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Effects of fat and/or methionine hydroxy analog added to a molasses-urea-based supplement on ruminal and postruminal digestion and duodenal flow of nutrients in beef steers consuming low-quality lovegrass hay[1]

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ABSTRACT: Five crossbred beef steers (initial BW = 338.6 ± 7.8 kg) fitted with ruminal and duodenal cannulas were used in a 5 × 5 Latin square design experiment to evaluate the effects of methionine hydroxy analog (MHA) and/or yellow grease (fat) added to a molasses-urea-based supplement on intake and characteristics of digestion. Steers were fed low-quality hay (long-stem lovegrass *Eragrostis curvula*: 3.3% CP, 76.8% NDF; DM basis) ad libitum and supplemented with 0.91 kg/d (as fed) of 1 of 4 supplements in a 2 × 2 + 1 factorial arrangement of treatments. Supplemental treatments were 1) control (no supplement, NC); 2) molasses-urea liquid supplement (U); 3) U containing (as-fed basis) 1.65% MHA (UM); 4) U containing (as-fed basis) 12% fat (UF); and 5) U containing (as-fed basis) 1.65% MHA and 12% fat (UMF). Total and forage OM intake (kg/d and as % of BW) increased ($P < 0.01$) with molasses-urea, decreased ($P \leq 0.04$) with MHA, and were not affected ($P = 0.61$) with fat supplementation. Total tract NDF digestibility increased ($P = 0.01$) with molasses-urea supplementation, and was less ($P = 0.01$) for fat than for nonfat supplementation. Total and microbial N flowing to the duodenum increased ($P = 0.01$) with molasses-urea supplementation. Although, total N flowing to duodenum was not affected ($P = 0.27$), microbial N decreased ($P = 0.01$), and non-ammonia nonmicrobial N (NANMN) increased ($P = 0.01$) with fat supplementation. Extent of in situ OM and NDF digestibility at 96 h increased ($P = 0.01$) with molasses-urea supplementation, but were not affected ($P \geq 0.14$) by either MHA or fat supplementation. Duodenal flow of total AA, essential AA, and nonessential AA increased ($P \leq 0.02$) with molasses-urea supplementation. Total and nonessential serum AA concentration decreased ($P < 0.01$) with molasses-urea supplementation. Total ruminal VFA concentration increased ($P = 0.01$) with molasses-urea supplementation, and was not affected ($P \geq 0.14$) by MHA or fat supplementation. Fat can be used in molasses-urea liquid supplements for cattle consuming low-quality forage to increase energy intake without negatively affecting forage intake or characteristics of digestion. However, adding MHA did not further improve the response to urea supplementation of cattle consuming low-quality forage. Conversely, the inclusion of MHA on urea supplement decreased forage intake.

Key words: beef cattle, digestion, fat, low-quality forage, methionine hydroxy analog

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INTRODUCTION

Feeding supplemental protein to ruminants consuming low-quality forage has been shown to increase DM intake and digestibility (Raleigh and Wallace, 1963; Hannah et al., 1991; Wickersham et al., 2008; Sawyer et al., 2012; Cappellozza et al., 2014). When cattle consuming low-quality forage have been supplemented with NPN, performance has generally been reduced compared with true protein supplements (Chizzotti et al., 2008; Wickersham et al., 2009). In contrast, some studies have reported improvement in body condition (Kalmbacher et al., 1995) and reproductive efficiency (Fordyce et al., 1997) when cows consuming low-quality forage were supplemented with NPN-based supplements compared with supplements containing cottonseed meal. One potential limitation of NPN-based supplements is a decreased supply of metabolizable AA flowing to the duodenum (Willms et al., 1991). This limitation might be overcome by adding specific ruminally undegraded AA to the supplement; however, data on its effect on digestion and performance of beef cattle consuming low-quality forage are limited.

Including fat increases energy density of supplements. Pate et al. (1995) added fat to a molasses-based liquid supplement and reported an increase in rate of gain of yearling heifers. Supplemental fat has also been shown to increase milk production (Banta et al., 2011) and reproductive efficiency (Lake et al., 2007) in cows. Beef cattle consuming low-quality forage may benefit from supplementation with urea, ruminally undegraded AA, and/or fat; however, the effect of combinations of these ingredients on ruminal and total tract digestion has not been examined. This study was designed to determine the effects of molasses-urea supplements with or without methionine hydroxy analog (**MHA**) and (or) yellow grease (**fat**) on intake and characteristics of digestion by steers consuming low-quality hay.

