

Effects of Supplementing Rumen-Protected Methionine on Production Performance, Apparent Digestibility, Blood Parameters, Ruminal Metabolites and Economic Effectiveness in Lactating Holstein Dairy Cows

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Abstract

Background: Because of disadvantages of excessive dietary crude protein (CP), decreasing dietary CP of dairy cows has attracted the worldwide attention. Rumen protected methionine (RPM) supplementation can allow lower CP diets and is beneficial to milk production performance, N efficiency of cows and environment. The aim of this study was to evaluate the effects of reducing dietary CP and supplementing RPM on production, digestibility of nutrients, blood parameters, ruminal metabolites and economic effectiveness in lactating Holstein dairy cows.

Results: A total of 96 lactating cows (63 ± 25 d in milk; 34.4 ± 5.74 kg/d of milk production; mean \pm SD) were randomly assigned to 1 of 2 treatments: diet containing 17.3% CP without RPM (control group; CON; $n = 49$); diet containing 16.4% CP with supplementing 15.0 g/d of RPM (treatment group; RPM; $n = 47$). No effect was observed of reducing dietary CP on milk yield and milk composition. The apparent digestibility of nutrients was similar between treatments. The results related to blood showed that cows in RPM group exhibited lower concentration of blood urea nitrogen than that in CON group ($P < 0.001$). Moreover, there were no differences between treatments on concentrations of aspartate transaminase, alanine transaminase, alkaline phosphatase, globulin and albumin. In ruminal metabolites, microbial crude protein (MCP) of dairy cows in RPM group was higher compared with CON group ($P = 0.006$). Ruminal volatile fatty acid (VFA) contents were not changed by treatments except that the concentrations of butyrate and isovalerate of RPM group were higher than that of CON group at 2 h after feeding ($P < 0.05$). In addition, supplying the diet of 16.4% CP with RPM supplementation to cows could reduce feeding cost by 0.5 \$/d per cow and boost net profits.

Conclusions: Lower dietary CP with RPM supplementation did not limit milk yield, milk composition and apparent digestibility of nutrients, and could improve nitrogen utilization of dairy cows and synthesis of MCP in rumen, change VFA production at 2 h after feeding, as well as boost the economic benefits of the dairy farms.

1. Background

To satisfy the protein requirement, cows sometimes are received excessive dietary crude protein (CP) and then excreted in the form of urea, which would be emitted into the atmosphere in the form of NO_2 [1], or be hydrolyzed to NH_3 and volatilized[2], ultimately harmful to the environment. In addition, some researchers noted high dietary CP has a negative impact on N utilization for dairy cows[3]. And there is no improvement in milk production and milk composition when dietary CP is increased up to 18%[2] or 18.8%[4]. Furthermore, 40% of feeding cost can be attributed to dietary protein[5], and excess protein in the diets will lead to high cost and reduce profit margins. In consideration of above-mentioned factors, decreasing dietary CP has attracted the attention from worldwide researchers. In fact, previous study has shown that ensuring ideal balance for AA, especially methionine (Met) and lysine (Lys)[2], is the key to formulate cow rations rather than for dietary protein[6].

Given that rumen undegradable protein (RUP) is significant to meet daily protein needs of cows especially during the lactation stage, an alternative strategy of feeding rumen-protected AA has been taken into consideration. Recently years, there have been some research that explored the effects of rumen-protected methionine (RPM) supplementation on dairy cows. Several studies indicated that RPM supplementation and decreasing dietary CP could increase milk production, milk protein, fat as well as lactose contents[7–10]. Recently research has shown that an increase in overall dry matter intake (DMI) could be found after parturition when cows fed lower dietary CP and RPM[11]. In addition, supplementing RPM may allow lower CP diets to improve N efficiency and reduce urinary N excretion[8, 12] and production of total gas and methane volume[13]. Meanwhile, a previous meta-analysis of dietary protein content noted that not overfeeding dietary CP is the most efficient means to increase efficiency of N utilization[14]. In general, reducing dietary CP levels with RPM supplementation would be beneficial to milk production performance and N efficiency of dairy cows, as well as environment. Other effects of RPM, especially in aspects of digestibility of nutrients, health, rumen function and farmer's profits need to be further explored.

