00. Mean BW changes were calculated as the difference between beginning and final BW. Another body weighing was done on the beginning of the urine spot sampling day and daily urinary N excretion was calculated using the relationship between MUN, BW, and urinary N developed by Wattiax and Karg (2004) so that urinary N (g/d) = 0.0283 × BW (kg) × MUN (mg/dl). N balance was calculated by N intake minus N excreted by milk, faeces and urine for 25 to 28 d. N intake was calculated from multiplying diet N concentration by DMI and Faeces N was estimated from apparent N digestibility.

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Table 6. Effect of reducing CP level and supplementation of rumen protected Met and Lys on dry matter intake and milk production and composition of cows.

	1[1]	2	3	SEM	*P*
Rectal temp. (°C)	38.8	38.9	38.8	0.18	ns
DMI (Kg/d)	24.5	25.2	24.9	1.31	ns
Milk yield (Kg/d)	39.1	39.3	38.7	1.93	ns
FCM 3.5% (Kg/d)	38.4	39.9	37.8	1.98	ns
Milk protein (%)	2.9[b]	3.08[a]	2.86[b]	0.26	*
Milk protein (Kg/d)	1.25[ab]	1.34[a]	1.11[b]	0.04	*
Milk fat (%)	3.46	3.59	3.35	0.17	ns
Milk fat (Kg/d)	1.4	1.4	1.3	0.07	ns
MUN (mg/dl)	16.8[a]	14.8[b]	11.8[c]	0.67	*
Milk N: N intake (%)	24.1[b]	25.8[ab]	28.9[a]	1.13	*

[1]1)17.5% CP Without RP AA, 2) 16% CP With RP Met, 3) 14.5% CP With RP Met and RP Lys.
[a, b, c] Least square means in a row with differing letters differ significantly ($P<0.05$).
ns: not significant, *$P \leq 0.05$.

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Statistical analysis

Data such as dry matter intake, milk yield and milk composition that included repeated measurements per cows were analyzed using the mixed model procedure of SAS (1998) as a repeated measurement analysis with model effects for time, treatment and time × treatment interaction effects as fixed effects, cow as random effect and initial milk at the beginning of the experiment and days in milk as covariates. Data that included only one sample per cow such as plasma metabolites, AA profile, N excretion and nutrient digestibility were analyzed by the general linear model for effect of treatments. The effects of factors were declared at $P < 0.05$ and trends were discussed at $P < 0.15$.

Results and Discussion

Intake and Lactation Performance

Ambient temperature, temperature-humidity relative index and cow' body temperature showed that cows were under heat stress (Table 1 and 6). The effects of hot, humid conditions are thought to be mediated through an effect on cow body temperature (West, 2003). Berman et al. (1985) suggested that the upper limit of ambient temperature at which Holstein cattle may maintain a stable body temperature is 25 to 26 ºC. Temperature-humidity index (THI) incorporates the combined effect of temperature and relative humidity (NOAA, 1976). In a classic work, Johnson et al., (1963) reported that milk yield and DMI exhibited significant declines when maximum THI reached 77. Later research determined that the critical values for minimum, mean and maximum THI were 64, 72 and 76, respectively (Igono et al., 1992). Dry matter intake (DMI), body weight change, milk yield, 3.5% FCM yield, milk fat percent and yield were not affected by rations (Table 6). However, there were significant differences in milk protein percent and yield, N efficiency for milk production and MUN between experimental treatments. The lack of effect of rations on DMI, BW change and milk yield is similar to the results of earlier studies under thermoneutral (Holter et al., 1982; Davidson et al., 2003; Broderick et al., 2008) and hot environmental conditions (Higginbotham et al., 1989; Arieli et al., 2004). Arieli et al. (2004) reported that reducing the CP level from 17.4 to 15 % under hot climate, with 74.7, 75.8 and 79.9 of average daily THI for their trials had no significant effect on milk production and composition. But, Higginbotham et al. (1989) showed that a moderate reduction in the CP level from 18.4 to 16.1 % under heat stress tended to higher FCM 3.5 % production. Broderick et al. (2008)

reported that lowering the CP level to 14.8% resulted in lower milk production that is not in agreement with the obtained result of present study. In order to formulate isoenergetic diets, earlier studies that investigated the effect of supplementing low protein diets, grains were used (Higginbotham et al., 1989; Leonardi et al., 2003; Arieli et al., 2004 Socha et al., 2005; Broderick et al., 2008). However, in the present study, fat powder was used to keep the energy density of diets equal. This may explain the different results in the study reported here.