MATERIALS AND METHODS

General

The New Mexico State University Institutional Animal Care and Use Committee approved all procedures and experimental protocols.

All procedures were conducted at the New Mexico State University Campus Livestock Research Center in Las Cruces, NM. Five 1.5-yr-old English crossbred steers (initial BW = 338.6 ± 7.8 kg) were used in this experiment. Steers were fitted with a 10 cm internal diameter ruminal cannula and a 1.8 cm internal diameter T-type duodenal cannula. Steers were housed in individual 10 × 30 m semienclosed pens. Pens were equipped with concrete feed bunks and automatic water dispensers.

Table 1. Nutrient composition of hay (Lovegrass; *Eragrostis curvula*) and molasses-urea-based supplements with methionine hydroxy analog (MHA) and (or) fat

		Supplements[1]			
		−FAT		+FAT	
Item	Hay	U	UM	UF	UFM
DM, %	94.8	62.7	62.3	58.9	58.4
Analyzed composition, % DM					
OM	93.2	80.5	80.8	82.83	83.1
CP	3.3	61.4	63.2	61.4	63.2
NPN	–	51.9	51.0	52.1	51.2
Ether Extract	2.7	0.4	0.4	18.0	18.0
NDF	76.8	–	–	–	–
ADF	44.2	–	–	–	–

[1]U = molasses-urea; UM = U plus 1.65% MHA; UF = U plus 12% yellow grease (fat); and UMF = U plus 1.65% MHA and 12% fat.

Experimental Design and Sampling

Steers were randomly allotted to 1 of 5 treatments in a 5 × 5 Latin square design experiment. Treatments were arranged as a 2 × 2 + 1 factorial with factors being MHA (Alimet; Novus International, St. Louis, MO) and yellow grease (**fat**) plus a negative control. Treatments were: 1) control (no supplement, **NC**); 2) molasses-urea supplement (**U**); 3) U containing (as-fed basis) 1.65% MHA (**UM**); 4) U containing (as-fed basis) 12% fat (**UF**); and 5) U containing (as-fed basis) 1.65% MHA and 12% fat (**UMF**). Chemical composition of hay and supplements are shown in Table 1. Steers had ad libitum access to mature long-stem lovegrass (*Eragrostis curvula*: 3.3% CP, 76.8% NDF, 44.2% ADF; chopped to pass through a 3.81-cm screen using a Bear Cat 5A; Western Bear Cat, Hastings, NE) hay fed twice daily (0800 and 1600 h) and were supplemented with 0.91 kg/d (as fed basis) of 1 of 4 supplements. The forage was selected to reflect the quality of dormant native range. Molasses-urea and MHA supplements were provided to ruminants to supply protein and increase DMI and digestibility, whereas fat was provided to increase the energy density of supplements.

Steer BW was determined at the initiation and completion of each 14-d period. Steers were dosed intraruminally with 7.5 g CrO_3 at each feeding throughout the experiment. In addition, supplement was delivered through the ruminal cannula before the morning feeding to ensure complete and rapid consumption. Forage and supplements were weighed daily and fed to steers on an individual basis and orts weighed daily before the morning feeding. Forage, orts, and supplements were

sampled at feeding. Forage samples were composited by period, whereas orts and supplement samples were composited by animal within period. Forage and orts were dried at 55°C in a forced-air oven for 48 h and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 2-mm screen. Supplement samples were stored (-20°C) until analyzed. Dry matter of supplement samples was determined by the Karl Fischer method (Thiex, and Van Erem, 2002; AquaStart Model V1B; EM Science, Gibbstown, NJ).

Each of the 5 experimental periods was 14 d in length with d 1 through d 7 for diet adaptation and d 8 through d 14 for sampling. Animals were haltered and tied during sample collection. Ruminal and duodenal samples were collected on d 8 and d 9 at 4-h intervals. On d 9, sampling times were advanced by 2 h so that every 2 h in a 24-h day was represented. Duodenal samples were collected (approximately 100 mL) and frozen (-20°C). Whole ruminal contents (approximately 500 g) were collected from several locations in the rumen (cranial to caudal) at the interface of the fiber mat and fluid layers. Approximately 250 g of ruminal contents were strained through 4 layers of cheesecloth, whereas the remainder was retained as whole contents and frozen (-20°C). Ruminal fluid pH was measured immediately using a combination electrode (Orion Research, Boston, MA). Ruminal fluid was then divided into two 10-mL aliquots; one aliquot was acidified with 0.5 mL 6 *N* HCl. Ruminal fluid samples were stored frozen (-20°C) in 15-mL centrifuge tubes.