Thus, the objective of the present study was to investigate the effects of reducing dietary CP levels and supplementing RPM on production performance, apparent digestibility of nutrients, blood parameters, ruminal metabolites and economic effectiveness in lactating Holstein dairy cows. We hypothesized that reducing dietary CP levels and supplementing RPM would not limit milk yield and composition, and could change rumen fermentation and farmer's profits.

2. Materials And Methods

2.1 Treatments, Experimental Diet and Cows management

This study was conducted at Shengsheng Dairy Farm in Luoyang City, Henan Province, China. Animal management and experimental procedures were approved by Animal Care Committee of China Agricultural University. A total of 96 lactating Holstein cows (63 ± 25 d in milk; 34.4 ± 5.74 kg/d of milk production; mean \pm SD) were randomly assigned to 1 of 2 dietary treatments. Experimental periods lasted 75 d and consisted of 15 d for diet adaptation and 60 d for data collection. Diets were fed as total mixed ration (TMR; Table 1): diet containing 17.3% CP without RPM (control group; CON; n = 49); diet containing 16.4% CP with supplementing 15.0 g/d of RPM (treatment group; RPM; n = 47). It is worth to mention that the diet of CON contains a Lys:Met ratio of 3.39:1, while that of treatment group was 2.84:1. The TMR were prepared by blending individual feed ingredients. All cows had free access to feed and water.

Table 1
The feed ingredients and chemical composition in experimental diets¹

Item	CON	RPM
Feed ingredients(% DM)		
Alfalfa	11.3	11.3
Oat hay	3.7	3.7
Corn silage	45.2	44.9
Whole cottonseed	2.5	5.0
Beet pulp	2.5	2.5
Molasses	3.5	3.5
Fat power	0.5	0.5
Sodium bicarbonate	0.4	0.4
Yeast	0.5	0.5
Steam flaked maize	1.3	3.7
Ground corn grain	12.1	10.0
Wheat bran	2.0	2.0
Corn gluten feed	1.5	1.5
Distillers dried grains with soluble	1.5	1.5
Vitamins and trace minerals	1.6	1.6
Whole soybean	0.8	0.8
Soybean meal	9.0	6.2
Cotton expeller	—	0.5

¹Treatments CON group and RPM group consists of total mixed ration containing 17.3 and 16.4% CP, respectively.

²Rumen-protected Met product from Evonik (China) Co., Ltd.

³DM dry matter, NE_L net energy for lactation, CP crude protein, NDF neutral detergent fiber, ADF acid detergent fiber, $peNDF$ physically effective neutral detergent fiber, NFC non-fiber carbohydrate, Met methionine, Lys lysine

⁴Estimated using the NRC (2001) model based on DM intakes

⁵ $NFC = 100 (\%NDF - NDIN \times 6.25) - \% CP - \% fat - \% ash$

Item	CON	RPM
Mepron ²	—	0.04
Chemical composition ³		
DM(%)	61.4	61.6
NE _L ⁴ (M.cal/kg)	1.7	1.7
CP(% DM)	17.3	16.4
NDF(% DM)	31.3	32.3
ADF(% DM)	18.4	19.0
peNDF(% DM)	23.3	24.6
NFC ⁵ (% DM)	35.5	36.0
Fat(% DM)	6.0	6.0
Met(% MP)	1.92	2.27
Lys(% MP)	6.51	6.45
Lys: Met ratio	3.39:1	2.84:1
¹ Treatments CON group and RPM group consists of total mixed ration containing 17.3 and 16.4% CP, respectively.		
² Rumen-protected Met product from Evonik (China) Co., Ltd.		
³ DM dry matter, NE _L net energy for lactation, CP crude protein, NDF neutral detergent fiber, ADF acid detergent fiber, peNDF physically effective neutral detergent fiber, NFC non-fiber carbohydrate, Met methionine, Lys lysine		
⁴ Estimated using the NRC (2001) model based on DM intakes		
⁵ NFC = 100 (%NDF – NDIN × 6.25) – % CP – % fat – % ash		

2.2. Sample Collection and Analysis

Cows were milked three times daily. Every 7 days in experimental period, milk was collected for analyzing for fat, true protein, lactose, milk urea nitrogen (MUN), somatic cell count (SCC), solids-not-fat (SNF), total solid (TS) and freezing point (FP) at the Luoyang Dairy Cow Center (Luoyang, China).

