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Lowering rumen-degradable protein maintained energy-corrected milk yield and improved nitrogen-use efficiency in multiparous lactating dairy cows exposed to heat stress

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ABSTRACT

The objective of this study was to examine the effect of reducing rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) proportions on feed intake, milk production, and N-use efficiency in primiparous and multiparous cows exposed to warm climates. Eighteen primiparous and 30 multiparous mid-lactation Holstein cows were used in a completely randomized design with a 2 × 2 factorial arrangement of treatments. Cows were randomly assigned to 1 of 4 dietary treatments formulated to contain 2 proportions of RDP (10 and 8%) and 2 proportions RUP (8 and 6%) of dry matter (DM) indicated as follows: (1) 10% RDP, 8% RUP; (2) 8% RDP, 8% RUP; (3) 10% RDP, 6% RUP; and (4) 8% RDP, 6% RUP. Protein sources were manipulated to obtain desired RDP and RUP proportions. Diets were isoenergetic and contained 50% forage and 50% concentrate (DM basis). Cows were individually fed the 10% RDP, 8% RUP diet 3 wk before treatment allocation. Cows were exposed to the prevailing Tennessee July and August temperature and humidity in a freestall barn with no supplemental cooling. Main effects and their interaction were tested using the Mixed procedure of SAS (least squares means ± standard error of the mean; SAS Institute Inc., Cary, NC). Observed values of nutrient intake and milk production were used to obtain NRC (2001) model predictions. Cows showed signs of heat stress throughout the study. Reducing from 10 to 8% RDP decreased dry matter intake (DMI; 0.9 kg/d) at 8% RUP, but increased DMI (2.6 kg/d) at 6% RUP in primiparous cows. Reducing from 10 to 8% RDP decreased milk yield (10%) at 8% RUP, but increased yield (14%) at 6% RUP. Treatments did not affect yield of energy-corrected milk. For multiparous cows, treatments did not affect DMI. Reducing from 10 to 8% RDP decreased yield of energy-corrected milk (3.4%) at 8% RUP, but increased yield (8.8%) at 6% RUP. Reducing from 10 to 8% RDP and 8 to 6% RUP both increased N-use efficiency for primiparous and multiparous cows. The NRC model underestimated metabolizable protein and RUP supply, and overestimated RUP requirements, resulting in predictive losses of milk yield 1.4 to 5.8 times greater than observed values. In summary, the reduction of RDP and RUP proportions did not affect DMI, whereas the RUP reduction at 10% RDP had a small negative effect on energy-corrected milk yield. However, reduction of RDP and RUP consistently improved N-use efficiency of heat-stressed multiparous cows. The reduction of RDP and RUP proportions reduced DMI and milk yield but did not affect energy-corrected milk yield in primiparous cows, indicating a limited supply of nutrients.

Key words: rumen degradable protein, rumen undegradable protein, heat stress

INTRODUCTION

Protein and AA requirements of lactating dairy cows have been studied to optimize milk production, increase dietary N captured in milk, and minimize excretion of N into the environment (Mutsvangwa et al., 2016). Cows fed ≥18.0% CP maximize milk production (NRC, 2001); however, high CP diets reduce dietary N captured in milk and increase N excretion into the environment (Colmenero and Broderick, 2006; Rius et al., 2012). Dairy cows convert one-third of dietary N into milk N, and excrete two-thirds of dietary N via urine and feces (Bequette et al., 1998; Hristov et al., 2004). Research has shown that as dietary CP increases from 15.1 to 18.4%, the efficiency of converting dietary N to milk N (i.e., N-use efficiency) decreases linearly (Broderick, 2003). However, recent work has reported improvements in N-use efficiency, when feeding lower than recommended RDP and RUP proportions, without a negative effect in milk production (Kalscheur et al., 2006; Wang et al., 2007; Rius et al., 2010). For example, a reduction of 2.8 percentage units in RDP increases N-use efficiency and capture of N in milk by 16% and reduces urinary N excretion by 29% (Kalscheur et al.,

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2006). Similarly, a reduction of 2.5 percentage units in RUP increases N-use efficiency and capture of N in milk by 15% and reduces urinary N excretion by 25% (Wang et al., 2007). Thus, reductions of RDP and RUP have been implemented to reduce urinary N excretion and increase N-use efficiency.

Previous research tested the effect of increasing dietary protein content to compensate for lower DMI of lactating cows exposed to warm climates. However, an increase in dietary protein reduced milk yield, N capture in milk, and N-use efficiency (Higginbotham et al., 1989; Huber et al., 1994; Arieli et al., 2004). Kamiya et al. (2005) investigated the effect of heat stress in lactating dairy cows on N utilization. Compared with thermoneutral conditions, heat stress increased the proportion of dietary N intake excreted in urine by 41% and reduced milk protein synthesis by 9%. Collectively, exposure to heat stress conditions reduces milk protein synthesis and exacerbates the poor capture of dietary N in milk N, and increases the excretion of dietary N in urine. Reducing dietary N may improve the capture of N in milk and the yield of milk during periods of heat stress.

Manipulation of RDP and RUP can be an effective nutritional strategy to maintain production and improve the utilization of dietary N in lactating cows exposed to heat stress. Researchers have indicated that a reduction of RDP from 10.8 to 8.5% of DM improved milk yield and did not affect DMI of heat-stressed cows (Zook, 1982; Taylor et al., 1991). Although N-use efficiency was not measured in these studies, a reduction of N intake combined with an increase in milk production is expected to improve the capture of dietary N in milk and N-use efficiency. In addition to RDP, a reduction of dietary CP from 16.7 to 15.1%, by manipulating RDP and RUP, did not affect DMI and milk production, but increased N retention in milk by 6% in heat-stressed cows (Arieli et al., 2004). Therefore, lowering RDP and RUP concentrations may maintain milk production and DMI, increase capture of dietary N in milk protein, and improve N-use efficiency in heat-stressed cows.

The effect of reduced RDP and RUP proportions on milk production and utilization of N in primiparous cows is unclear; however, the utilization of additional RUP differs in primiparous when compared with multiparous cows. Flis and Wattiaux (2005) reported that milk protein yield increased in response to additional RUP fed to primiparous and multiparous cows. However, multiparous cows retained more of the additional RUP, whereas primiparous cows showed a greater urinary excretion of the additional RUP. Moreover, urinary N excretion increased in response to additional RUP more in primiparous compared with multiparous cows (33 vs. 6.5 g/d). Therefore, the objectives of this study were to (1) assess the effects of low fractions of RDP and RUP on milk production and N-use efficiency in primiparous and multiparous cows exposed to heat stress conditions and (2) evaluate the predictive ability of the NRC (2001) model on milk production for cows fed low RDP and RUP. We hypothesized that reduction of RDP or RUP would sustain milk production and improve N-use efficiency in heat-stressed dairy cows.