Fecal samples were collected at 0700 and 1900 h on d 8 through d 12 and frozen (-20°C). Fecal samples were dried in a forced-air oven (55°C) for 72 h and ground in a Wiley mill to pass through a 2-mm screen. Dried fecal samples were then composited by animal within period on an equal weight basis.

Beginning on d 11, hay in situ digestibilities were determined. Within each animal and period, duplicate 10 × 20 cm, 50 ± 15 µm pore size; Dacron bags (Ankom, Fairport, NY) containing 5 g of air-dried hay obtained from a representative hay sample and ground on a Wiley mill to pass through a 2-mm screen were used to represent 2, 6, 10, 16, 24, 48, 72, and 96 h of digestion. To achieve the various incubation times, each set of duplicate bags was inserted into a mesh bag in the rumen at the appropriate time interval, and all bags were removed at the same time to ensure that rinsing conditions would be the same for all bags. Also, a set of duplicate Dacron bags representing 0 h were soak for 15 min and rinsed with the incubated Dacron bags. Bags were rinsed together in tap water (25°C) in a 20-L plastic bucket until the rinse water was clear on 3 successive rinses. Bags were then rinsed individually until contents were at the bottom of the bag (Vanzant et al., 1996). Bags were kept frozen (-20°C) until drying at 60°C in a forced-air oven for 48 h.

Serum samples were collected on d 14 at 4 and 23 h postsupplementation via coccygeal venipuncture into evacuated serum separator tubes (Corvac Serum Separator Tube; Tyco Healthcare Group LP, Mansfield, MA). Tubes were allowed to coagulate at ambient temperature for 30 min and were centrifuged at 1,500 × *g* for 15 min. Serum was then decanted and frozen (-20°C) until analyzed.

Laboratory Methods

Whole ruminal contents were composited by animal within period on an equal wet-weight basis, and a bacteria-rich pellet was obtained from 2,000 g of the composite of whole ruminal contents as described by Bock et al. (1991). The bacterial pellet was lyophilized and analyzed for purine content using a modification (2 *M* $HClO_4$ for the extraction procedure) of the procedure described by Zinn and Owens (1986), N content by micro-Kjeldahl, and ash (942.05; AOAC, 1997). The purine to N ratio of bacteria was used to calculate microbial N flow to the duodenum.

Frozen samples of duodenal digesta were thawed, mixed thoroughly, subsampled, and refrozen (-20°C). One subsample was lyophilized (VirTis Lyotroll; SP. Scientific, Gardiner, NY), ground with a microgrinder (Model CM4; Salton/Maxim Housewares Inc., Mt. Prospect, IL) and composited by animal within period on an equal-weight basis. Dry matter concentration of duodenal contents was determined from moisture loss during freeze-drying. Forage, orts, supplements, duodenal, and fecal samples were analyzed for DM, ash, N, and ether extract (**EE**). Methods 930.15, 990.02, 942.05, and 920.39 were used to determine DM, ash, N, and EE (AOAC, 1997). Also, forage, orts, duodenal, and fecal samples were also analyzed for NDF (Goering and Van Soest, 1970). Lyophilized duodenal contents were analyzed for AA by separation with high-precision liquid chromatography, and postcolumn derivatization with ninhydrin (AOAC, 1990), whereas purines (modification of Zinn and Owens, 1986), and ammonia (Broderick and Kang-Meznarich, 1980) were determined in a subsample of composited duodenal contents that was not lyophilized. Also, duodenal and fecal samples were analyzed for Cr with an air-plus-acetylene flame using atomic absorption spectroscopy (Williams et al., 1962).

Acidified samples of ruminal fluid were thawed and centrifuged at 1,500 × *g* for 15 min and analyzed for ammonia concentration by the phenol-hypochlorite method (Broderick and Kang-Meznarich, 1980). Another 8 mL of nonacidified ruminal fluid was thawed and centrifuged at 1,500 × *g* for 15 min and added to 2 mL of ice-cold metaphosphoric acid for VFA analysis. Concentration of ruminal fluid VFA was determined

by gas chromatography (Erwin et al., 1961). Serum was analyzed for cholesterol (Procedure 352; Sigma Diagnostics, St. Louis, MO) NEFA (C kit, ACS-ACOD method; Wako Chemicals USA, Inc., Richmond, VA). The AA in the digesta was determined using the EZ: faast method for AA analysis (Phenomenex, Torrance, CA), and protein hydrolyzed by GC-FID kit (Phenomenex). Briefly, 2 to 4 mg of dry sample was mixed with 6 *N* HCl (containing 4% thioglycolic acid) in a freeze-dried ampoule (8 × 145 mm) and heat sealed under vacuum. The sample was then hydrolyzed for 24 h at 110°C on cooling, the tube was opened and neutralized with 200 uL of EZ: faast reagent (2 carbonate). A 25-uL aliquot was used for derivatization.