The experimental diets were fed as TMR three times daily. The amounts of feed offered was adjusted daily to 3 to 5% orts. The TMR samples were collected every 19 days to determine the composition. Samples dried at 65°C for 48h until a constant weight was obtained. Then, feed composites were ground through a 1-mm screen (KRT-34; KunJie, Beijing, China) and analyzed for DM using method 950.15 of

Association of Official Analytical Chemists[15]. Nitrogen was analyzed by the method 984.13[15] and crude protein (CP) was measured by multiplying 6.25 by the nitrogen content. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured according to the method described by Van Soest et al.[16]. Ash (method 942.05), calcium (Ca, method 965.09), and total phosphorus (P, method 965.17) were measured according to the AOAC[17].

On d 0, d 30, d 60 of the experimental period, blood samples were randomly collected from the 15 cows of each group from tail vein of cows using 10 mL blood collection heparin-coated tubes (Vacutainer; aosaite Medical Instrument; Shandong) before morning feeding. Blood samples were immediately centrifuged at 3,500×g at 4°C for 15 min to obtain plasma samples, which stored at - 20°C for further analysis. Concentrations of total protein (TP), albumin (ALB), Globulin (GLB), blood urea nitrogen (BUN), cholesterol (CHO), Aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP) were measured by testing organization (People's Liberation Army 534 hospital, China).

Fecal samples were collected from 15 cows each group randomly and collected for 4 times (0300, 0800, 1300 and 1800) each day on d 58, d 59 and d 60. The fecal samples of each cow were evenly mixed to make a simple composite and taken to about 200 g, adding 10% tartaric acid of 1/4 fecal weight to fix nitrogen. These fecal grab samples were held at 65°C in a forced-air oven until completely dried, and were ground to pass a 1-mm screen (KRT-34; KunJie, Beijing, China). Fecal samples were analyzed for DM, NDF, ADF, CP, calcium, phosphorus, Ash as described earlier. The acid-insoluble ash (AIA) was used as an internal marker to determine the apparent digestibility of nutrients. TMR, orts and fecal samples were analyzed according to the procedure by Van Keulen and Young[18]. The apparent digestibility of nutrients was calculated as follows:

Apparent Digestibility of Nutrients = $[1 - (Ad \times Nf) / (Af \times Nd)] \times 100$.

where Ad = AIA in the diets (g/kg); Af = AIA in the feces (g/kg); Nd = the concentration of a nutrient in the diet (g/kg); Nf = the concentration of a nutrient in the feces (g/kg).

On the last day of the experiment, 15 cows from each group were selected randomly and were collected samples of rumen fluid. Samples were collected before morning feeding and 2, 4, 6, 8, 12 h after morning feeding by oral sampler. Rumen fluid samples was filtered with four layers of gauze, centrifuged at 4000 r/min for 15min and took 1mL supernatant, which was acidified with 4.5mL 0.2mol/L HCL for later analysis of ammonium nitrogen. Meanwhile, 4mL supernatant was added 1 mL 25% metaphosphate acid and were stored at - 20°C for later analysis of microbial crude protein (MCP), Ammonia N (NH₃-N) and volatile fatty acid (VFA) including acetate, propionate, butyrate, valerate, isobutyrate and isovalerate (using Shimadzu GC-7A gas chromatograph, Japan).

2.3. Statistical Analysis

Dairy cow was used as the experimental unit, and all data were analyzed using SAS (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). The milk yield, milk composition, apparent digestibility of nutrients, blood parameters, and ruminal metabolites were analyzed using PROC MIXED procedure of SAS. The

milk yield, milk composition and blood parameters were calculated by averaging the three samples collected in d 0, d 30 and d 60 and then analyzed. The model of ruminal metabolites included the fixed effects of treatment, time, and time × treatment interaction, and cow within treatment as a random effect. Degrees of freedom were counted using the Kenward-Roger approximation option of the MIXED procedure. In order to explicate the repeated measures within-subject, the covariance structures were executed for each repeated variable on the basis of best fit determined by the Akaike information criterion. A significant difference between treatment group and control group was declared at $P < 0.05$, tendencies were considered when $0.05 \leq P < 0.10$, a highly significant difference indicated at $P \leq 0.01$.

