



Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short Communication

Effects of replacement of alfalfa silage with corn silage and supplementation of methionine analog and lysine-HCl on milk production and nitrogen feed efficiency in early lactating cows

Chen Yanting^a, J.H. Harrison^{b,*}, L.D. Bunting^c^a Department of Animal Science, Washington State University, Pullman, WA, USA^b Department of Animal Science, Washington State University, Puyallup, WA, USA^c Archer Daniels Midland Co., Decatur, IL, USA

ARTICLE INFO

Keywords:

Corn silage
Alfalfa silage
Nitrogen efficiency
Milk fat
Early lactation

ABSTRACT

The objective of this experiment was to study effects of replacement of alfalfa silage with corn silage in diets with sufficient metabolizable protein (MP) and balanced metabolizable lysine and methionine on milk production and nitrogen feed efficiency in early lactating cows. Thirty-six cows were blocked by similar body weight at calving, parity and predicted transmitting ability of milk yield, and randomly assigned into 3 dietary treatments from calving to 15 wk postpartum. Dietary treatments included 1) alfalfa silage diet (AF, 186 g/kg CP), 2) a diet replacing alfalfa silage with corn silage and supplemented with rumen undegradable protein and 2-hydroxy-4-methylthio-butanoic acid (HMB) (CS-M, 160 g/kg CP), 3) CS-M + lysine-HCl (CS-ML, 160 g/kg CP). Metabolizable protein was similar among treatments and approximate 110 g/kg of DM. Metabolizable Met and Lys were 2.2 and 6.3% of MP in AF; and were 2.5 and 6.2% of MP in CS-M; and were 2.5 and 6.9% of MP in CS-ML. Dry matter intake, MP intake, body weight and body condition score were not affected by treatments. Nitrogen intake was less (~ 100 g/d) in CS-M and CS-ML than AF, but milk yield, milk protein percentage and yield were similar among treatments. The percentage and yield of milk fat and milk fat corrected milk were greater in AF and CS-ML than CS-M. From 8 to 15 wk postpartum, CS-M had a moderate milk fat depression (milk fat < 30 g/kg). Compared with AF, CS-M and CS-ML had greater nitrogen feed efficiency and less milk urea nitrogen. Concentrations of lysine and leucine in blood were greater in CS-M and CS-ML, and valine tended to be greater in CS-M and CS-ML than AF. Overall, the replacement of alfalfa silage with corn silage in the diet with adequate MP and balanced metabolizable lysine and methionine could improve nitrogen feed efficiency without compromising milk production in early lactation. In addition, unbalanced ratio of metabolizable Lys to Met could depress milk fat percentage and yield.

1. Introduction

Serious nitrogen (N) pollution and high cost of protein feeds require dairy cows to produce milk efficiently (Ruddy et al., 2006;

Abbreviations: N, nitrogen; MP, metabolizable protein; RDP, rumen degradable protein; RUP, rumen undegradable protein; AA, amino acids; DM, dry matter; TMR, total mixed ration; ADF, acid detergent fiber; NDF, neutral detergent fiber; ADIN, acid detergent insoluble nitrogen; NDIN, neutral detergent insoluble nitrogen; CP, crude protein; BCS, body condition score; BW, body weight; SNF, solids non-fat; SCC, somatic cell count; MUN, milk urea nitrogen

* Corresponding author.

E-mail address: jhharrison@wsu.edu (J.H. Harrison).

<https://doi.org/10.1016/j.anifeedsci.2018.06.007>

Received 29 January 2018; Received in revised form 11 June 2018; Accepted 16 June 2018

0377-8401/© 2018 Published by Elsevier B.V.

USDA-NASS, 2015). Since alfalfa silage has high degradable protein and fermentable fibers (Broderick, 2001), alfalfa silage is commonly used in dairy ration to benefit milk production (Benchaar et al., 2007). However, because of alfalfa silage also containing excess of rumen degradable protein and less non-fermentable carbohydrates, feeding alfalfa silage also greatly increases urinary N excretion without effectively improving N feed efficiency in milk protein synthesis (Hassanat et al., 2013). Compared with alfalfa silage, corn silage is a less cost and contains relatively balanced degradable N and fermentable carbohydrates (Benchaar et al., 2007; Broderick, 2001, 1985). In previous studies, replacement of alfalfa silage with corn silage was observed to decrease urinary N excretion and increase N feed efficiency (Broderick, 1985; Dhiman and Satter, 1997; Valadares et al., 1999), but milk and milk protein yields are often decreased in the replacement partially due to the deficient rumen undegradable protein (RUP) in corn silage diets (Broderick, 1985; Dhiman and Satter, 1997). Therefore, supplementation of RUP source to increase metabolizable protein (MP) could be beneficial to the success of replacement of alfalfa silage with corn silage.