MATERIALS AND METHODS

Animals, Housing, and Treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Tennessee. Eighteen primiparous (140 ± 49 DIM) and 30 multiparous (126 ± 47 DIM) mid-lactation Holstein cows were used from the East Tennessee AgResearch and Education Center (**ETREC**) dairy herd and housed in a freestall barn at the ETREC dairy facility. Prior to treatments, cows were stratified into 4 groups based on DIM, milk production, BCS, and parity and then randomly assigned to 1 of the 4 treatments for 21 d (n = 12 cows/treatment) in a completely randomized design with a 2 × 2 factorial arrangement of the treatments. Dietary treatments were 2 proportions of RDP (10 and 8%) and 2 proportions of RUP (8 and 6%) of DM indicated as follows: (1) 10% RDP, 8% RUP (**10D:8U**); (2) 8% RDP, 8% RUP (**8D:8U**); (3) 10% RDP, 6% RUP (**10D:6U**); and (4) 8% RDP, 6% RUP (**8D:6U**). Dietary treatments were formulated to meet NRC (2001) recommendations for a mid-lactation dairy cow exposed to heat stress (West, 2003) with 622 kg of BW (BCS = 3.0), producing 35 kg of milk/d containing 3.5% fat and 3.2% protein, and consuming 21 kg/d of DM. Diets contained 50% forage and 50% concentrate on a DM basis (Table 1). Ruminally degradable and undegradable protein proportions were manipulated by varying the amounts of soybean meal, rumen-protected soybean meal (SoyPLUS, West Central Cooperative, Ralston, IA), fish meal, blood meal, and urea. Soybean hulls and rumen-protected fat (BergaFat, Berg+Schmidt Feed, Libertyville, IL) were included to maintain equal energy content of the treatments. Diets were mixed at 0900 h and fed once daily as a TMR to each individual cow using an electronic feeding system (American Calan Inc., Northwood, NH). Feed was offered to achieve 5 to 10% refusals. Cows were fed the 10D:8U diet during a pre-treatment period of 21 d to acclimate to a common diet, followed by their respective dietary treatments. Cows were milked twice daily at 0900 and 1900 h in a double-8 herringbone parlor, and milk production was automatically recorded at each milking. Body weights and BCS, assessed by the

Table 1. Composition of experimental diets (% of DM basis)

Ingredient	Experimental diet			
	8% RUP		6% RUP	
	10% RDP	8% RDP	10% RDP	8% RDP
Corn silage	45.0	45.0	45.0	45.0
Wheat silage	1.50	1.50	1.50	1.50
Clover hay	3.50	3.50	3.50	3.50
Corn grain, ground, dry	27.0	27.0	27.0	27.0
Soybean meal, solvent (48% CP)	12.1	2.70	5.60	5.80
Soybean hulls	—	2.40	6.40	6.60
Protected soybean meal[1]	5.60	11.9	2.60	4.00
Urea	—	—	0.75	—
Blood meal, ring dried	1.00	1.00	1.00	0.50
Fish meal, menhaden	0.80	0.50	0.70	0.40
Rumen-protected methionine[2]	0.35	0.35	0.35	0.35
Rumen-stable fat[3]	0.30	1.10	2.50	2.30
Salt	0.35	0.35	0.35	0.35
Calcium phosphate (mono-)[4]	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.70	0.70	0.70	0.70
Potassium carbonate	0.50	0.50	0.50	0.50
Calcium carbonate	0.60	0.60	0.60	0.60
Magnesium oxide	0.15	0.15	0.15	0.15
Calcium propionate	0.01	0.01	0.01	0.01
Trace mineral and vitamin mix[5]	0.45	0.45	0.45	0.45

[1] SoyPLUS, West Central Cooperative (Ralston, IA).

[2] Adisseo MetaSmart (Alpharetta, GA), as pelletable form of methionine.

[3] Berg+Schmidt Feed BergaFat (Libertyville, IL).

[4] Contained 16.4% Ca and 21.6% P.

[5] AgCentral Cooperative (Athens, TN); formulated to provide (per kg of dietary DM): 6.0×10^5 IU of vitamin A, 1.5×10^5 IU of vitamin D, 2.8×10^6 IU of vitamin E, 25 mg of Cu, 3.0×10^2 mg of Fe, 1.1×10^2 mg of Mn, and 2.1×10^2 mg of Zn.

same experienced participant (1 = emaciated to 5 = extremely obese; Edmonson et al., 1989), were recorded once weekly after the 0900 h milking.

Environmental Management

Environmental temperature (°C) and relative humidity (%) were measured at 10-min intervals using HOBO Pro v2 Series probes (Onset Computer Corporation, Bourne, MA). Temperature-humidity index (**THI**) was assessed based on the following equation from Dikmen and Hansen (2009), where T = environmental temperature (°C) and RH = relative humidity (%):

$$\mathrm{THI} = (1.8 \times \mathrm{T} + 32) - [(0.55 - 0.0055 \times \mathrm{RH}) \times (1.8 \times \mathrm{T} - 26)].$$

Throughout the pre-treatment period, the barn was managed to prevent elevated environmental temperature (e.g., fans were set to come on at 20°C). To mimic the daily fluctuation of temperature observed in summer months in Tennessee, throughout the treatment period all cows experienced prevailing Tennessee July and August summer temperatures from 1000 to 2000 h for which heat abatement was not provided (THI ranged from 74 to 82). At 2000 to 1000 h, fans were set to come on at 20°C and the THI ranged from 69 to 76. Monitoring thermal load during the treatment period was accomplished through measurements of core body temperature (rectal and vaginal temperature) and respiration rate. Rectal temperatures were measured twice daily at 1000 and 1500 h using a GLA M700 battery-operated digital read-out thermometer (GLA Agricultural Electronics, San Luis Obispo, CA; accuracy ± 0.1°C). Respiration rates were measured 3 times weekly at 1000 and 1500 h by counting flank movements for 15 s and reported as breaths per minute. Furthermore, vaginal temperatures were measured in 2 groups of 24 animals each (primiparous, n = 9; multiparous, n = 15) on d 1, 2, 3, 4, 5, 11, 12, 13, 14, and 15 and on d 6, 7, 8, 9, 10, 17, 18, 19, 20, and 21 of the experiment. Vaginal temperatures were assessed every 10 min using temperature loggers (DS1922L Thermochron iButton Device, Maxim Integrated, San Jose, CA; accuracy ± 0.5°C) inserted into a modified blank controlled internal drug release devices (Elanco, Greenfield, IN; Dikmen et al., 2008; Burdick et al., 2012).

Sample Collection and Analyses

Forage, concentrate, and TMR samples were collected twice weekly and stored at −20°C. Samples of forage and concentrate mixes from each treatment were dried at 60°C and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Weekly pooled samples were chemically analyzed (Table 2) for total N according to AOAC International (1999; method 990.03) using a CN628 Carbon/Nitrogen Determinator (Leco, Saint Joseph, MI); for NDF according to Van Soest et al. (1991); for ADF according to AOAC International (1999; method 973.18); for starch according to AOAC International (1999) using a YSI 2700 Select Biochemistry Analyzer (Yellow Springs, OH); for ether extract by AOAC International (1999; method 2003.05); and for minerals according to AOAC International (1999) using inductively coupled plasma spectrometry (Thermo iCAP 6300, Waltham, MA). Milk samples were collected from each a.m. and p.m. milkings on d 18, 19, and 20 of the pre-treatment and treatment periods. Individual milk samples were analyzed for fat, protein, lactose, SNF, and MUN by Fourier transform mid-infrared analysis (Foss MilkoScan, Eden Prairie, MN; United DHIA, Radford, VA). Milk SCC was analyzed by flow cytometry (Foss Fossomatic FC; United DHIA). Energy-corrected milk was calculated by using the equation provided by Tyrrell and Reid (1965). Nitrogen utilization was assessed through calculated predicted values for urinary N, fecal N, and N-use efficiency using the equations provided by Wattiaux and Karg (2004).