3. Results And Discussion

3.1. Milk Yield, and Milk Composition

The results of the milk yield and milk composition are shown in Table 2. Milk yield and milk composition were not limited by the diet of lower protein with RPM supplementation. Meanwhile, SCC and MUN values for RPM diet decreased numerically and that of milk fat increased numerically. There was a trend for lower SNF with decreased CP feeding and RPM supplementation ($P = 0.09$).

Table 2
Effects of adding Rumen-Protected methionine on milk yield, milk composition of dairy cows

Item ²	Treatment ¹		SEM	P-value ³
	CON	RPM		
Milk yield, kg/day	36.8	36.8	0.07	1.00
FP (°F)	31.0	40.0	0.94	0.40
SCC (cell/mL)	153,100	115,900	0.42	0.38
Milk composition, %				
Fat	3.49	3.80	0.03	0.24
Protein	3.39	3.34	0.004	0.36
Lactose	4.85	4.87	0.01	0.82
TS	12.01	11.97	0.04	0.32
SNF	8.59	8.41	0.01	0.09
MUN (mg/dL)	15.44	14.82	0.07	0.35
¹ Treatments CON group and RPM group consists of total mixed ration containing 17.3 and 16.4% CP, respectively				
² FP freezing point, SCC somatic cell count, TS total solid, SNF solids-not-fat, MUN milk urea nitrogen				
³ Probability of a significant effect of diet				

Our findings implied that decreasing dietary CP to 16.4% with RPM supplementation and relatively constant fermentable carbohydrate failed to have any negative effect on the milk yield and milk composition. The results are consistent with other researchers who found there were no changes in milk production performance when keeping the dietary AA balanced, and CP of the diets in these studies varied from 15.6–18%[2], from 14.8–18.6%[8], from 14.6–18.3%[19], from 13–17%[9]. Moreover, several trials have demonstrated that with RPM supplementation, milk yield[10, 20] and milk protein yield[21] increased. In contrast, a study reported that milk yield or milk composition like fat, lactose and SNF decreased significantly with diet of 11% CP, which may due to 11% CP content was not enough to meet the milk production needs of dairy cows[9]. Furthermore, a report noted that compared with the diet of 17.3% CP, SNF yield in milk was lower in that of 16.1% CP[8], which was accorded with another study where reported that reduced dietary CP (from 17.1–15.8%) could decrease milk SNF yield[22]. These studies indicated that lower dietary CP could result in less yield of milk SNF, which may account for the result of the current study.

In addition, no significant treatment effect was observed on MUN, while decreased with RPM group numerically in the current study. A previous study found that MUN concentration was lower when feeding

the low-protein diet and supplementing rumen-protected essential amino acids[23]. A report elsewhere observed that compared with 18% CP diet, the MUN values decreased with 16.4% and 15.6% CP diets supplementing RPM[2]. However, some researchers noted that there was no significant change in MUN value if the dietary CP content changed by 1%[24]. Therefore, the similar content of MUN in our study could be explained by that the difference in dietary CP between the two experimental groups was 0.9% (< 1%). Hence, reduced MUN in RPM group may benefit to conception rates of dairy cows[25]. SCC level, an indicator of animal mammary disease, often increases when an infection of the mammary gland occurs[26, 27]. In the current study, SCC values did not change and was numerically lower in RPM group. And in these two groups the SCC values were both within the normal levels (100,000 ~ 314,000 cells/mL) [28], which indicated the health condition of dairy cows may not be affected by treatments.

3.2 Blood Parameters

The effects of RPM supplementation on the blood parameters contents are shown in Table 3. The BUN value decreased ($P < 0.001$) with RPM supplementation. GLB was numerically higher in cows consuming lower protein diet supplemented RPM; however, this difference was not statistically significant. Other blood parameters did not exhibit any difference by treatment.