Lysine and Met have been identified as limited amino acids (AA) in milk protein synthesis (Rulquin et al., 1993; Schwab et al., 1992a, 1992b). A meta-analysis conducted by Robinson (2010) reported that cows fed diets with balanced metabolizable Met and Lys produced 1.4 kg/d more milk with 2.7% of increase in milk protein percentage. Therefore, we hypothesized that the replacement of alfalfa silage with corn silage in diets with adequate MP and balanced metabolizable Met and Lys could improve N feed efficiency without compromising milk and milk protein productions in early lactation.

2. Material and methods

2.1. Animals

Animals were cared for and handled according to the guidelines of the Washington State University Animal Care and Use Committee. Thirty-six Holstein cows including 15 primiparous and 21 multiparous cows were blocked by similar body weight at calving, parity and predicted transmitting ability of milk yield, and randomly assigned into 3 dietary treatments from calving to 15 wk postpartum. Prior to the experiment, cows were trained to adapt to individual feeding system. Each treatment had 12 cows. Cows were housed in a free stall barn with free access to water and milked twice daily (0010 and 0022). Cows were fed individually once a day (0012) through a Calan head gate system (American Calan, Northwood, NH, USA). Feed intake was adjusted daily to allow refusals to 50 to 100 g/kg of as feed intake.

2.2. Diets and treatments

Ingredient composition of dietary treatments is summarized in Table 1. Dietary treatments included an alfalfa silage diet (AF); replacement of alfalfa silage with corn silage and supplemented with RUP and a Met analog (CS-M); and CS-M + Lys – HCl (CS-ML). Briefly, approximately 266 g/kg of alfalfa silage and 17 g/kg of soybean meal in AF were replaced with 291 g/kg of corn silage in CS-M and CS-ML. Rumen protected fish meal (Prolak; H.J. Baker & Bro. Inc., Westport, CT, USA) and soy protein (Arsoy; ADM, Decatur, IL, USA) as RUP sources to increase the MP, Lys and Met in CS-M and CS-ML. Besides that, HMB (2-hydroxyl-4-methylthino-butanoic acid; Alimet, Novus, St Charles, MO, USA) and Lys – HCl were supplemented in the CS-M and CS-ML diets to change the ratio of metabolizable Lys to Met. HMB is a Met analog and converted to L-Met in body tissues (Lobley et al., 2006). HMB and Lys – HCl were mixed with mineral and vitamin premix before incorporated into the TMR. Metabolizable protein, Lys and Met in diets were estimated by AMTS.Cattle.Pro (CNCPS model version 4.1.4; AMTS LLC, New York, NY, USA).

2.3. Sample collection and laboratory analysis

The TMR was sampled weekly and dried in a forced air oven at 55 °C for dry matter (DM) analysis. Dried samples were ground through 1 mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA), and composited monthly. The composites were sent to Cumberland Valley Analytical Service (Hagerstown, MD, USA) for chemical analyses, including crude protein (CP) (984.13), crude fat (954.02), acid detergent fiber (ADF) (973.18), ash (942.05) and minerals (AOAC, 2000) according to AOAC (2000). In addition, neutral detergent fiber (NDF) (Van Soest et al., 1991), soluble CP (Krishnamoorthy et al., 1982), lignin (Goering and Van Soest, 1970), neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN) (Leco FP-528, Nitrogen Analyzer, Leco Instruments Inc., St. Joseph, MI, USA) were also analyzed.

Milk samples were obtained weekly and collected from the a.m. (0011) and p.m. (0023) milking, and mixed together based on the proportion of weight. Milk samples were immediately sent to the local DHIA laboratory (Burlington, WA, USA) for milk protein, fat, lactose, solids non-fat (SNF), and somatic cell counts (SCC) analyses by a Fossomatic 4000 Combi mid-infrared analyzer (Eden Prairie, MN, USA). Milk samples obtained from 6 cows in each treatment at 2, 4, 6, 8, 10 and 12 wk postpartum were analyzed for MUN (Crocker, 1966). Body weight was recorded weekly every morning before milking, and body condition score (BCS) was estimated by 2 individuals weekly. Estimation of BCS was utilized a 5 point scoring system with 0.25 point increment (Edmonson et al., 1989). NE_l was estimated according to the guidelines of NRC (2001).

Blood samples were taken from the coccygeal tail vein into heparinized tubes at 2 and 6 wk postpartum. Blood samples were mixed with 500 ul of 10% sulfosalicylic acid and 5 uM norvaline solution, and centrifuged at 14,000 x g for 10 min at 4 °C. Supernatant plasma was frozen at -20 °C until for AA analyses. The AA concentration was analyzed by Waters Ultra Performance Liquid Chromatograph Mass Trak AA analysis solution developed by Waters Corporation (ACQUITY Ultra Performance LC, Milford, MA, USA).