Statistical Analysis

Nutrient and DMI, yields of milk and ECM, milk composition, N utilization, BW, BCS, rectal and vaginal temperature, and respiration rate data were analyzed using the Mixed model procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). For milk yield and DMI, data collected throughout the 21 d were analyzed to determine temporal changes in response to treatments. In addition, milk yield, DMI, and all collected data throughout the last 7 d of the treatment period were used in the statistical analysis and to obtain overall treatment LSM and SEM (Zanton, 2016). Daily DMI and milk yield data throughout the treatment period were analyzed as repeated measures in time. Nonrandom time constraints were expected in these dependent variables, thus repeated measures were used for experimental days. The autoregressive variance-covariance structure was selected based on Akaike's information criterion (**AIC**) and corrected AIC fit statistics, including expected relationships between days. The most suitable covariance structure was chosen from observation of the smallest AIC and corrected AIC values. Observations from the last 7 d of the pre-treatment period were included as a covariate adjustment in the model for DMI and milk yield and from the last 3 d for milk components. The model included

$$\mathrm{Y_{ijkl}} = \mu + \mathrm{D_i} + \mathrm{U_j} + \mathrm{A(D \times U)_{ijk}} + \mathrm{T_l} \\ + \text{interactions} + \beta(\chi)_{\mathrm{ijkl}} + \mathrm{e_{ijkl}},$$

where $\mathrm{Y_{ijkl}}$ = dependent variable; μ = the overall mean; $\mathrm{D_i}$ = the fixed effect of the ith RDP treatment (i = 10% and 8% RDP); $\mathrm{U_j}$ = the fixed effect of the jth RUP treatment (j = 8 and 6% RUP); $\mathrm{A(D \times U)_{ijk}}$ = the random effect of the kth animal in the ith and jth treatment; $\mathrm{T_l}$ = the fixed effect of lth day as repeated measures; interactions = the fixed effects of all D, U, and T interactions; $\beta(\chi)_{\mathrm{ijkl}}$ = the covariate effect; and $\mathrm{e_{ijkl}}$ = the random error. Variables of interest were ana-

Table 2. Observed chemical composition of the feed ingredients used in the experimental diets (% of DM basis)

	Ingredient[1]						
Item	Corn silage	Wheat silage	Clover hay	Concentrate mix A	Concentrate mix B	Concentrate mix C	Concentrate mix D
DM, % of feed	33.2	23.3	82.6	88.6	88.4	89.4	88.8
NDF	39.7	60.8	55.2	9.47	12.7	14.2	16.7
ADF	24.9	37.1	32.2	4.03	6.05	7.70	9.50
CP	7.70	13.3	13.0	26.5	23.7	23.8	20.0
NFC	44.1	14.4	19.7	—	—	—	—
Calcium	0.17	0.31	0.66	1.39	1.46	1.47	1.42
Phosphorus	0.21	0.33	0.31	0.65	0.59	0.53	0.53
Magnesium	0.16	0.16	0.23	0.37	0.39	0.35	0.36
Potassium	0.88	3.09	2.41	1.62	1.50	1.38	1.37
Sodium	0.007	0.013	0.025	0.78	0.73	0.78	0.72

[1] Concentrate mix A was used to formulate the 10% RDP and 8% RUP diet, concentrate B was used to formulate the 8% RDP and 8% RUP diet, concentrate C was used to formulate the 10% RDP and 6% RUP diet, and concentrate D was used to formulate the 8% RDP and 6% RUP diet.

lyzed in primiparous and multiparous cows, separately. For a minimum power of 0.80 in the present experimental design, 45 observations per treatment were needed to determine significance differences among treatments. Thus, for primiparous cows, the number of observations, mainly BW and BCS data, limited the ability to detect differences among treatment groups. Least significant differences with the slice option of SAS were reported when D × U interactions were significant. Interaction terms between treatments were removed from the model whenever deemed nonsignificant. Significant differences were declared at $P \leq 0.05$ and trends were declared at $0.05 < P \leq 0.10$. All results are reported as least squares means (±SEM). Two multiparous cows (treatments 10D:8U and 8D:6U) were removed from the study due to unexpected health issues (i.e., mastitis).

NRC Model Analysis

The NRC (2001) model was assessed using least squares means from multiparous cows. Least squares means of DMI, milk yield, milk composition, BW, and BCS from each dietary treatment were used as inputs to assess the model predictions for accuracy of MP. The chemical composition of the diets was set to actual ingredient values listed in Table 2, and observed DMI was used to set the feeding rate for each ingredient. Tabular values were used to calculate the nutrient content of each concentrate grain mix and compared with the observed values of the mixes reported in Table 2. An adjustment was required because the tabular values of CP, NDF, and ADF in NRC (2001) differed from values obtained by chemical analysis of concentrates. Soybean meal, rumen-protected soybean meal, soybean hulls, and blood and fish meal were the major contributors of CP in concentrate mixes A, B, C, and D; therefore, the tabular CP content of these ingredients was adjusted to reflect the measurements of CP in the concentrate mixes. The tabular CP content was increased from 46.6 to 51.3% of DM for rumen-protected soybean meal and from 13.8 to 16.8% of DM for soybean hulls, whereas CP value was reduced from 95.5 to 86.0% of DM for blood meal. The 3.0-percentage-unit CP adjustment for soybean hulls likely reflects formulation errors at the feed mill. The NDF and ADF content of soybean hulls, soybean meal, and rumen-protected soybean meal were adjusted in a similar manner to reflect the values obtained by the chemical analysis of the concentrate mixes. The NDF content of soybean hulls and soybean meal was increased from 60.3 to 63.3% of DM and from 9.8 to 10.3% of DM, whereas the NDF content for protected soybean meal was reduced from 17.6 to 15.0% of DM. The ADF tabular values of soybean hulls and rumen-protected-soybean meal were reduced from 44.6 to 40.1% of DM and from 10.6 to 9.5% DM, whereas the ADF value for soybean meal increased from 6.2 to 6.8% of DM.

RESULTS

The formulated composition of experimental diets is presented in Table 1. Actual nutrient composition of individual feed ingredients is reported in Table 2. The observed nutrient contents of diets are reported in Table 3. The observed NDF, ADF, NFC, and NE_L contents of the experimental diets differed from the values formulated due to variation in the chemical composition of the ingredients and formulation variation. The observed energy content in the diets was slightly different than predicted, possibly due to variation in dietary starch and fat across the diets. As anticipated, the CP, RDP, and RUP differences among experimental diets remained relatively within 2.0 percentage units of their counterparts.

Nutrient Intake and Milk Production

A RDP × RUP interaction (Figure 1A; $P < 0.01$) was observed such that lowering RUP from 8 to 6% at 10% RDP decreased DMI of primiparous cows ($P < 0.01$; 19%; Table 4); however, DMI did not change at 8% RDP. Relative to 10% RDP, the 8% RDP treatment reduced DMI at 8% RUP but increased DMI at 6% RUP. Primiparous cows fed the 10D:8U diet had the greatest DMI, but cows fed the 10D:6U diet had the lowest DMI. Compared with 10% RDP, the 8% RDP treatment decreased CP intake (0.54 kg/d) at 8% RUP but did not affect CP intake at 6% RUP (RDP × RUP interaction, $P < 0.01$). Compared with 10% RDP, the 8% RDP treatment increased NDF and ADF intake (1.0 and 0.63 kg/d, respectively) at 6% RUP but did not affect NDF nor ADF intake at 8% RUP (RDP × RUP interaction, $P < 0.01$). Compared with 10% RDP, the 8% RDP treatment decreased initial and final BW (85 and 64 kg, respectively) at 6% RUP but did not affect BW at 8% RUP (RDP × RUP interaction, $P \leq 0.04$). Compared with 10% RDP, the 8% RDP treatment decreased DMI as a percentage of BW at 8% RUP; however, DMI as a percentage of BW was increased with reduced RDP at 6% RUP (RDP × RUP interaction, $P \leq 0.04$).

The RDP × RUP interaction did not affect the DMI of multiparous cows (Table 4; Figure 1B). However, compared with 8% RUP treatments, a tendency was observed for the 6% RUP treatment to reduce DMI (0.35 kg/d; $P = 0.08$). As expected, reducing RDP from 10 to 8% decreased ($P < 0.01$) CP intake (0.45 kg/d). Similarly, reducing RUP from 8 to 6% decreased ($P <$

0.01) CP intake (0.52 kg/d). Compared with 8% RUP, the 6% RUP treatment increased ($P < 0.03$) NDF and ADF intake (0.33 and 0.29 kg/d, respectively). Treatments did not affect initial or final BW and DMI as a percentage of BW.