Table 3
Effects of adding Rumen-Protected methionine on the blood parameters of dairy cows

Item ²	Treatment ¹		SEM	P-value ³
	CON	RPM		
ALT (U/L)	26.62	27.00	0.25	0.88
AST (U/L)	88.38	99.00	0.99	0.28
TP (g/L)	73.74	74.20	0.14	0.75
ALB (g/L)	28.55	28.06	0.09	0.59
GLB (g/L)	45.19	46.13	0.18	0.61
ALB: GLB ratio	0.64	0.62	0.004	0.49
BUN (mmol/L)	5.30	4.14 ^b	0.03	< 0.001
CHO (mmol/L)	5.65	5.16	0.05	0.30
ALP (U/L)	56.69	53.58	0.61	0.63
¹ Treatments CON group and RPM group consists of total mixed ration containing 17.3 and 16.4% CP, respectively				
² TP total protein, ALB albumin, GLB globulin, BUN blood urea nitrogen, CHO cholesterol, AST aspartate transaminase, ALT alanine transaminase, ALP alkaline phosphatase				
³ Probability of a significant effect of diet				

The significant decline of BUN in our findings accords with several earlier research. Bahrami-Yekdangi et al. conducted research in 2013, 2016 respectively. They found plasma urea concentrations were lower in cows consuming low-protein diets with RPM supplementation in 2013[2]. In another study, they also observed that BUN concentrations had a trend of a linear decline ($P=0.06$) with reduced dietary CP and rumen degradable protein (RDP)[29]. These results may be explained by the fact that low-protein diets and ameliorating the balance of dietary AA, especially the ratio of Lys to Met, could enhance the protein utilization of dairy cows[23]. Moreover, it's worth to mention that some researchers indicated higher BUN may lead to saturation of transfer process because of the inhibitory effect of increased $\text{NH}_3\text{-N}$ contents in the rumen or a limitation of urease activity[30]. Reduced BUN concentrations might stimulate rumen urea transfer and increase urea transport rates[31]. Several research also noted the negative effect of high MUN or BUN value on reproductive performance of cows[32, 33]. Therefore, these evidences indicate that reasonable BUN decline has a positive impact on the nitrogen utilization of dairy cows. A previous study supported that RPM supplementation could decrease inflammation of dairy cows, which may attribute to there is an association between RPM and galectin expression and secretion[34]. However, the findings from the abovementioned study differed from our results in that there was no difference in GLB contents, an indicator of immune system[35], between the two groups. We have no explanation for this result, and further experimentation may be required.

3.3 Apparent digestibility of nutrients

The results of the apparent digestibility of nutrients of dairy cows are shown in Table 4. The total apparent digestibility of DM, CP, NDF, ADF, ash, calcium and phosphorus were not affected by treatment diet, despite an overall reduction in lower dietary CP of 16.4%. Furthermore, apparent minerals digestibility, calcium and phosphorus, were numerically higher in cows consuming lower protein diets; however, these differences were not statistically significant.

Table 4

Effects of adding Rumen-Protected methionine on the apparent digestibility of nutrients of dairy cows

Item ² (%)	Treatment ¹		SEM	P-value ³
	CON	RPM		
DM	65.09	64.44	0.32	0.83
CP	71.43	72.65	0.30	0.70
NDF	48.60	41.35	0.51	0.13
ADF	38.88	44.59	0.69	0.38
Ash	53.43	55.17	0.34	0.59
Calcium	42.75	44.24	0.51	0.76
Phosphorus	45.96	48.50	0.65	0.68
¹ Treatments CON group and RPM group consists of total mixed ration containing 17.3 and 16.4% CP, respectively				
² DM dry matter, NE_L net energy for lactation, CP crude protein, NDF neutral detergent fiber, ADF acid detergent fiber, $peNDF$ physically effective neutral detergent fiber				
³ Probability of a significant effect of diet				