Calculations

Organic matter flow to the duodenum and total fecal OM output was determined by dilution of the daily dose of Cr in duodenal contents or feces, respectively. Duodenal flow and fecal output of nutrients were calculated as the concentration of each nutrient in duodenal or fecal contents multiplied by duodenal OM flow or total fecal OM output, respectively. Apparent ruminal digestibility of nutrients was calculated as intake of each nutrient minus the amount of each nutrient flowing from the rumen divided by intake of each nutrient. True ruminal OM digestibility was determined by subtracting bacterial OM from duodenal OM flow. Apparent total tract digestibility was calculated as intake minus fecal output divided by intake of each nutrient. Postruminal digestion was calculated as total tract digestion minus ruminal digestion. Lag time, rate, and extent of OM, NDF, and CP in situ digestibility were calculated as outlined by Wilkerson (1992).

Daily N flow at the duodenum was partitioned into microbial N, ammonia N, and nonammonia, nonmicrobial N (**NANMN**). Microbial N was determined by multiplying the purine concentration in the duodenum by the purine to N ratio in the ruminal bacterial pellet (Zinn and Owens, 1986). Flow of NANMN was determined by subtraction of the microbial and ammonia N from total N. Microbial efficiency was calculated by dividing g of microbial N flow by kg of OM truly fermented in the rumen.

Statistics

Data were analyzed as a 5 × 5 Latin square design using the Mixed procedures of SAS (SAS Inst. Inc., Cary, NC). The model included supplement and period as fixed effects and steer as the random effect. Sample collection was repeated for 2 steers in a sixth period due to extremely low intakes in period one, data of period one was eliminated for those 2 steers, and their data of period six was used for the statistical analysis. In addition, 1 steer was removed from period 2 due to extremely low intake. Therefore, $n = 5$ for NC, UM, UF, and UMF and $n = 4$ for U. Time-dependent variables (ruminal ammonia, VFA, and pH) were evaluated using repeated measures analysis using the AR(1) first-order autoregressive covariance structure in the Mixed procedures of SAS. The model included period, steer, supplement, collection time, supplement × time, and steers × period × supplement; the random variable was steer. Means were separated using preplanned single degree of freedom contrasts. Contrasts were 1) supplement vs. no supplement; 2) supplements with MHA vs. supplements without MHA; 3) supplements with fat vs. supplements without fat; and 4) the interaction of MHA and fat. Results were considered significant when $P \leq 0.05$, and a trend was considered when $P = 0.05$ to 0.10.

RESULTS

Intake and Digestibility of OM, NDF, EE, and N

Effects of liquid supplements containing MHA and (or) fat on intake and digestibility of OM, NDF, EE, and N in steers consuming low-quality forage are presented in Table 2. Interactions of MHA × fat were not observed ($P \geq 0.16$). Initial BW was not different between supplemented and not supplemented ($P = 0.89$), whereas supplemented steers tended ($P = 0.08$) to lose less BW than NC steers. Total and forage OM intake was greater ($P < 0.01$) for supplemented steers, both in kg/d and as percent of BW, compared with NC steers. Steers supplemented without MHA had greater total and forage OM intake in kg/d ($P < 0.01$) and as a percent of BW ($P = 0.02$) than steers supplemented with MHA. Intake of NDF, EE, and N was greater ($P = 0.01$) for supplemented steers compared with NC. Also, intake of NDF, EE, and N was greater ($P \leq 0.02$) for supplemented steers receiving no MHA than for those receiving supplements with MHA. Moreover, intake of EE was greater ($P = 0.01$) for steers receiving supplements containing fat than those receiving supplements without fat. Supplemented steers showed greater ($P = 0.01$) OM and NDF flow to the small intestine than NC steers. Also, NDF flowing to the small intestine decreased ($P = 0.03$) for steers supplemented with MHA as compared with steers supplemented with no MHA.