2.4. Statistical analyses

Data were analyzed as a randomized complete block design with repeated measurements using PROC MIXED procedure of SAS version 9.4 according to the following model:

$$Y_{ijkm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_m(\gamma_k) + (\alpha\beta)_{ij} + \varepsilon_{ijkm}$$

Where Y is the observation on cow k at sampling time j given treatment i; μ is the overall mean; α_i is the fixed effect of treatment i (AF, CS-M or CS-ML); β_j is the fixed effect of sampling time (week); $(\alpha\beta)_{ij}$ is the fixed effect of interaction between treatment i and sampling time j; γ_k is the random factor of block. $\delta_m(\gamma_k)$ is the random effect of cow k nested within block of m. ε_{ijkm} is the residual term. Estimation of parameters was used as a restricted maximum likelihood. The AR (1) covariance structure was used in the model. The Kenward-Roger option of the MODEL statement was used to adjust the degrees of freedom. Multiple comparison was performed with TUKEY methods. SLICE option was used in LSMEANS statement to compare three treatments within each week. If $P < 0.05$, it was considered as a significant difference between treatments. If $0.05 \leq P < 0.1$, it was considered as a trend.

3. Results and discussion

Chemical composition of diets is presented in Table 1. Mainly because of replacement of alfalfa silage with corn silage, dietary CP in CS-M and CS-ML was reduced from 186 to 160 g/kg of DM relative to AF. After supplementation of RUP source, MP was similar

Table 1
Ingredient and chemical compositions of diets fed to experimental cows.

Items	AF ¹	CS-M ¹	CS-ML ¹
Ingredients (g/kg of DM)			
Alfalfa hay	166	185	185
Alfalfa silage	266	0	0
Corn silage	0	291	291
Soybean meal	17	0	0
Whole cottonseed	102	102	102
Barley ²	74	108	104
Corn ²	103	45	33
Wheat ²	157	133	133
Peas ²	57	58	58
Molasses	17	0	0
Corn gluten meal	16	29	29
Beef tallow	1.6	3.3	3.2
Calcium carbonate	5	4.8	4.8
Dicalcium phosphate	3.4	3.3	3.3
Sodium bicarbonate	7.7	7.4	7.4
Magox ³	1.6	1.6	1.6
Zinpro 4-Plex ⁴	0.5	0.5	0.5
Trace mineral premix ⁵	5	4.8	4.8
Bloat Guard ⁶	0.4	0.4	0.4
Maxi-Bond ⁷	0.2	0.2	0.2
Vitamin premix ⁸	0.3	0.3	0.3
Arsoy ⁹	0	0	23.4
Prolak ¹⁰	0	21.4	9.8
Alimet ¹¹	0	1.2	1.5
Lys-HCl ¹²	0	0	3.3
Chemical composition (g/kg DM)			
CP	186	160	160
ADF	274	298	329
NDF	389	412	447
ADIN	16	16	17
NDIN	24	24	24
Soluble CP	75	51	54
Lignin	65	65	72
Ash	84	61	59
Crude fat	46	47	52
NFC	319	344	307
NE _L , Mcal/kg	1.5	1.5	1.5
RDP	108	94	96
MP ¹³	110	112	110
Metabolizable Met, % DM ¹³	0.24	0.27	0.27
Metabolizable Met from supplemental RUP and HMB, % DM ¹⁴	0	0.05	0.05

(continued on next page)

Table 1 (continued)

Items	AF ¹	CS-M ¹	CS-ML ¹
Metabolizable Lys, % DM ¹³	0.7	0.69	0.76
Metabolizable Lys from supplemental RUP and HCl-Lys, % DM ¹⁴	0	0.07	0.14
Metabolizable Met, % MP ¹³	2.2	2.5	2.5
Metabolizable Lys, % MP ¹³	6.3	6.2	6.9
Lys : Met ratio in MP ¹³	2.9 : 1	2.5 : 1	2.8 : 1

¹ AF = alfalfa silage diet with 186 g/kg CP; CS-M = corn silage diet (160 g/kg CP) with adequate MP and 1.2 g/kg HMB; ML = corn silage diet (160 g/kg CP) with adequate MP, 1.5 g/kg HMB and 3.3 g/kg HCl-Lys.

² Ingredients were ground before mixed into the TMR.

³ Magox (Premier Magnesia LLC, Conshohocken, PA) contained 562 Mg (g/kg DM).

⁴ Zinpro 4-Plex (Zinpro Corp., Eden Prairie, MN) contained approximately Zn, 2530 mg/kg; Cu, 8800 mg/kg; Mn, 1400 mg/kg; Co, 1500 mg/kg on DM basis.

⁵ Trace mineral premix approximately contained 393 g/kg Na; 607 g/kg Cl; Fe, 2000 mg/kg; Zn, 3500 mg/kg; Cu, 350 mg/kg; Mn, 1800 mg/kg; Se, 90 mg/kg; Co, 60 mg/kg; I, 100 mg/kg on DM basis.

⁶ Bloat Guard was used to prevent bloat in cattle, and contained 53 g/kg of poloxalene (Phibro Animal Health, Teaneck, NJ).