Some researchers mentioned that digestibility of OM, NDF, and ADF is associated to ruminal NH_3 -N and CP contents of diets[8]. An earlier research observed lower ruminal organic matter (OM) digestion on the 9% RDP diet, an insufficient level[36]. And inadequate RDP could result in a NH_3 -N deficiency in rumen, which could lower fiber digestion and harm microbial growth[37]. However, in our research, diets contained 16.4% or 17.3% CP with or without RPM supplementation did not have negative impacts on nutrients digestion, which also accords with some previous observations, where apparent digestibility of DM, OM, NDF, CP and starch were not different whether or not RPM was added, and digestibility of ADF would elevate by adding RPM[38, 39]. These above-mentioned results may be attributed to the sufficient dietary CP and rational ruminal NH_3 -N concentrations, which were above the minimum requirement (≥ 5 mg/dL) of ruminal NH_3 -N concentration for rumen microbial growth[40].

3.4 Ruminal fermentation

Effects of experimental diets on ruminal metabolites concentrations are shown in Table 5. The results showed that MCP in the RPM group was higher than that in CON group ($P=0.006$). All ruminal metabolites contents were affected by time significantly ($P<0.05$), but no differences were found between treatments. Treatment \times time interaction effects ($P<0.05$) were observed for ruminal butyrate and isovalerate concentrations, with cows fed RPM having higher butyrate and isovalerate contents at 2h

after feeding, as shown in Fig. 1. It is worth to mention that the mean content of NH₃-N was numerically higher in treatment diet; however, the effect was not statistically significant.

Table 5
Effects of adding Rumen-Protected methionine on the Ruminal metabolites of dairy cows

Items ²	Treatment ¹		SEM ³	P-value ⁴		
	CON	RPM		Treatment	Time	Treatment × Time
MCP	116	135	5.33	0.01	< 0.001	0.56
Ammonia nitrogen (mg/dL)	7.85	9.60	0.49	0.15	0.001	0.24
VFA (mmol/L)						
Acetate	38.55	40.48	1.67	0.62	0.002	0.11
Propionate	19.38	19.99	1.22	0.78	< 0.001	0.09
Butyrate	9.25	10.00	0.43	0.37	< 0.001	0.03
Valerate	1.54	1.45	0.07	0.52	0.018	0.10
Isobutyrate	1.03	1.06	0.07	0.82	< 0.001	0.74
Isovalerate	1.84	1.66	0.09	0.28	< 0.001	< 0.001
Acetate:propionate ratio	2.55	2.79	0.21	0.63	0.007	0.99
¹ Treatments CON group and RPM group consists of total mixed ration containing 17.3 and 16.4% CP, respectively.						
² MCP microbial crude protein, VFA volatile fatty acid						
³ Standard error of the least squares means						
⁴ Probability of a significant effect of diet						

A previous study noted that with the development of RPM technology, most of protected Met is able to escape ruminal degradation, while a small proportion of Met is still released into the rumen and may alter the community composition of microbiota in rumen and their metabolism[11]. Several researchers have shown that unprotected methionine promoted ruminal bacteria growth rate and protein synthesis in vitro[41, 42]. And the study also found that rumen degradable methionine could increase the abundance of *Selenomonas ruminantium*[11], which is Gram-negative bacteria that could account for up to 51% of the ruminal total viable bacterial counts[43]. Some earlier scholars noted this strain was a typical ureolytic gastrointestinal tract anaerobe[44–46]. In the current study, we found that ruminal MCP content increased significantly in the PRM group ($P < 0.01$), which agreed with the increase of ruminal NH₃-N concentration numerically, though. Based on the above-mentioned research, a possible explanation for our results might be that a small fraction of methionine RPM contained could be degraded in the rumen

and then increase the bacteria populations in the rumen, of course. Therefore, more peptides and AA in the rumen were degraded to $\text{NH}_3\text{-N}$ via microbial deamination[41, 47] and then synthesize MCP better. The similar results were also observed in another study[13].