A RDP × RUP interaction (Figure 2A; $P < 0.01$) was observed such that lowering RUP from 8 to 6% decreased milk yield ($P < 0.01$; 16.9%; Table 5) at 10% RDP but had no effect at 8% RDP in primiparous cows. However, treatments did not affect ECM yield. Compared with 8% RUP, the 6% RUP treatment decreased ($P \leq 0.04$) lactose percentage (0.06 percentage units) and milk protein yield (8.0%). Compared with 10% RDP, the 8% RDP treatment increased milk protein percentage (0.13 percentage units) at 8% RUP but had no effect at 6% RUP (RDP × RUP interaction, $P = 0.01$). Compared with 10% RDP, the 8% RDP treatment tended to increase milk fat percentage (0.56 percentage units) only at 8% RUP (RDP × RUP interaction, $P = 0.09$). Compared with 10% RDP, the 8% RDP treatment decreased ($P < 0.01$) MUN concentration (28%), and compared with 8% RUP, the 6% RUP treatment decreased ($P < 0.01$) MUN concentration (29%).

For multiparous cows, a trend was observed for a RDP × RUP interaction on milk yield when the 21-d treatment period was analyzed ($P = 0.06$; Figure 2B). A RDP × RUP interaction was observed on yields of milk and ECM ($P = 0.03$; Table 5) such that lowering from 8 to 6% RUP decreased yields of milk (15%) and ECM (8.6%) at 10% RDP but sustained yields at 8% RDP. Relative to 10% RDP, the 8% RDP treatment did not reduce yields of milk and ECM at 8% RUP and 6% RUP treatments. Compared with 10% RDP, the 8% RDP treatment decreased milk lactose yield (6.0%) and milk protein yield (6.3%) at 8% RUP but had no effects at 6% RUP (RDP × RUP interaction, $P \leq 0.03$). Compared with 8% RUP, the 6% RUP treatment decreased ($P < 0.01$) milk lactose percentage (0.08 percentage units). Compared with 8% RUP, the 6% RUP treatment increased ($P < 0.01$) milk protein percentage (0.19 percentage units). Treatments did not affect milk fat yield; however, reducing from 8 to 6% RUP increased ($P = 0.01$) milk fat percentage (0.31 percentage units). Compared with 10% RDP, the 8% RDP treatment decreased ($P < 0.01$) MUN concentration (36%), and compared with 8% RUP, the 6% RUP treatment decreased ($P < 0.01$) MUN concentration (26%).

Nitrogen Utilization

Compared with 10% RDP, the 8% RDP treatment decreased ($P < 0.01$) predicted urinary N excretion in

Table 3. Observed nutrient composition of experimental diets and nutrient balance and allowable milk as predicted from the NRC (2001) model using the chemical analysis of feed[1]

	Experimental diet			
	8% RUP		6% RUP	
Item	10% RDP	8% RDP	10% RDP	8% RDP
DM,[2] %	48.1	47.8	48.7	48.1
RDP, % of DM	9.80	7.80	9.70	7.80
RUP, % of DM	7.80	8.10	5.90	6.00
NDF, % of DM	25.4	26.9	28.3	28.7
ADF, % of DM	15.2	16.1	17.0	17.2
NFC, % of DM	47.9	47.2	45.0	46.7
Crude fat, % of DM	3.60	4.70	5.70	5.60
NE_L, Mcal/kg	1.69	1.70	1.68	1.69
RDP required, g/d	2,124	2,015	2,041	2,027
RDP supplied, g/d	1,991	1,505	1,896	1,497
RDP balance, g/d	−133	−510	−145	−529
RUP required, g/d	1,438	1,622	1,378	1,653
RUP supplied, g/d	1,594	1,567	1,168	1,171
RUP balance, g/d	156	−55.0	−210	−482
MP required, g/d	2,436	2,324	2,280	2,307
MP supplied, g/d	2,573	2,275	2,104	1,899
MP balance, g/d	137	−48.0	−176	−408
MP allowable milk, kg/d	42.3	36.0	29.4	26.2
NE_L allowable milk, kg/d	38.8	36.2	34.9	35.0

[1] Values predicted from NRC (2001) using actual chemical analysis of CP, NDF, and ADF of feed ingredients, DMI, milk yield, and components for each treatment. Requirements were set based on observed performance variables (i.e., DMI, milk yield, milk component yields, BW, and BCS).

[2] Actual DM of TMR for each treatment.

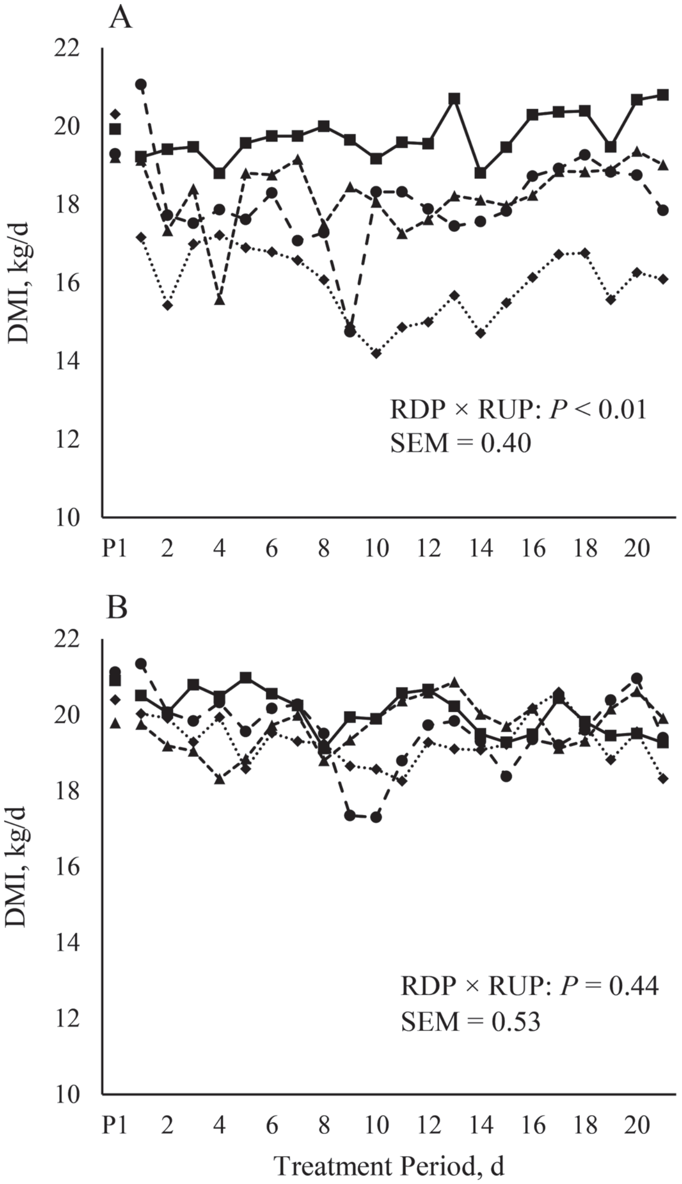

Figure 1. Effect of varying RDP and RUP proportions on DMI (P1, mean of 7-d pre-treatment period). Values are LSM ± pooled SEM for (A) primiparous and (B) multiparous cows. All cows were fed 10% RDP, 8% RUP throughout the pre-treatment period. Legend: 10% RDP, 8% RUP (–■–); 8% RDP, 8% RUP (- -▲- -); 10% RDP, 6% RUP (···◆···); 8% RDP, 6% RUP (- -●- -).

primiparous (42.3 g/d) and multiparous cows (67.4 g/d; Table 6). Similarly, compared with 8% RUP, the 6% RUP treatment decreased ($P < 0.01$) predicted urinary N excretion in primiparous (47.4 g/d) and multiparous cows (48.4 g/d). Compared with 8% RUP, the 6% RUP treatment decreased ($P < 0.01$) predicted fecal N excretion (47.0 g/d) in primiparous cows. Compared with 10% RDP, the 8% RDP treatment increased ($P < 0.01$) N-use efficiency for primiparous (10%) and multiparous cows (10%), and compared with 8% RUP, the 6% RUP treatment increased ($P < 0.01$) N-use efficiency in primiparous (13%) and multiparous cows (9.0%).