True ruminal OM digestibility was greater ($P = 0.05$) for NC than for supplemented steers. Although total tract OM digestibility was not affected ($P = 0.53$) by supplementation, total tract digestibility of NDF, EE, and N was greater ($P = 0.01$) for supplemented steers that for NC. Also, total tract digestibility of EE was greater ($P = 0.01$) for steers receiving supplements

Table 2. Effects of liquid supplements containing methionine hydroxy analog (MHA) and (or) fat on intake and digestibility of OM, NDF, ether extract, and N in steers consuming low-quality forage

	Supplemental treatment [1]						Contrast OSL[2]			
		−Fat		+Fat						
Item	NC	U	UM	UF	UFM	SE[3]	1	2	3	4
Animal replicates	5	4	5	5	5					
Initial BW, kg	343	343	346	346	331	9.1	0.89	0.46	0.49	0.30
BW change, kg	−26.4	−5.7	−17.8	−6.1	−5.2	9.8	0.08	0.52	0.49	0.46
Intake										
Total OM, kg/d	3.24	4.71	4.04	4.62	4.16	0.18	0.01	0.01	0.92	0.54
Total OM, % of BW	0.97	1.36	1.17	1.34	1.25	0.05	0.01	0.02	0.61	0.36
Forage OM, kg/d	3.24	4.24	3.58	4.18	3.72	0.18	0.01	0.01	0.85	0.54
Forage OM, % of BW	0.97	1.22	1.04	1.21	1.11	0.05	0.01	0.02	0.56	0.42
NDF, kg/d	2.61	3.51	2.96	3.43	3.06	0.16	0.01	0.01	0.95	0.42
Ether extract, g/d	80.9	113.8	95.5	275.9	259.1	5.5	0.01	0.01	0.01	0.88
N, g/d	17.7	57.6	53.6	57.5	54.8	1.45	0.01	0.02	0.68	0.62
Flow to the duodenum										
OM, kg/d	1.98	2.74	2.33	2.48	2.47	0.16	0.01	0.16	0.70	0.17
NDF, kg/d	1.47	1.81	1.58	1.94	1.64	0.12	0.03	0.03	0.42	0.76
Ether extract, g/d	111.2	121.3	100.5	158.3	179.5	17.6	0.11	0.75	0.01	0.33
Digestibility, % of intake										
Apparent ruminal										
OM	38.3	41.8	42.4	44.7	41.3	2.3	0.10	0.36	0.87	0.25
NDF	43.2	48.7	46.8	43.3	46.1	1.9	0.13	0.79	0.13	0.19
Ether extract	−40.3	−3.3	−8.0	45.0	27.7	11.5	0.01	0.32	0.01	0.56
N	−65.7	21.5	22.8	28.0	26.4	11.4	0.01	0.99	0.63	0.88
True ruminal OM	56.6	53.0	51.9	50.3	54.2	2.0	0.05	0.45	0.95	0.18
Post ruminal										
OM	9.7	7.6	7.9	3.5	12.1	3.2	0.53	0.14	0.99	0.16
NDF	5.3	7.2	6.6	7.5	5.2	2.0	0.50	0.42	0.78	0.62
Ether extract	81.9	42.0	52.34	25.4	48.6	11.4	0.01	0.30	0.35	0.93
N	131.5	56.3	57.3	48.1	51.8	13.6	0.01	0.85	0.58	0.91
Total tract										
OM	48.1	49.3	50.2	48.2	52.3	3.1	0.53	0.37	0.87	0.55
NDF	48.5	55.8	53.4	50.8	51.3	1.1	0.01	0.32	0.01	0.16
Ether extract	41.6	38.7	44.3	70.4	76.3	1.1	0.01	0.01	0.01	0.43
N	65.8	77.7	80.1	76.1	78.2	2.9	0.01	0.40	0.49	0.96

[1]NC = negative control, U = molasses-urea, UM = U plus 1.65% methionine hydroxy analog (MHA), UF = U plus 12% yellow grease (fat), and UMF = U plus 1.65% MHA and 12% fat.

[2]Observed significance level for contrasts: 1) = supplement vs. no supplement; 2) = supplements containing MHA vs. supplements containing no MHA; 3) = supplements containing fat vs. supplements containing no fat; and 4) = interaction of MHA and fat.

[3]Standard error of least squares means.

containing MHA compared with those receiving supplements without MHA. Moreover, total tract digestibility of NDF was less ($P = 0.01$) and of EE was greater ($P = 0.01$) for steers receiving supplements containing fat than for those receiving supplements without fat.