Although no effects were found in VFA concentration during the whole period, cows fed RPM had higher ruminal butyrate and isovalerate contents at 2h after feeding, which is consistent with other studies [13, 48]. A previous research described that ruminal VFA production depended on substrates, bacterial populations and fiber degradation[49]. Some researchers noted that bacterial populations changed in fermenter fluid if rumen inoculum accepted different dietary CP with or without RPM in vitro, and abundance of *Ruminococcus albus* was the highest as dietary low protein with high RPM level[13]. In addition, another study started the diets which had well-balanced essential AA profiles especially RPM evaluated the abundance of ruminal fibrinolytic bacterial (*R. flavefaciens*, *F. succinogenes*, and *R. albus*) [50]. Thus, we speculated the increase of ruminal butyrate and isovalerate contents at 2h after feeding was because abundance of cellulolytic species changed in rumen when cows fed RPM and subsequently produced more VFA. However, a limitation of the current study is analysis of ruminal specific bacteria species was not included in the design.

3.5 Feeding cost

The price of TMR ingredients and the cost of feeding formulations are shown in Table 6. A previous study noted that the optimal levels of dietary RPM could boost the profits on dairy farm[51]. In the current study, the cost per kg of TMR was lower in RPM group, and the mean DMI of RPM group is 43.55 kg/d per cow while that of CON group is 42.83 kg/d per cow (not shown in the table). We calculated that decreasing dietary CP from 17.3–16.4% with RPM supplementation could save about 0.5 \$/d per cow in feeding cost (RPM vs. CON; 11.19 vs. 11.69 \$/d per cow). That meant, decreasing dietary CP from 17.3–16.4% with RPM supplementation can save about \$180,000 per year in a dairy herd of 1000 cows farm without limiting milk production performance.

Table 6
The price of total mixed ration ingredients and the cost of feeding formulations

Ingredients	Price(\$/kg)	Treatment ¹			
		CON		RPM	
Item	Price(\$/kg)	composition(kg)	cost(\$)	composition(kg)	cost(\$)
Alfalfa	0.38	4.50	1.71	4.50	1.71
Oat hay	0.25	1.50	0.38	1.50	0.38
Corn silage	0.08	18.0	1.39	18.0	1.39
Whole cottonseed	0.37	1.00	0.37	2.00	0.74
Beet pulp	0.35	1.00	0.35	1.00	0.35
Molasses	0.23	1.40	0.32	1.40	0.32
Fat power	1.35	0.20	0.27	0.25	0.34
Sodium bicarbonate	0.29	0.15	0.04	0.15	0.04
Yeast	1.38	0.15	0.21	0.15	0.21
Steam flaked maize	0.32	0.50	0.16	1.50	0.48
Ground corn grain	0.02	5.00	0.10	4.00	0.08
Wheat bran	0.01	0.80	0.01	0.80	0.01
Corn gluten feed	0.01	0.60	0.01	0.60	0.01
Distillers dried grains with soluble	0.03	0.60	0.02	0.60	0.02
Mineral and Vitamin premix	0.04	0.65	0.02	0.65	0.02
Whole soybean	0.04	0.30	0.01	0.30	0.01
Soybean meal	1.54	3.60	5.54	2.50	3.85
Cotton expeller	0.03	0.00	0.00	0.20	0.01
RPM ²	21.5	0.00	0.00	0.02	0.32
Total cost		40.0	10.9	40.0	10.3

¹Treatments CON group and RPM group consists of total mixed ration containing 17.3 and 16.4% CP, respectively

²RPM rumen-protected methionine

Ingredients	Treatment ¹			
	CON		RPM	
Cost per kg	1.0	0.273	1.0	0.257
¹ Treatments CON group and RPM group consists of total mixed ration containing 17.3 and 16.4% CP, respectively				
² RPM rumen-protected methionine				

4. Conclusion

In this study, compared with 17.3% dietary CP, milk yield and milk composition were not limited by the lower dietary CP (16.4%) with RPM supplementation of 15.0g/d per cow. In fact, decreasing dietary CP from 17.3–16.4% could reduce BUN concentration, which made a positive impact on the nitrogen utilization of dairy cows. In addition, higher ruminal MCP could be found in RPM group and concentrations of butyrate and isovalerate in the rumen of RPM group were higher than that of CON group at 2h after feeding. In economic terms, supplying the diet of 16.4% CP with RPM supplementation to cows could reduce feeding cost by 0.5 \$/d per cow and boost net profits. In general, RPM may make a positive effect on dairy cows and several research programs are still needed to explore RPM more to discover its other vital roles in dairy cow life.