⁷ Maxi-Bond, steam conditioner (AgResearch, Joliet, IL).

⁸ Vitamin premix contained 6810 KIU/kg vitamin A; 5000 KIU/kg vitamin D.

⁹ Arsoy (ADM, Quincy, IL) contained approximately 350 g/kg of CP on DM basis.

¹⁰ Pro-Lak (H.J. Baker & Bro. Inc., Westport, CT) was an animal marine blended protein product, and contained approximately 769 g/kg CP on DM.

¹¹ Alimet (Novus International, St Charles, MO) was 2-hydroxyl-4-methylthio-butanoic acid.

¹² HCl-Lys (ADM, Decatur, IL) contained 985 g/kg of Lys on DM.

¹³ Metabolizable protein and metabolizable Met (M-Met), Lys (M-Lys) were estimated by AMTS.Cattle.Pro (Version 4.1.4; AMTS LCC, New York, NY) using measured chemical composition of dietary ingredients and values in the feed library.

¹⁴ Contents and intestinal bioavailability of Lys and Met in Arsoy, Prolak, Alimet and HCl-Lys were provided by manufactures: Arsoy contained 350 g/kg of CP, 45 g/kg Lys and 12 g/kg Met in CP, and the bioavailability of Lys and Met in the small intestine was 800 g/kg on DM. Prolak contained 740 g/kg of CP, 60 g/kg of Lys and 16 g/kg of Met in CP, and the bioavailability of Met and Lys in the small intestine was 800 g/kg. Alimet contained 880 g/kg of HMB, and 400 g/kg of HMB was absorbed and converted to Met. HCl-Lys contained 985 g/kg of Lys and the bioavailability of Lys in the small intestine was 80 g/kg.

among treatments and approximate 110 g/kg of DM. The metabolizable Met and Lys in AF were about 2.2 and 6.3% of MP. NRC (2001) suggested that the optimum metabolizable Met and Lys for maximum milk protein response were 2.4 and 7.2% of MP. Due to the recommendation limited by profitability in practice, Schwab et al. (2003) also suggested that the applicable recommendation for metabolizable Met and Lys were 2.2 and 6.8% of MP. In AF, the metabolizable Met was less than the recommendation of NRC (2001), and metabolizable Lys was less than the recommendations of both NRC (2001) and Schwab et al. (2003). Compared with AF, metabolizable Met in CS-M and CS-ML was increased to 2.5%, and metabolizable Lys in CS-ML was increased to 6.9% of MP. Therefore, all treatments contained adequate MP, but CS-ML contained much more balanced metabolizable Met and Lys relative to AF and CS-M.

Interaction between dietary treatment and lactation week was not observed ($P \geq 0.05$) in the data results, so our interpretation mainly focused on the treatment effects. The DMI and MP intake were similar among treatments, but N intake was approximately 100 g/d less ($P < 0.001$) in CS-M and CS-ML than AF (Table 2). The BW and BCS were not affected by treatments. Compared with AF, CS-M and CS-ML had similar milk yield, milk protein yield and percentage. Therefore, replacement of alfalfa silage with corn silage in diets with adequate MP and metabolizable Met and Lys could maintain the milk and milk protein yields.

Interestingly, milk fat percentage was 6 g/kg of unit less (32 vs 38 g/kg, $P = 0.01$) in CS-M than AF and CS-ML, resulting in less milk fat yield (1.1 vs 1.3 kg/d, $P < 0.001$) and fat corrected milk ($P = 0.03$) in CS-M. Compared with CS-ML, CS-M also tended to have approximately 4.6 kg/d less ($P = 0.08$) energy corrected milk (Table 2). Specifically, the milk fat percentage in CS-M was less than 30 g/kg from 9 to 15 wk postpartum (Fig. 1). Although dietary protein and AA affecting milk fat synthesis has not been fully explored, feeding diets with unbalanced ratio of metabolizable Lys to Met was previously recorded to decrease milk fat percentage and yield. Rogers et al. (1989) fed the diets with a decreased ratio of metabolizable Lys to Met from 3.0 : 1 to 2.5 : 1, and observed that the milk fat percentage of cows fed diets with the ratio less than 2.8 : 1 was decreased to less than 30 g/kg. Consistent with the previous study, the ratio of metabolizable Lys to Met in CS-M (2.5 : 1) was much less than the ratio in AF (2.9 : 1) and CS-ML (2.8 : 1). NRC (2001) and Schwab et al. (2003) suggested that an optimum ratio of metabolizable Lys to Met for maximum milk protein percentage and yield responses is 3.0 : 1. Therefore, our study suggested that the unbalanced ratio of metabolizable Lys to Met could affect milk fat percentage beside with milk protein.