It has previously been observed that Met supplementation of dairy cow diets improves milk protein concentration (Berthiaume et al., 2006; Patton, 2010). In the present study, feeding a diet with 16% CP and RP Met produced a higher milk protein percentage and yield compared to other experimental treatments. This is in agreement with earlier studies (Leonardi et al., 2003; Broderick et al., 2008). It was suggested that the optimum Lys to Met ratio in dairy cow diets for milk protein synthesis is 3 to 1 (Schwab et al., 1992; NRC, 2001). Also, Met and Lys are often the first two limiting AA for milk protein production by dairy cows (NRC, 2001). In the present study, supplementing 14.5% CP diet with Lys and Met and also balancing Lys to Met ratio in 3 to 1 still could not prevent depression of milk protein concentration and yield. These results may suggest other AA to be limiting for milk protein synthesis in diets with CP level lower than 15% as proposed earlier (Piepenbrink et al., 1996; Broderick et al., 2008). Reducing the CP level from 17.5 to 14.5% improved conversion of dietary protein into milk protein from 24.12 to 28.93%, which is similar to what others have found under hot environmental temperature (Higginbotham et al., 1989; Arieli et al., 2004).

Table 7. Effect of reducing CP level and supplementation of Rumen protected Met and Lys on apparent nutrient digestibility and N Excretion.

	1[1]	2	3	SEM	*P*
Apparent digestibility (%)					
DM	75.2[a]	72.1[ab]	68.9[b]	1.9	*
OM	76.7[a]	74.0[ab]	71.3[b]	1.7	*
CP	76.9[a]	74.4[ab]	70.6[b]	1.7	*
EE	83.4	82.5	83.4	2.3	ns
NDF	63.9[a]	58.3[b]	56.8[b]	1.3	*
NFC	89.9	90.0	87.3	1.1	ns
N intake (g/d)	674[a]	637[ab]	547[b]	38.0	*
N in milk (g/d)	161	163	159	7.4	ns
N in feces (g/d)	157.4	159.0	159.1	14.3	ns
N in urine (g/d)	247[a]	204[b]	177[b]	13.0	*
N balance (g/d)	101[a]	104[a]	36.7[b]	18.5	*
Creatinine (mg/dl)	75.4[b]	81.4[ab]	83.3[a]	2.4	*
Urine volume (L/d)	23.2[a]	20.2[ab]	19.1[b]	1.3	ns

[1]1)17.5% CP Without RP AA, 2) 16% CP With RP Met, 3) 14.5% CP With RP Met and RP Lys.

[a, b, c] Least square means in a row with differing letters differ significantly ($P<0.05$).

ns: not significant, $*P \leq 0.05$.

Apparent Nutrient Digestibility and N Excretion

Reducing dietary CP concentration significantly decreased apparent DM, OM, CP and NDF digestibility (Table 7). N intake, N excreted in urine, N balance, urine volume and creatinine concentration in urine spot samples were affected significantly by dietary treatments. However, N excreted in feces and milk was not different in experimental groups.

In cows that were not heat stressed, reduced nutrient digestibility in response to reduced dietary CP was observed in other studies (Cunningham et al., 1996; Broderick, 2003; Groff and Wu, 2005; Broderick et al., 2008). In these studies, the reduced NDF or DM digestibility was attributed mostly to reduced RDP supply of low protein diets, but some of this reduction can be attributed to increased amounts of grain and hence starch content in the low protein diets. In the present study, the amount of grain was fixed among diets and wheat bran was used as filler in place of protein supplements that were removed in the two lower protein diets. It has been reported that the minimum ammonia concentration in rumen fluid for activity of mixed ruminal bacteria and especially fibrolytic bacteria is 5 mg/dl (Satter and Slyter, 1975; Slyter and Roffler, 1975). Therefore, it could be assumed that the ruminal ammonia concentration in this study was not limiting (7.17 mg/dl for the low protein diet, unpublished data). Rations with more CP content may stimulate digestion of some nutrients especially fiber.