Environmental Management and Temperature Responses

Fluctuation of THI observed daily is presented in Figure 3. By design throughout the study, daily THI remained elevated. Temperature-humidity index peaked at 81.8 and averaged 81.0 ± 0.35 from 1200 to 2000 h (i.e., moderate heat stress). Temperature-humidity index averaged 74.9 ± 0.35 from 2000 to 1200 h. Along with THI, core body temperatures and respiration rates differed between the a.m. and p.m. measurements (means not shown), indicating that cows showed signs of moderate heat stress. Morning rectal and vaginal temperatures remained at 38.8 and 39.2°C, respectively, and a.m. respiration rates remained at 63.9 breaths/min (Table 7). Afternoon rectal and vaginal temperatures remained at 39.7 and 39.7°C, respectively, and p.m. respiration rates remained at 87.1 breaths/min. Treatments did not affect rectal temperature and respiration rate. However, a RDP × RUP interaction ($P = 0.04$) was observed whereby lowering from 8 to 6% RUP in the 10% RDP diet increased p.m. vaginal temperature (0.4°C) of multiparous cows.

NRC Model Analysis

Observed chemical composition of ingredients and treatment means for DMI and milk production were used to obtain NRC (2001) predictions (Table 3). Body weight and BCS changes between initial and final measurements had no effect on NRC (2001) model predictions. In the 10D:8U diet, NE_L was limiting for allowable milk. Treatments did not meet RDP requirements; however, by design, treatments with low RDP proportions resulted in similar RDP deficiencies. Relative to the 10D:8U diet, the 8D:8U diet resulted in a RDP deficiency (377 g) and an increase in RUP requirement (184 g). Compared with the 10D:8U diet, the reduction of 2 points in dietary RDP predicted a NE_L-allowable milk yield decline of 2.6 kg (10D:8U − 8D:8U). Furthermore, the MP-allowable milk yield decline was 6.3 kg. In comparison, observed milk yield was reduced by 2.0 kg. The model overestimated the loss by 0.8 kg of milk (2.8 − 2.0 kg), indicating that the observed response was 71% of the predicted response. Compared with the 10D:6U diet, the 8D:6U diet resulted in a RDP deficiency (384 g) and an increase in RUP requirement (275 g), which resulted in deficiencies of RUP (482 g) and MP (408 g). Compared with the 10D:6U diet, the reduction of 2 points of dietary RDP predicted an allowable milk yield decline of 3.2 kg (10D:6U − 8D:6U); however, observed milk yield increased by 2.3 kg. Therefore, the model had a prediction error for allowable milk yield on the direction and the magnitude of this response.

Relative to the 10D:8U diet, the 10D:6U diet predicted a decline of RUP supplied (426 g). The model predicted an allowable milk yield decline of 9.4 kg (10D:8U − 10D:6U); however, observed milk yield declined by 6.0 kg. Relative to the 8D:8U diet, the 8D:6U diet predicted a decline of RUP supplied (396 g). The model predicted an allowable milk yield decline of 9.8 kg (8D:8U − 8D:6U); however, observed milk yield declined by 1.7 kg. Compared with the 10D:8U diet, the 8D:6U diet predicted an allowable milk yield decline of 12.6 kg; however, observed milk yield declined by 3.7 kg.

DISCUSSION

Health, production, and utilization of nutrients are affected under hyperthermic conditions, causing a burden to the dairy industry. Even with new technologies and advances in heat abatement techniques, warm climates continue to present problems for lactating dairy cows (Ravagnolo et al., 2000). Reduction of RDP and RUP can potentially reduce some of the negative effects of heat stress on dairy cows (Kassube et al., 2017). Herein, the effects of RDP and RUP proportions are evaluated on intake, milk production, and N-use efficiency for primiparous and multiparous dairy cows exposed to heat stress.

Feed Intake and Milk Production

The DMI remained low throughout the treatment period due to an increase in THI. In the present study, treatments affected DMI in primiparous cows. A 2-percentage-point reduction of RUP proportions decreased DMI by 3.7 kg at 10% RDP, whereas a 2-percentage-point reduction of RDP decreased DMI by 0.9 kg at 8% RUP. A 2-percentage-point reduction in the form of RUP had a 4 times greater negative effect on DMI than a 2-percentage-point reduction in the form of RDP. In agreement with DMI results, treatments also affected CP intake, but lowering RUP proportions reduced CP intake by a greater magnitude (0.4 to 1.0 kg) relative to lowering RDP proportions (0.0 to 0.5 kg). When RDP or RUP was decreased in the 10D:8U treatment milk production declined, but lowering RUP resulted in a 40% greater loss of milk yield than lowering RDP (5.9 vs. 3.5 kg of milk yield loss; 10D8U − 10D:6U vs. 10D8U − 8D:8U). In fact, reduction of RDP at 6% RUP increased milk production (29.0 vs. 33.3 kg/d). In agreement with the current results, Bruckental et al. (1989) reported that a 4-percentage-unit reduction of

Table 4. Least squares means of intake, BW, and BCS for heat-stressed Holstein cows fed varying amounts of RDP and RUP (LSM ± SEM)

	Experimental diet					Effect (*P*-value)		
	8% RUP		6% RUP					
Item	10% RDP	8% RDP	10% RDP	8% RDP	SEM	RDP (D)	RUP (U)	D × U
Primiparous, no.	5	4	4	5				
Intake, kg/d								
DM	19.7^{a}	18.8^{b}	16.0^{c}	18.6^{b}	0.30	0.01	<0.01	<0.01
CP	3.53^{a}	2.99^{b}	2.54^{c}	2.60^{c}	0.05	<0.01	<0.01	<0.01
NDF	5.50^{b}	5.55^{b}	4.86^{c}	5.86^{a}	0.09	<0.01	0.10	<0.01
ADF	3.27^{b}	3.30^{b}	2.96^{c}	3.59^{a}	0.06	<0.01	0.83	<0.01
Initial BW, kg	577^{b}	599^{ab}	640^{a}	555^{b}	18.6	0.10	0.60	<0.01
Final BW, kg	591^{ab}	616^{ab}	627^{a}	563^{b}	21.1	0.35	0.70	0.04
Initial BCS	2.43	2.44	2.46	2.35	0.14	0.70	0.82	0.66
Final BCS	2.50	2.41	2.40	2.34	0.14	0.58	0.53	0.91
DMI, % of BW	3.32^{a}	2.99^{b}	2.79^{b}	3.22^{a}	0.08	0.48	0.04	<0.01
Multiparous, no.	7	8	8	7				
Intake, kg/d								
DM	20.4	19.3	19.6	19.4	0.38	0.43	0.08	0.70
CP	3.65	3.18	3.11	2.68	0.07	<0.01	<0.01	0.76
NDF	5.63	5.81	6.01	6.08	0.13	0.35	0.02	0.69
ADF	3.35	3.47	3.66	3.73	0.08	0.25	<0.01	0.77
Initial BW, kg	663	613	638	625	27.7	0.24	0.80	0.47
Final BW, kg	700	628	642	635	22.8	0.08	0.24	0.14
Initial BCS	2.33	2.19	2.23	2.04	0.12	0.13	0.27	0.82
Final BCS	2.52	2.16	2.28	2.08	0.17	0.10	0.33	0.63
DMI, % of BW	3.21	3.09	3.06	3.05	0.11	0.48	0.32	0.60

$^{a–c}$Values within a row with differing superscripts denote RDP by RUP interactions ($P \leq 0.05$).