Compared with AF, N feed efficiency (milk N / intake N) was increased approximately 15.1% (29.1 vs 33.7%, $P < 0.01$), and the concentration of MUN was decreased approximately 27.5% (18.9 vs 13.8 mg/dl, $P < 0.001$) in CS-M and CS-ML. As MUN is positively correlated with N excretion in urine, N pollution would be attenuated by cows fed the CS-M and CS-ML diets (Kauffman and St-Pierre, 2001). Also, the less MUN could be mainly attributed to the replacement of alfalfa silage with corn silage in CS-M and CS-ML.

Table 2
Dry matter intake and milk performance of cows affected by experimental diets.

Item ⁵	AF ¹	CS-M ¹	CS-ML ¹	SEM	Treat	Week	Treat × Week
DMI, kg/d	20.5	20.4	20.4	0.95	0.99	< 0.001	0.24
N intake, kg/d	0.61 ^a	0.52 ^b	0.52 ^b	0.03	< 0.001	< 0.001	0.11
MP intake, kg/d ²	2.26	2.29	2.25	0.11	0.87	< 0.001	0.25
BW, kg/d	649.1	673.8	664.2	22.8	0.44	< 0.001	0.18
BW change, kg/d	18.3	25.7	7.3	10.19	0.44	< 0.001	0.16
BCS	3.31	3.26	3.24	0.05	0.59	< 0.001	0.93
BCS change / wk	-0.25	-0.27	-0.26	0.04	0.95	< 0.001	0.92
Milk yield, kg/d	35.8	35.3	37.5	2.30	0.68	< 0.001	0.60
ECM, kg/d ³	37.5 ^{ab}	34.1 ^b	38.8 ^a	2.08	0.08	< 0.001	0.25
3.5% FCM, kg/d ⁴	37.2 ^a	33.2 ^b	38.6 ^a	2.08	0.03	< 0.001	0.29
Protein, g/kg	30.8	30.8	30.7	0.7	0.10	< 0.001	0.95
Protein yield, kg/d	1.10	1.08	1.13	0.07	0.71	< 0.001	0.52
Fat, g/kg	38.2 ^a	32.1 ^b	37.9 ^a	1.6	0.01	< 0.001	0.50
Fat yield, kg/d	1.34 ^a	1.11 ^b	1.38 ^a	0.07	< 0.01	0.015	0.36
Lactose, g/kg	50.8 ^a	49.4 ^b	49.5 ^b	0.5	0.05	< 0.001	0.45
Lactose yield, kg/d	1.82	1.74	1.86	0.11	0.67	< 0.001	0.51
SNF, g/kg	90.3	89.7	89.6	0.9	0.73	< 0.001	0.30
SNF, kg/d	3.23	3.16	3.34	0.20	0.71	< 0.001	0.62
SCC, × 10 ³ , cells / ml	320.9	221.1	384.3	142.0	0.66	0.29	0.05
MUN, mg/dl	18.9 ^a	13.2 ^b	14.3 ^b	0.77	< 0.001	0.04	0.47
Milk yield / DMI, kg/kg	1.79	1.76	1.85	0.07	0.61	0.01	0.92
Milk N / N intake, %	29.1 ^a	33.1 ^b	34.5 ^b	1.01	< 0.01	< 0.001	0.98
MP balance, g/d ²	82.6 ^{cd}	152.2 ^c	30.2 ^d	46.5	0.15	< 0.001	0.54
NE _L balance, Mcal/d ²	-5.1 ^a	-1.9 ^b	-5.8 ^a	0.83	< 0.001	< 0.001	0.76

¹ AF = alfalfa silage diet with 186 g/kg CP; CS-M = corn silage diet (160 g/kg CP) with adequate MP and 1.2 g/kg HMB; ML = corn silage diet (160 g/kg CP) with adequate MP, 1.5 g/kg HMB and 3.3 g/kg HCl-Lys.

² Estimated according to NRC (2001).

³ Energy corrected milk = milk yield × 0.327 + milk protein yield × 7.65 + milk fat yield × 12.95 (Sjaunja, 1990).

⁴ 3.5% Fat corrected milk = 0.432 × milk yield + 16.22 × fat yield.

⁵ Different superscripts indicate significant difference between dietary treatments.

Dhiman and Satter (1997) and Brito and Broderick (2006) observed that the replacement of alfalfa silage with corn silage decreased the concentration of NH₃ in the rumen and urea N in blood and milk. Since alfalfa silage had greater RDP and less degradable carbohydrates than corn silage, more NH₃ could be emitted from the rumen fermentation and converted to urea in the liver and excreted into urine and milk (Hristov et al., 2004). The increased supply of metabolizable Lys and Met could also be beneficial to the increased N feed efficiency through decreasing catabolism of AA in organs, such as liver and mammary gland (Lee et al., 2012a, 2012b). Noftsker and St-Pierre (2003) observed that MUN was less for cows fed isonitrogenous diets with greater metabolizable Met.