Abbreviations

DM: Dry matter; NEL: Net energy for lactation; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; peNDF: Physically effective neutral detergent fiber; NFC: Non-fiber carbohydrate; Met: Methionine; Lys: Lysine; FP: Freezing point; SCC: Somatic cell count; TS: Total solid; SNF: Solids-not-fat; MUN: Milk urea nitrogen; TP: Total protein; ALB: Albumin; GLB: Globulin; BUN: Blood urea nitrogen; CHO: Cholesterol; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; MCP: Microbial crude protein, VFA: Volatile fatty acid; TMR: Total mixed ration; RDP: Rumen degradable protein

Declarations

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Authors' contributions

Conceived the idea and designed the experiments: ZJC and YHZ. Implemented the experiment: YXL. Performed the analysis: JLW, BCY, MYD, CYL. Wrote the manuscript: JLW and SL. All authors read and approved the final manuscript.

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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Figures

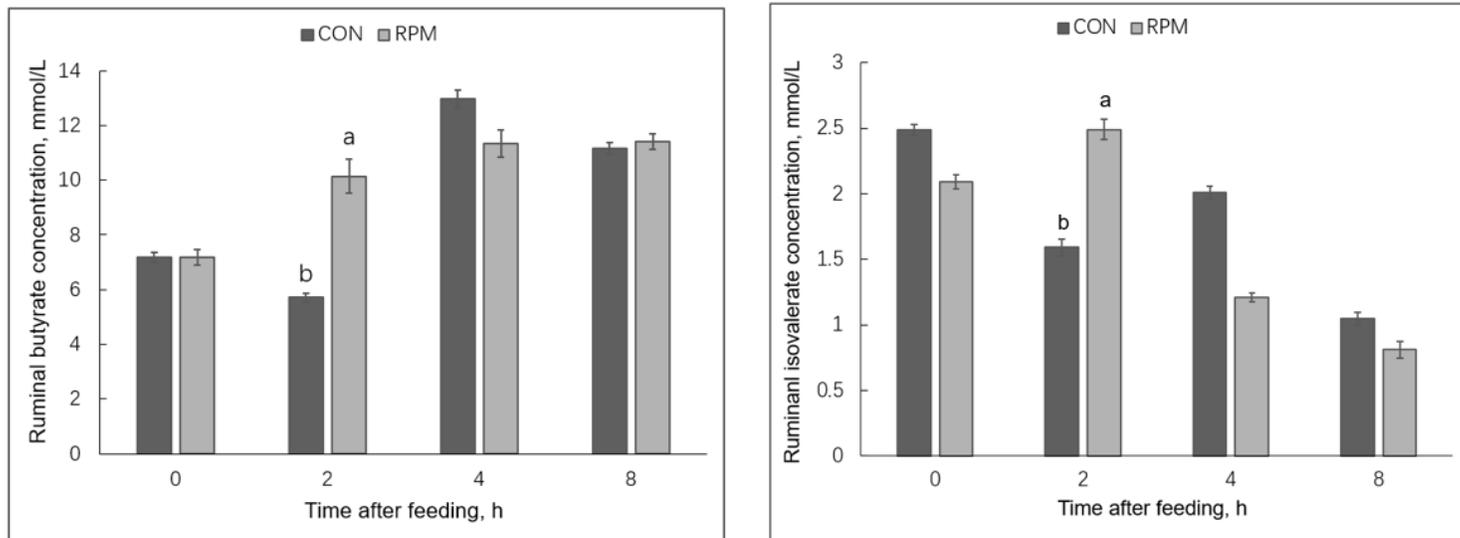


Figure 1

Ruminal butyrate and isovalerate concentrations in Holstein cows fed a control diet (CON) containing 17.3% CP or treatment diet containing 16.4% CP with RPM supplementation (RPM) at 0h, 2h, 4h and 8h after feeding. a,bLeast squares means within a row with different superscripts differ ($P < 0.05$). Bars indicate standard error of the means.