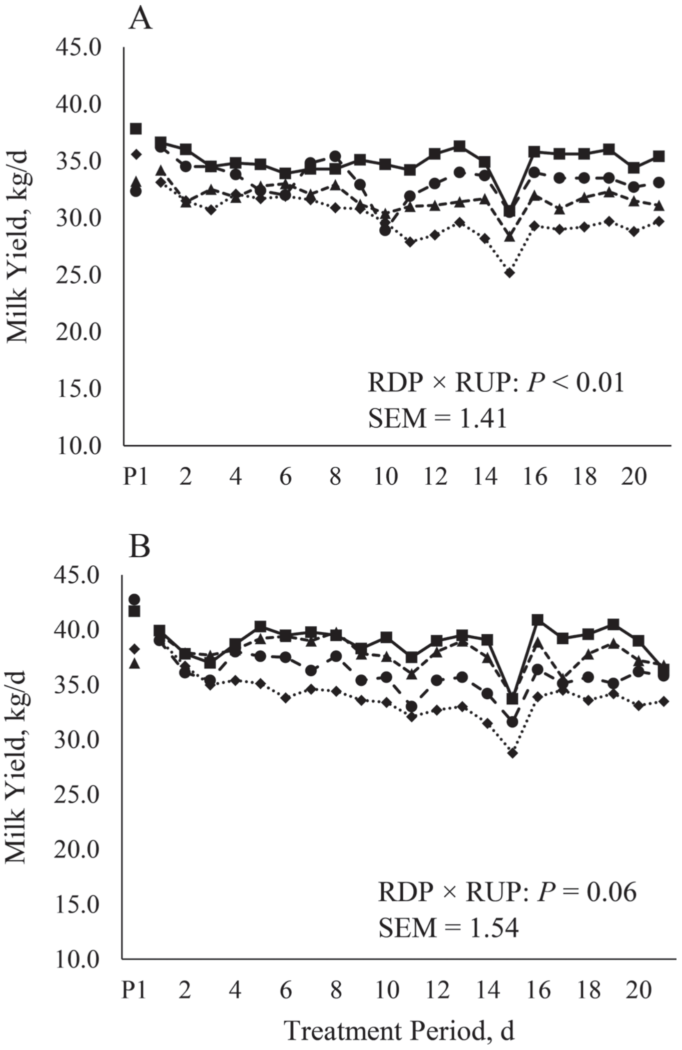

Figure 2. Effect of varying RDP and RUP proportions on milk yield (P1, mean of 7-d pre-treatment period). Values are LSM ± pooled SEM for (A) primiparous and (B) multiparous cows. All cows were fed 10% RDP, 8% RUP throughout the pre-treatment period. Legend: 10% RDP, 8% RUP (–■–); 8% RDP, 8% RUP (- -▲- -); 10% RDP, 6% RUP (···◆···); 8% RDP, 6% RUP (- -●- -).

RUP (from 21 to 17% CP of DM) by removing amount of soybean meal and fish meal reduced milk yield in primiparous cows (31.2 vs. 29.4 kg/d). Conversely, milk yield was depressed in cows when fed a 10% excess of RDP diet compared with NRC (2001) recommended amounts. In the present study, the reduction of RDP and RUP likely limited the supply of MP in primiparous cows (NRC, 2001). Microbial protein yield and rumen bypass protein to the duodenum may have been reduced in response to lowering RDP and RUP proportions. In conclusion, our results indicate that a 2-percentage-point reduction of RUP in a 10D:8U diet likely compromised the supply of MP; however, a 2-percentage-point reduction of RDP in a 10D:6U diet likely improved MP supply in heat-stressed primiparous cows.

Compared with recommended RDP proportion (NRC, 2001), the reduction of RDP did not reduce milk component percentages or yields in primiparous cows. However, lower RUP proportions reduced lactose percentage and milk protein yield, and tended to reduce yields of milk fat and lactose. Treatments did not affect yields of ECM; however, the low RUP diets resulted in numerically lower yields of ECM. In agreement with the present results, Bruckental et al. (1989) showed that a reduction of RUP reduced percentages and yields of milk fat, but a reduction of RUP with less soybean meal did not affect yields of milk protein. Reductions on yields of milk components in the lower RUP diets indicate that precursors of milk fat and protein were reduced and limited production of primiparous cows.

Although the reduction of RDP and RUP reduced intake of CP by 0.5 kg/d, treatments did not affect DMI of multiparous cows. In agreement with the current results, Arieli et al. (2004) reported no change in DMI when a 0.3 kg/d reduction in CP intake occurred in heat-stressed multiparous cows (16.7 vs. 15.1% of CP). Taylor et al. (1991) reported that the reduction of RDP from 10.8 to 8.5% did not affect DMI in heat-stressed cows. Collectively, the reduction of RDP and RUP proportions did not limit DMI of heat-stressed multiparous cows. Additionally, higher dietary fat in diets with 6% RUP did not negatively affect DMI in multiparous cows. The BW and BCS of multiparous cows were not affected by treatments, and the difference between initial and final BW and BCS within each diet was negligible. Body condition score is a crude estimate of energy reserves but is not an accurate measurement to detect small differences in adipose tissue mobilization. These data indicate that the biological responses observed are driven by intake of nutrients because body reserves were not affected by treatments. The present results should benefit dairy producers by reducing the cost of feeding cows exposed to periods of heat stress.

In our study, regardless of RUP proportion, reduction of RDP did not reduce milk yield of multiparous cows and, in fact, supported a numerical increase at 6% RUP proportion. However, diets with 8% RUP supported greater milk yield compared with 6% RUP diets. In agreement with the current study, a 9.7% RDP (16.1% CP) diet compared with a 12.0% RDP (18.4% CP) diet increased milk yield (28.4 vs. 26.5 kg/d) of multiparous cows exposed to warm climates (Higginbotham et al., 1989). Similarly, reduction of a 2.6% RDP proportion (14.9% CP) diet sustained milk yield in multiparous cows compared with a 17.5% CP diet (Mutsvangwa et al., 2016). Interestingly, in the current study, a 2-percentage-point decline in dietary

Table 5. Least squares means of milk production and composition for heat-stressed Holstein cows fed varying amounts of RDP and RUP (LSM ± SEM)

	Experimental diet					Effect (*P*-value)		
	8% RUP		6% RUP					
Item	10% RDP	8% RDP	10% RDP	8% RDP	SEM	RDP (D)	RUP (U)	D × U
Primiparous, no.	5	4	4	5				
Milk yield, kg/d	34.9^{a}	31.4^{bc}	29.0^{c}	33.3^{b}	0.74	0.60	0.03	<0.01
ECM,[1] kg/d	30.5	30.5	26.1	28.9	2.00	0.51	0.16	0.49
Lactose, kg/d	1.62	1.48	1.36	1.49	0.07	0.92	0.09	0.07
True protein, kg/d	1.01	0.98	0.89	0.94	0.04	0.80	0.04	0.25
Fat, kg/d	0.89	1.02	0.72	0.85	0.07	0.15	0.07	0.95
SNF, kg/d	2.93	2.73	2.50	2.71	0.12	0.97	0.08	0.10
Lactose, %	4.79	4.73	4.72	4.68	0.02	0.06	0.03	0.57
True protein, %	3.02^{b}	3.15^{a}	3.14^{a}	3.03^{ab}	0.05	0.83	0.94	0.01
Fat, %	2.65	3.21	2.65	2.69	0.17	0.11	0.16	0.09
SNF, %	8.66	8.74	8.72	8.58	0.05	0.54	0.35	0.05
MUN, mg/dL	11.2	8.34	8.19	5.61	0.52	<0.01	<0.01	0.82
SCC, × 1,000 cells/mL	129	167	101	159	42.2	0.27	0.69	0.82
Multiparous, no.	7	8	8	7				
Milk yield, kg/d	39.1^{a}	37.1^{ab}	33.1^{c}	35.4^{bc}	0.96	0.69	<0.01	0.03
ECM, kg/d	34.9^{a}	33.7^{ab}	31.9^{b}	34.7^{ab}	0.89	0.38	0.28	0.03
Lactose, kg/d	1.84^{a}	1.73^{b}	1.53^{c}	1.65^{bc}	0.04	0.98	<0.01	0.01
True protein, kg/d	1.12^{a}	1.05^{b}	1.01^{b}	1.06^{ab}	0.02	0.61	0.04	0.03
Fat, kg/d	1.05	1.06	1.03	1.15	0.05	0.35	0.41	0.71
SNF, kg/d	3.30^{a}	3.10^{b}	2.83^{c}	3.01^{bc}	0.07	0.89	<0.01	0.01
Lactose, %	4.70	4.70	4.62	4.62	0.02	0.99	<0.01	0.86
True protein, %	2.90	2.88	3.17	2.98	0.06	0.08	<0.01	0.13
Fat, %	2.75	2.95	3.14	3.17	0.12	0.52	0.01	0.16
SNF, %	8.47	8.45	8.65	8.46	0.05	0.04	0.06	0.10
MUN, mg/dL	11.7	7.98	9.17	5.46	0.54	<0.01	<0.01	0.98
SCC, × 1,000 cells/mL	78.8	86.0	71.9	160	28.9	0.11	0.28	0.17

[a–c]Values within a row with differing superscripts denote RDP by RUP interactions ($P \leq 0.05$).