Compared with AF, concentration of Lys in blood was greater (P = 0.04) in CS-M and CS-ML, but the concentration of Met was not affected by treatments (Table 3). HMB is converted to Met in the body tissues, so the less response of concentration of Met in blood was also observed in the previous studies (Johnson-VanWieringen et al., 2007; Lobley et al., 2006). Interestingly, the concentrations of branched chain AA, Leu (P = 0.02) and Val (P = 0.09) were greater in CS-M and CS-ML than AF. Muscle contains abundant branched chain AA, and muscle mobilization could result in the increased blood concentration of Leu and Val (Phillips et al., 2003). However, the blood concentration of 3-methylhistidine, an indicator of muscle mobilization (Houweling et al., 2012), was not

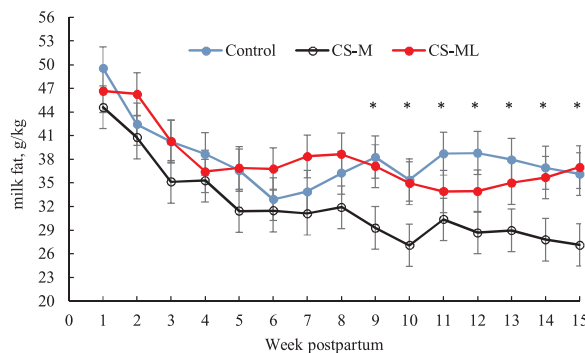


Fig. 1. Milk fat percentage affected by cows fed alfalfa silage diet with 186 g/kg CP (AF), corn silage diet (160 g/kg CP) with adequate MP and 1.2 g/kg HMB (CS-M) or with adequate MP, 1.5 g/kg HMB and 3.3 g/kg HCl-Lys (CS-ML) in 15 wk postpartum. * P < 0.05 (AF, CS-ML vs CS-M). Color figure is only available online.

Table 3
Concentrations of AA in plasma affected by cows fed experimental diets¹.

Item (μM) ²	AF	CS-M	CS-ML	SEM	Treat	Week	Trt x Wk
Ala	243	256	259	16.62	0.76	0.056	0.86
Arg	47.6	58.8	56.3	4.38	0.19	0.55	0.37
Asp	96.2	110	73.5	20.00	0.60	< 0.01	0.95
Cys	5.34	7.04	6.38	0.57	0.13	0.36	0.39
Glu	95.6	137	292	112.8	0.43	0.43	0.26
Gly	511	606	535	33.2	0.13	< 0.05	0.87
His	87.9	96.5	95.7	4.05	0.27	0.93	0.74
Ile	91.2	97.3	115.2	8.95	0.16	0.72	0.71
Leu	145 ^a	178 ^b	206 ^b	13.2	0.02	0.35	0.73
Lys	115 ^a	134 ^b	143 ^b	7.34	0.04	0.95	0.82
Met	24.5	26.0	24.5	1.62	0.72	0.64	0.57
Phe	35.9	40.4	40.7	1.99	0.18	0.8	0.65
Ser	113	113	102	7.41	0.49	0.16	0.26
Thr	110	120	124	7.39	0.40	0.32	0.79
Val	220	250	281	19.1	0.09	0.21	0.58
Tyr	47.6	49.9	56.8	3.74	0.21	0.13	0.99
Trp	15.9	18.8	17.7	2.53	0.68	0.068	0.71
3-M-His ³	4.4	4.0	3.9	0.45	0.23	< 0.01	0.56
NH3	94.6	81.6	85.3	6.21	0.33	0.6	0.41

¹ AF = alfalfa silage diet with 186 g/kg CP; CS-M = corn silage diet (160 g/kg CP) with adequate MP and 1.2 g/kg HMB; ML = corn silage diet (160 g/kg CP) with adequate MP, 1.5 g/kg HMB and 3.3 g/kg HCl-Lys.

² Different superscripts indicate significant difference between dietary treatments.

³ 3-methyl-histidine.

affected by treatments, so the increased concentration of Leu and Val in CS-M and CS-ML was likely caused by other reasons. Apelo et al. (2014) observed that the concentration of branched chain AA in blood was increased in cows fed the diets with increased contents of metabolizable Lys and Met. Because of peripheral tissues preferentially utilizing branched chain AA as substrates for energy supply, additional supplies of Lys and Met may spare some branched chain AA and not to be catabolized in the peripheral tissues (Goodwin et al., 1987; Nichols et al., 2016).

4. Conclusions

Overall, replacement of alfalfa silage with corn silage in diets with adequate MP and balanced metabolizable Lys and Met improved N feed efficiency without having negative impacts on milk production in the first 15 wk postpartum. Also, diets with an unbalanced ratio of metabolizable Met to Lys could depress milk fat synthesis in a long term feeding.

Conflict of interest

None.

Acknowledgements

The authors thank the staff of Kott Dairy Center in Washington State University for care of the animals and ADM company for financial support.