The difference in N intake observed was due to different N concentration among diets because DMI between treatments was not different. Decreased urinary N excretion in response to reducing dietary CP that was observed in this study is in agreement with other studies (Dinn et al., 1998; Leonardi et al., 2003; Broderick et al., 2008). Reducing dietary CP from 17.5 to 14.5% decreased urine output from 23.18 to 19.03 L/d, but there was no significant difference between 17.5% CP diet and 16% CP diet. These results agree with results of Leonardi et al. (2003) and Broderick et al. (2008). Dinn et al. (1998) measured urine output through total collection and reported that urine output increased approximately 2 L/d for every 1 percentage unit increment in dietary protein. These reductions in urinary N excretion and urine output may be more useful for hot ambient conditions.

Plasma Metabolites and Free Amino Acid Concentrations

There were no significant differences among rations in plasma metabolites, except for BUN (Table 8). Also, Plasma concentrations of nonessential AA were not significantly different among cows (Table 9). But, plasma concentrations of Val and Lue were significantly lower for 14.5% CP ration than others. Concentrations of Met in plasma were higher ($P < 0.05$) for cows fed rations supplemented with RP Met.

The lack of effect of low protein diets supplemented with RP Met or Lys on energy indicators of plasma (glucose, NEFA and BHBA) are in agreement with other studies under thermoneutral (Dinn et al., 1998; Socha et al., 2005) and hot climate conditions (Higginbotham et al., 1989; Arieli et al., 2004). When CP level was decreased from 17.5 to 14.5%, BUN concentrations decreased from 19.4 to 15.2 mg/dl. This result is in agreement with earlier findings (Higginbotham et al., 1989; Piepenbrink et al., 1996; Socha et al., 2005; Broderick et al., 2008). Oldham (1984) stated that decreased milk yield of heat stressed cows that fed high protein diets is due to energy costs associated with synthesizing and excreting urea.

Lysine availability at the small intestine would not appear to be limiting factor for cows fed any of the rations based on a comparison of plasma Lys concentrations. Methionine was not limiting in the two diets containing lower protein percentages because plasma AA concentrations increased only when supply was greater than demand (Clark, 1975). Plasma Met concentrations in the lower protein diets was higher than in the 17.5% CP diet. This result is in agreement with result of Dinn et al. (1998) and it is suggested that supplying Met by RP Met product may be more effective than some RUP sources. NRC (2001) has proposed that a ratio of Met or Lys to MP (as % of MP) is the correct measure of Met and Lys adequacy. The committee has suggested that the Met and Lys content should be 2.54 and 7.4% of MP, respectively. However, Lapierre et al. (2006) suggested that because of competing metabolic fates, expressing requirements as ratios lacks sufficient precision. Patton (2009) suggested that adding gram quantities of RP Met to fulfill the Met requirement, irrespective of MP percentage, was the best method to describe requirements for practical applications. The lack of treatment effect on non essential AA (NEAA) in this study is in contrast to the studies in which a negative effect of reducing CP level on some NEAA was found (Dinn et al., 1998; Davidson et al., 2003). This contrary may be attributed to the sampling procedure between the present study and earlier studies. Because they used one blood sampling at 4-5 h after feeding, but in the present study, a serial blood sampling was done at 2, 6 and 10 h after feeding for determination plasma free AA concentrations. Also, the supply of NEAA should not have affected the efficiency of N utilization in the cows because shortages of individual NEAA can be met by making them from essential AA or other NEAA (NRC, 2001).

Table 8. Effect of reducing CP level and supplementation of Rumen protected Met and Lys on plasma metabolites.

	1[1]	2	3	SEM	*P*
BHBA (µmol/l)	0.69	0.65	0.59	0.1	ns
NEFA (µmol/l)	0.36	0.43	0.40	0.1	ns
BUN (mg/dl)	19.4[a]	17.0[ab]	15.2[b]	1.1	*
Triglycerids (mg/dl)	7.75	8.00	8.50	1.6	ns
Cholesterol (mg/dl)	110	134	146	19	ns
Glucose (mg/dl)	62.8	64.5	65.5	4.5	ns

[1]1)17.5% CP Without RP AA, 2) 16% CP with RP Met, 3) 14.5% CP with RP Met and RP Lys.
[a, b, c] Least square means in a row with differing letters differ significantly ($P<0.05$).
ns: not significant, $*P \leq 0.05$.

Table 9. Effect of reducing CP level and supplementation of Rumen protected Met and Lys on plasma free amino acid concentration.