[1]Energy-corrected milk calculated in equation derived from Tyrrell and Reid (1965).

RUP reduced milk yield at 10% RDP (i.e., 10D:6U vs. 10D:8U). However, Bruckental et al. (2002) reported a comparable RUP reduction from 8 to 6% but did not reduce milk yield of multiparous cows. Interestingly, in the current study, cows consuming diets with 16% CP (i.e., 10D:6U and 8D:8U) maintained similar CP intakes

Table 6. Nitrogen efficiency of heat-stressed Holstein cows fed varying amounts of RDP and RUP (LSM ± SEM)

	Experimental diet					Effect (*P*-value)		
	8% RUP		6% RUP					
Item	10% RDP	8% RDP	10% RDP	8% RDP	SEM	RDP (D)	RUP (U)	D × U
Primiparous, no.	5	4	4	5				
Intake N, g/d	561^{a}	483^{b}	419^{c}	420^{c}	11.7	<0.01	<0.01	<0.01
Milk N, g/d	162	157	141	151	5.90	0.93	0.03	0.20
Predicted urine N,[1] g/d	187	139	134	97.3	9.08	<0.01	<0.01	0.56
Predicted fecal N,[2] g/d	213	184	136	167	16.7	0.96	0.01	0.10
N-use efficiency,[3] %	29.0	33.0	33.7	36.2	1.19	0.01	<0.01	0.58
Multiparous, no.	7	8	8	7				
Intake N, g/d	576	516	494	435	16.5	<0.01	<0.01	0.52
Milk N, g/d	178^{a}	169^{ab}	159^{b}	170^{ab}	3.84	0.69	0.13	<0.01
Predicted urine N, g/d	214	143	162	98.2	10.3	<0.01	<0.01	0.74
Predicted fecal N, g/d	186	203	169	174	4.59	0.58	0.24	0.77
N-use efficiency, %	31.6	32.8	32.4	37.8	1.39	<0.01	<0.01	0.18

[a–c]Values within a row with differing superscripts denote RDP by RUP interactions ($P \leq 0.05$).

[1]Predicted urine N output = 0.0283 × MUN (mg/dL) × BW (kg); Wattiaux and Karg (2004).

[2]Predicted fecal N output = N intake – predicted urinary N – milk N.

[3]N-use efficiency = 100 × milk N/intake N.

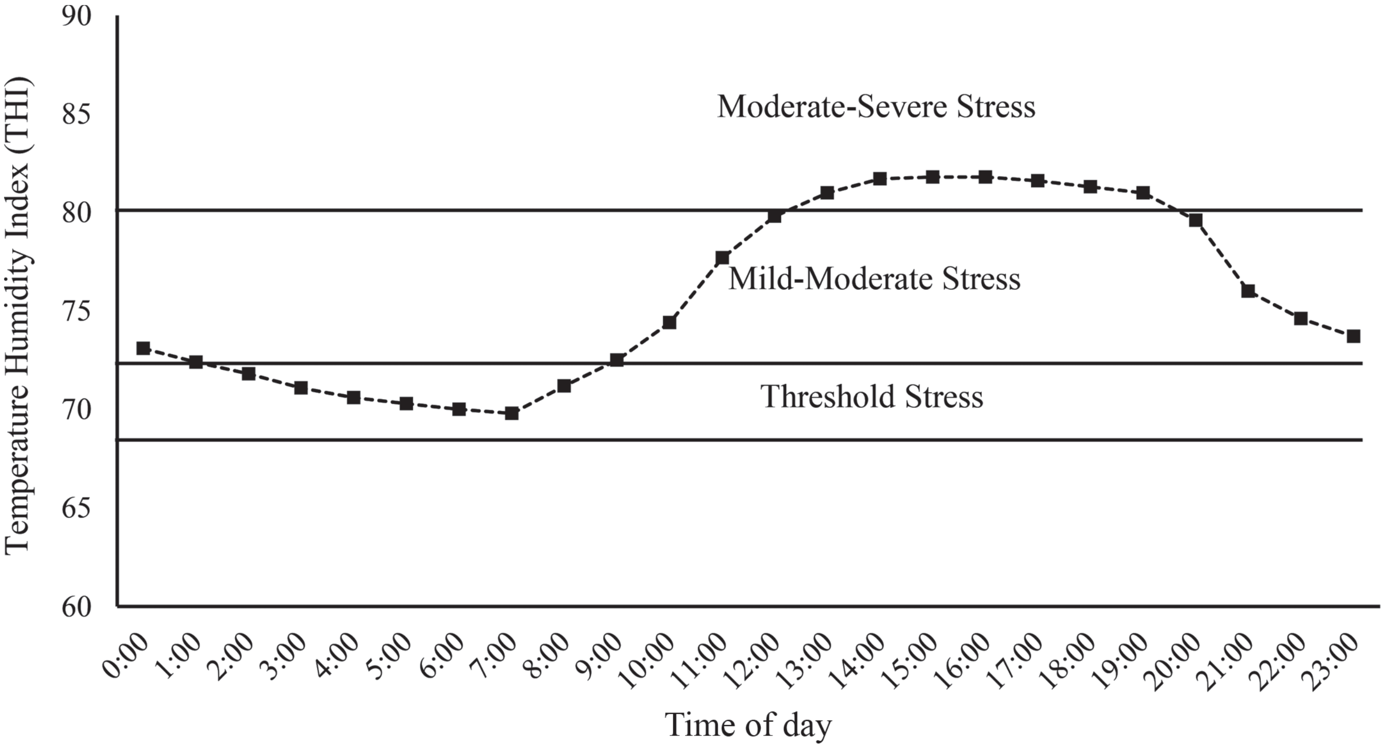

Figure 3. Cows experienced a circadian pattern of daily summer temperatures and relative humidity.

Table 7. Body temperature and respiration rate (RR) in heat-stressed Holstein cows fed varying amounts of RDP and RUP in a.m. and p.m. values[1] (LSM ± SEM)

	Experimental diet					Effect (*P*-value)		
	8% RUP		6% RUP					
Item	10% RDP	8% RDP	10% RDP	8% RDP	SEM	RDP (D)	RUP (U)	D × U
Primiparous, no.	5	4	4	5				
Rectal, °C								
a.m.	38.9	38.6	38.9	38.9	0.32	0.63	0.72	0.75
p.m.	39.6	39.4	39.9	39.6	0.20	0.29	0.32	0.96
Vaginal, °C								
a.m.	38.9	39.2	39.1	39.1	0.22	0.36	0.87	0.55
p.m.	39.5	39.8	39.6	39.7	0.22	0.37	0.99	0.62
RR, breaths/min								
a.m.	64.4	64.4	63.8	63.3	4.08	0.96	0.84	0.96
p.m.	86.4	93.8	87.3	87.8	3.53	0.35	0.53	0.41
Multiparous, no.	7	8	8	7				
Rectal, °C								
a.m.	38.8	39.0	38.8	38.8	0.15	0.55	0.46	0.32
p.m.	39.7	40.0	39.6	39.7	0.20	0.37	0.29	0.51
Vaginal, °C								
a.m.	39.0	39.3	39.4	39.3	0.14	0.35	0.15	0.11
p.m.	39.6^{b}	39.9^{ab}	40.0^{a}	39.8^{ab}	0.13	0.66	0.40	0.04
RR, breaths/min								
a.m.	60.9	66.6	63.9	64.2	4.15	0.49	0.95	0.53
p.m.	85.2	90.1	79.7	86.5	4.55	0.23	0.35	0.84

[a,b] Values within a row with differing superscripts denote RDP by RUP interactions ($P \leq 0.05$).