References

- AOAC, 2000. Official Methods of Analysis of the Association of Official Analytical Chemists. Arlington, VA.
- Apelo, S.I.A., Bell, A.L., Estes, K., Ropelewski, J., Veth, M.J., de, Hanigan, M.D., 2014. Effects of reduced dietary protein and supplemental rumen-protected essential amino acids on the nitrogen efficiency of dairy cows. *J. Dairy Sci.* 97, 5688–5699. <http://dx.doi.org/10.3168/jds.2013-7833>.
- Benchaar, C., Petit, H.V., Berthiaume, R., Ouellet, D.R., Chiquette, J., Chouinard, P.Y., 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *J. Dairy Sci.* 90, 886–897.
- Brito, A.F., Broderick, G.A., 2006. Effect of varying dietary ratios of alfalfa silage to corn silage on production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 89, 3924–3938. [http://dx.doi.org/10.3168/jds.S0022-0302\(06\)72435-3](http://dx.doi.org/10.3168/jds.S0022-0302(06)72435-3).
- Broderick, G.A., 1985. Alfalfa silage or hay versus corn silage as the sole forage for lactating dairy cows. *J. Dairy Sci.* 68, 3262–3271.
- Broderick, G.A., 2001. Maximizing utilization of alfalfa protein: the example of the lactating dairy cow. *Qual. Lucerne Medics Anim. Prod. Zaragoza Spain CIHEAM-IAMZ* 183–192.
- Crocker, C.L., 1966. Rapid determination of urea nitrogen in serum or plasma without deproteinization. *Am. J. Med. Technol.* 33, 361–365.
- Dhiman, T.R., Satter, L.D., 1997. Yield response of dairy cows fed different proportions of alfalfa silage and corn silage¹. *J. Dairy Sci.* 80, 2069–2082. [http://dx.doi.org/10.3168/jds.S0022-0302\(97\)76152-6](http://dx.doi.org/10.3168/jds.S0022-0302(97)76152-6).
- Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.* 72, 68–78. [http://dx.doi.org/10.3168/jds.S0022-0302\(89\)79081-0](http://dx.doi.org/10.3168/jds.S0022-0302(89)79081-0).
- Goering, H.K., Van Soest, P.J., 1970. Forage fiber analysis. *Agricultural Handbook No. 379*. US Dep. Agric. Wash. DC, pp. 1–20.