	1[1]	2	3	SEM	*P*
Nonessential AA (µmol/l)					
Ala	224	222	218	8.4	ns
Ser	76	79	80	2.9	ns
Asp	20	21	21	1.8	ns
Asn	46	48	51	2.8	ns
Glu	81	81	76	6.4	ns
Gln	212	213	195	8.9	ns
Tyr	61	64	61	5.5	ns
Gly	152	159	173	18.0	ns
Total NEAA	852	865	852	19.1	ns
Essential AA (µmol/l)					
Val	267[a]	262[a]	209[b]	15.8	*
Leu	127[a]	113[a]	64[b]	17.4	*
Ilu	159	163	138	6.9	ns
Lys	55	66	70	5.5	ns
Met	15[b]	22[a]	22[a]	1.9	*
Phe	63	69	61	3.2	ns
Thr	70	71	70	5.9	ns
Arg	83	79	77	6.5	ns
His	50	45	42	2.8	ns
Total EAA	888	889	762	34.0	ns

[1]1)17.5% CP Without RP AA, 2) 16% CP With RP Met, 3) 14.5% CP With RP Met and RP Lys.
[a, b, c] Least square means in a row with differing letters differ significantly ($P<0.05$).
ns: not significant, $*P \leq 0.05$.

Conclusion

The ultimate goal of feeding low protein diets and supplying ruminally protected AA is to improve perform-

mance of dairy cattle, improve N efficiency and reduce N output to environment. Obtaining these goals, especially under hot environmental conditions, will be useful for producers, cows and environment. In this study, cows that were heat stressed and fed low protein diets with Met or Met and Lys had similar DM intake and produced similar amount of milk to cows in the high protein treatment. Milk protein percentage and yield were highest for the 16% CP diet and lowest for the 14.5% CP diet. Balancing diets for the Lys to Met ratio suggested by Schwab (1992) could not prevent the depression of milk protein yield in the 14.5% CP diet. Also, N efficiency for milk protein production improved and urinary N excretion decreased in the two lower protein diets. Results of this study showed that balancing low protein diets with RP AA could be an alternative for high protein diets in dairy cows under hot environmental temperature.

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تاثیر متوازن کردن جیره های کم پروتئین با متیونین و لیزین بر عملکرد تولیدی گاوهای شیرده هلشتاین در اوایل دوره شیردهی تحت شرایط گرم محیطی

ح. عبدی بنمار، ک. رضایزدی، م. دهقان بنادکی و ر. کچوئی سفیددشتی

نویسنده مسئول، پست الکترونیک: rezayazdi@ut.ac.ir

چکیـده هدف از این مطالعه، بررســی تاثیر متوازن کردن جیره های کم پروتئین با متیونین و لیزین بر عملکرد تولیدی گاوهای شـیرده هلشـتاین در اوایل دوره شـیردهی تحت شرایط گرم محیطی بود. تعداد ۲۱ رأس گاو شیرده هلشتاین در اوایل دوره شیردهی به سه جیره آزمایشی شامل ۱) جیره حاوی پروتئین بالا با ۱۷/۵ درصد پروتئین خام، ۲) جیره حاوی پروتئین متوسـط با ۱۶ درصـد پروتئین خام همراه با ۱۲ گرم در روز متیونین محافظت شـده شـکمبه ای، ۳) جیره حاوی پروتئین پایین با ۱۴/۵ درصــد پروتئین خام همراه با ۱۴ گرم در روز متیونین محافظت شــده شــکمبه ای و ۵ گرم لیزین محافظت شـده شکمبه ای تخصیص داده شدند. جیره های آزمایشی اثری بر شیرخام تولیدی و درصد چربی شیر نداشتند ولی درصد پروتئین شیر، راندمان استفاده از نیتروژن برای تولید شیر، نیتروژن اوره ای شیر و خون بطور معنی داری تحت تاثیر قرار گرفتند. غلظت های پلاسـمایی اسـیدهای آمینه غیر ضروری توسط تیمارهای آزمایشی تحت تاثیر قرار نگرفتند ولی غلظت پلاسمایی متیونین، والین و لوسین بطور معنی داری تحت تاثیر قرار گرفت. این نتایج پیشنهاد می کند که جیره های کم پروتئین به همراه اســیدهای آمینه محافظت شــده شــکمبه ای می توانند به عنوان جایگزینی برای جیره های با پروتئین بالا تحت شرایط گرم محیطی مد نظر قرار گیرند.