[1] Differences among a.m. and p.m. temperatures were significant for rectal and vaginal temperatures and RR ($P < 0.01$). Means not shown.

(3.1 and 3.2 kg/d), but the diet with the recommended proportion of RDP combined with 6% RUP triggered a 4.0-kg reduction of milk yield (33.1 to 37.1 kg/d). It is possible that a 2-percentage-point RDP reduction did not reduce microbial protein yield and provided a balance supply of AA in MP (NRC, 2001). Therefore, a 2-percentage-point RDP reduction did not limit MP supply of heat-stressed multiparous cows; however, a 2-percentage-point RUP reduction at a recommended RDP level (NRC, 2001) likely reduced MP supply and milk yield.

Remarkably, the reduction of both RDP and RUP (8D:6U) resulted in similar yields of ECM in comparison with the recommended proportion of RDP (10D:6U; NRC, 2001), high proportion of RUP (8D:8U), or both (10D:8U). In agreement with the current results, Kalscheur et al. (2006) reported that an 8.2% RDP diet sustained milk protein yield compared with a 9.6% RDP diet. Wang et al. (2007) reported similar milk protein yield in cows fed 6.6% RUP compared with those fed 7.9% RUP. Under the conditions of this study, the 8D:6U treatment provided a balanced supply of AA in MP, which sustained high yields of ECM. Additionally, the balanced supply of AA reduces AA oxidation and plasma urea concentrations (Bach et al., 2000; Kassube et al., 2017). The latter contention is supported by lower MUN values observed from cows in the present study. Likely, cows fed the 8D:6U treatment partitioned a greater proportion of circulating AA toward milk production compared with the 10% RDP and 8% RUP cows (Bequette et al., 1998, 2000).

Milk fat percentage was concerning with overall low concentrations. Similarly, low milk fat percentages have previously been reported in high-producing dairy cows exposed to a warm climate (Higginbotham et al., 1989; Taylor et al., 1991; Arieli et al., 2004). For multiparous cows in the current study, reduction of RUP was consistently associated with higher milk fat percentages. This response could be a result of greater amounts of soybean hull in their diets, which in turn resulted in a greater intake of NDF. Furthermore, contents of rumen-protected fat were greater in 6% RUP compared with 8% RUP treatments. Greater contents of rumen-protected fat likely promoted milk fat synthesis. However, treatments did not affect milk fat yield, indicating a dilution response of milk fat content in cows fed the 6% RUP treatment. In summary, these results indicate that reduction of RUP did not affect milk fat yield.

Nitrogen Utilization

As expected in primiparous cows, the 6% RUP treatment sustained lower N intake at both levels of RDP; however, the 8% RDP treatment reduced N intake only at 8% RUP. Consequently, lowering RUP reduced milk protein N yield in primiparous cows. Multiparous cows fed either 10% RDP or 8% RUP had greater dietary N intake compared with cows fed 8% RDP or 6% RUP. However, milk protein N yield was not affected in cows fed diets with 8% RDP and 6% RUP at 8% RDP. In agreement with the present results, Rius et al. (2010) reported that reduction of RUP did not limit milk protein N yield. Additionally, Flis and Wattiaux (2005) showed that excess RDP and RUP did not increase yields of milk protein N compared with recommended amounts. Thus, low proportions of RDP, relative to recommended proportions of RDP, reduced N intake but probably did not limit microbial protein supply in MP of primiparous and multiparous cows in the current study.

Reduction of RDP and RUP reduced predicted urinary N excretion in primiparous and multiparous cows. In agreement to the present study, reduction of RDP and RUP reduced urinary and fecal N excretion in dairy cows in previous studies (Castillo et al., 2001; Cyriac et al., 2008; Rius et al., 2010). Factors contributing to these results may include lower rumen ammonia production (Agle et al., 2010), catabolism of AA in splanchnic tissues (Bahrami-Yekdangi et al., 2014), and urinary urea-N excretion (Colmenero and Broderick, 2006). Total N intake correlates with systemic partitioning of N, and with reduced dietary N intake urinary N excretion decreases exponentially ($R^2 = 0.74$; Castillo et al., 2001). Therefore, findings in the current study suggest that reduced RDP and RUP repartitioned N away from urinary excretion. Consequently, the N-use efficiency increased and, compared with the 10D:8U diet, more so in cows fed 8D:6U (25%, primiparous and 20%, multiparous). This increase in N-use efficiency was achieved by reducing urinary N output by $\geq$27% when compared with the higher RDP and RUP treatments. In agreement with these results, N-use efficiency increased when CP decreased from 17.5 to 14.5% of DM in lactating cows (Mutsvangwa et al., 2016). In summary, feeding low proportions of RDP and RUP could be a useful nutritional intervention to reduce urinary excretion of N in primiparous and multiparous cows exposed to heat stress.

NRC Model Analysis

The NRC (2001) model was evaluated for the ability to predict milk yield when dietary RDP and RUP were reduced to limit protein supply in multiparous cows. The model predicted milk yield for the 10D:8U diet within acceptable error (i.e., 0.3-kg difference between predicted and observed milk yields), and a small predicted deficiency of RDP requirement was compensated by an excess of dietary RUP. As dietary RDP is reduced, a predicted decline in rumen microbial yields is expected. By design, a decline in RDP elicited a RDP reduction of 486 g at 8% and 399 g at 6% RUP proportions. In response to low dietary RDP, the NRC (2001) model predicted an increase of RUP requirement by 13% (184 g) and by 20% (275 g) and a reduction of MP supply by 12% (298 g) and 10% (205 g) at 8 and 6% RUP proportions. In agreement with the current results, a previous report stated the NRC (2001) model underpredicted MP supplied and overpredicted RUP requirements in diets with 8.8 and 7.6% of RDP (Cyriac et al., 2008). Therefore, in the current study, the model most likely underestimated MP supplied and overestimated the RUP requirement.

The 2-percentage-point reduction of dietary RUP percentage had negligible effects on RUP requirements. However, the reduction of RUP caused a decline of RUP and MP supplies greater at 10% RDP (426 g of RUP and 469 g of MP) compared with that at 8% RDP (396 g of RUP and 376 g of MP). For the 2 point reduction of dietary RUP, the model overestimated a loss of milk yield 1.6 to 5.8 times greater than the actual loss (10D:8U − 10D:6U and 8D:8U − 8D:6U). Upon reduction of 2 percentage points of both RDP and RUP, the model overestimated a loss of milk yield 3.4 times greater (10D:8U – 8D:6U). In agreement with the current results, previous research indicated that the model overestimated a loss of milk yield in response to a similar reduction of dietary RUP at medium and high dietary CP levels (Rius et al., 2010). The utilization of AA in postabsorptive tissues is not fully represented in the model. For example, lactating cows and goats increase the capture of AA in the mammary gland in response to a decline of MP (Bequette et al., 2000; Raggio et al., 2004). Collectively, efforts to improve the accuracy of the NRC (2001) model should be directed toward supply of RUP and MP and the efficiency in the conversion of AA from MP into milk.

CONCLUSIONS

This study indicates that dietary reduction of RDP and RUP sustained yields of milk protein and ECM compared with higher amounts of RDP and RUP in heat-stressed multiparous cows; however, feeding lower RDP and RUP proportions to primiparous cows compromised production. Milk production response in multiparous cows fed 8D:6U was a consequence of improved efficiency of conversion of dietary N into milk protein. Lowering the excretion of N into the environment improves the environmental stewardship of the dairy industry. The NRC (2001) model overestimated requirements of RUP, underestimated supply of MP, and failed to predict accurate changes in milk yield in response to reduced RDP and RUP. Feeding primiparous cows separately from multiparous cows may be a management strategy to optimize milk production and N-use efficiency with varying diets of RDP and RUP proportions.

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