- Goodwin, G.W., Gibboney, W., Paxton, R., Harris, R.A., Lemons, J.A., 1987. Activities of branched-chain amino acid aminotransferase and branched-chain 2-oxo acid dehydrogenase complex in tissues of maternal and fetal sheep. *Biochem. J.* 242, 305–308.
- Hassanat, F., Gervais, R., Julien, C., Massé, D.I., Lettat, A., Chouinard, P.Y., Petit, H.V., Benchaar, C., 2013. Replacing alfalfa silage with corn silage in dairy cow diets: effects on enteric methane production, ruminal fermentation, digestion, N balance, and milk production. *J. Dairy Sci.* 96, 4553–4567. <http://dx.doi.org/10.3168/jds.2012-6480>.
- Houweling, M., van der Drift, S.G.A., Jorritsma, R., Tielens, A.G.M., 2012. Technical note: quantification of plasma 1- and 3-methylhistidine in dairy cows by high-performance liquid chromatography-tandem mass spectrometry. *J. Dairy Sci.* 95, 3125–3130. <http://dx.doi.org/10.3168/jds.2011-4769>.
- Hristov, A.N., Etter, R.P., Ropp, J.K., Grandeen, K.L., 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. *J. Anim. Sci.* 82, 3219–3229. <http://dx.doi.org/10.2527/2004.82113219x>.
- Johnson-VanWieringen, L.M., Harrison, J.H., Davidson, D., Swift, M.L., von Keyserlingk, M.A.G., Vazquez-Anon, M., Wright, D., Chalupa, W., 2007. Effects of rumen-undegradable protein sources and supplemental 2-hydroxy-4-(methylthio)-butanoic acid and lysine-HCl on lactation performance in dairy cows. *J. Dairy Sci.* 90, 5176–5188. <http://dx.doi.org/10.3168/jds.2006-741>.
- Kauffman, A.J., St-Pierre, N.R., 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey Cows1. *J. Dairy Sci.* 84, 2284–2294. [http://dx.doi.org/10.3168/jds.S0022-0302\(01\)74675-9](http://dx.doi.org/10.3168/jds.S0022-0302(01)74675-9).
- Krishnamoorthy, U., Muscato, T.V., Sniffen, C.J., Soest, P.J.V., 1982. Nitrogen fractions in selected feedstuffs. *J. Dairy Sci.* 65, 217–225. [http://dx.doi.org/10.3168/jds.S0022-0302\(82\)82180-2](http://dx.doi.org/10.3168/jds.S0022-0302(82)82180-2).
- Lee, C., Hristov, A.N., Cassidy, T.W., Heyler, K.S., Lapiere, H., Varga, G.A., de Veth, M.J., Patton, R.A., Parys, C., 2012a. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.* 95, 6042–6056. <http://dx.doi.org/10.3168/jds.2012-5581>.
- Lee, C., Hristov, A.N., Heyler, K.S., Cassidy, T.W., Lapiere, H., Varga, G.A., Parys, C., 2012b. Effects of metabolizable protein supply and amino acid supplementation on nitrogen utilization, milk production, and ammonia emissions from manure in dairy cows. *J. Dairy Sci.* 95, 5253–5268. <http://dx.doi.org/10.3168/jds.2012-5366>.
- Lobley, G.E., Wester, T.J., Calder, A.G., Parker, D.S., Dibner, J.J., Vázquez-Añón, M., 2006. Absorption of 2-hydroxy-4-methylthiobutyrate and conversion to methionine in lambs. *J. Dairy Sci.* 89, 1072–1080.
- Nichols, K., Kim, J.J.M., Carson, M., Metcalf, J.A., Cant, J.P., Doelman, J., 2016. Glucose supplementation stimulates peripheral branched-chain amino acid catabolism in lactating dairy cows during essential amino acid infusions. *J. Dairy Sci.* 99, 1145–1160. <http://dx.doi.org/10.3168/jds.2015-9912>.
- Noftsker, S., St-Pierre, N.R., 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. *J. Dairy Sci.* 86, 958–969. [http://dx.doi.org/10.3168/jds.S0022-0302\(03\)73679-0](http://dx.doi.org/10.3168/jds.S0022-0302(03)73679-0).
- NRC, 2001. *Nutrient Requirements of Dairy Cattle*, 7th rev. ed. ed. Natl. Acad. Press, Washington, DC.
- Phillips, G.J., Citron, T.L., Sage, J.S., Cummins, K.A., Cecava, M.J., McNamara, J.P., 2003. Adaptations in body muscle and fat in transition dairy cattle fed differing amounts of protein and methionine hydroxy analog. *J. Dairy Sci.* 86, 3634–3647.
- Robinson, P.H., 2010. Impacts of manipulating ration metabolizable lysine and methionine levels on the performance of lactating dairy cows: a systematic review of the literature. *Livest. Sci.* 127, 115–126. <http://dx.doi.org/10.1016/j.livsci.2009.10.003>.
- Rogers, J.A., Peirce-Sandner, S.B., Papas, A.M., Polan, C.E., Sniffen, C.J., Muscato, T.V., Staples, C.R., Clark, J.H., 1989. Production responses of dairy cows fed various amounts of rumen-protected methionine and lysine. *J. Dairy Sci.* 72, 1800–1817. [http://dx.doi.org/10.3168/jds.S0022-0302\(89\)79297-3](http://dx.doi.org/10.3168/jds.S0022-0302(89)79297-3).
- Ruddy, B.C., Lorenz, D.L., Mueller, D.K., 2006. *County-Level Estimates of Nutrient Inputs to the Land Surface of the Conterminous United States, 1982-2001*. US Department of the Interior, US Geological Survey.
- Rulquin, H., Pisulewski, P.M., Vérité, R., Guinard, J., 1993. Milk production and composition as a function of postruminal lysine and methionine supply: a nutrient-response approach. *Livest. Prod. Sci.* 37, 69–90.
- Schwab, C.G., Bozak, C.K., Whitehouse, N.L., Mesbah, M.M.A., 1992a. Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation 1, 2. *J. Dairy Sci.* 75, 3486–3502.
- Schwab, C.G., Bozak, C.K., Whitehouse, N.L., Olson, V.M., 1992b. Amino acid limitation and flow to the duodenum at four stages of lactation. 2. Extent of lysine limitation 1, 2. *J. Dairy Sci.* 75, 3503–3518.
- Schwab, C.G., Ordway, R.S., Whitehouse, N.L., 2003. Amino acid balancing in the context of MP and RUP requirements. In: *Proc. Four-State Appl. Dairy Nutr. Manage. Conf.*, Lacrosse, WI. Midwest Plan Service. Iowa State University, Ames. pp. 25–34.
- Sjaunja, L., 1990. A nordic proposal for an energy-corrected milk (ECM) formula. In: 27th Sess. Int. Comm. Rec. Product. Milk Anim. 2–6 July 1990 Paris Fr. USDA-NASS, 2015. "Agricultural Prices". Released May 28, 2015, and Accessed May 29, 2015 <http://usda.mannlib.cornell.edu/usda/nass/AgriPric//2010s/2015/AgriPric-05-28-2015.pdf> [WWW Document]. <http://usda.mannlib.cornell.edu/usda/nass/AgriPric/2010s/2015/AgriPric-05-28-2015.pdf>.
- Valadares, R.F.D., Broderick, G.A., Valadares Filho, S.C., Clayton, M.K., 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives1. *J. Dairy Sci.* 82, 2686–2696.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597. [http://dx.doi.org/10.3168/jds.S0022-0302\(91\)78551-2](http://dx.doi.org/10.3168/jds.S0022-0302(91)78551-2).