Nitrogen Flow to the Duodenum and Microbial Efficiency

Table 3 presents the effects of liquid supplements containing MHA and (or) fat on N fractions flowing to the duodenum and microbial efficiency in steers consuming low-quality forage. Total, microbial, and ammonia N flowing to duodenum was greater ($P = 0.01$) for supplemented steers than for NC. Microbial N and microbial efficiency were greater ($P = 0.01$) and NANMN bypassing the rumen was less ($P = 0.01$) for steers receiving supplements without fat than those receiving supplements with fat.

Rate and Extent of Nutrient Digestibility

Table 4 presents the effects of liquid supplements containing MHA and (or) fat on in situ lag time, rate, and extent of nutrient digestibility in steers consuming low-quality forage. There were no supplementation

Table 3. Effects of liquid supplements containing methionine hydroxy analog (MHA) and (or) yellow grease (fat) on N fraction flowing to the duodenum and microbial efficiency in steers consuming low-quality forage

	Supplemental treatment[1]						Contrast OSL[2]			
	−Fat			+Fat						
Item	NC	U	UM	UF	UFM	SE[3]	1	2	3	4
Duodenal flow, g/d										
Total N	27.99	45.15	41.50	40.89	40.02	2.78	0.01	0.37	0.27	0.58
Microbial N	21.01	39.56	38.27	28.60	30.57	2.73	0.01	0.94	0.01	0.61
Ammonia N	0.95	2.19	2.07	1.81	2.29	0.16	0.01	0.24	0.58	0.06
NANMN [4]	6.03	3.40	1.16	10.48	7.37	2.13	0.84	0.19	0.01	0.82
Microbial efficiency [5]	12.60	15.77	18.67	12.51	13.75	1.58	0.11	0.17	0.01	0.57

[1]NC = negative control, U = molasses-urea, UM = U plus 1.65% methionine hydroxy analog (MHA), UF = U plus 12% fat, and UMF = U plus 1.65% MHA and 12% fat.

[2]Observed significance level for contrasts: 1) = supplement vs. no supplement; 2) = supplements containing MHA vs. supplements containing no MHA; 3) = supplements containing fat vs. supplements containing no fat; and 4) = interaction of MHA and fat.

[3]Standard error of least squares means.

[4]Non-ammonia, non-microbial N.

[5]Microbial efficiency, g of bacterial N/g of OM truly fermented.

effects on lag time or rate of digestion for OM, NDF, and N ($P \geq 0.40$). However, 96 h in situ disappearance of OM and NDF was greater ($P = 0.01$) for supplemented steers than for NC.

Duodenal Flow and Serum Concentrations of AA

Table 5 shows daily flows of total AA, essential AA (**EAA**), nonessential AA (**NEAA**), and methionine (**Met**) to the duodenum and serum concentrations of total AA, EAA, NEAA, and Met. Duodenal flow of total AA ($P < 0.02$), EAA ($P < 0.01$), NEAA ($P = 0.05$), and Met ($P = 0.01$) was greater for steers supplemented compared with NC steers.

Serum concentrations of total and NEAA were lesser ($P = 0.01$) and concentrations of EAA tended ($P = 0.08$) to be lesser for supplemented steers compared with NC steers (Table 5). Serum Met concentration was not affected ($P = 0.13$) by supplementation.

Ruminal pH, Ammonia, and VFA Concentration

The effects of liquid supplements effects on ruminal pH, ammonia concentration, and VFA profiles are summarized in Table 6. Ruminal pH and isovalerate concentration decreased ($P = 0.01$) and ammonia concentration and total VFA production increased ($P = 0.01$) with supplementation. Also, ruminal pH and propionate concentration were greater ($P = 0.01$) for MHA supplementation compared with no MHA supplementation. Acetate:propionate ratio decreased ($P = 0.02$) with fat supplementation as compared with nonsupplemental fat. Molar proportions of propionate and butyrate tended ($P = 0.07$) to increase and isobutyrate decreased ($P = 0.01$) with supplementation compared with NC.

Serum Cholesterol and NEFA Concentrations

Serum cholesterol and NEFA concentrations in steers consuming low-quality forage and supplemented with liquid supplements containing MHA and (or) fat are presented in Table 7.

Concentration of serum cholesterol increased ($P = 0.01$) for steers supplemented with fat as compared with no fat supplementation at 4 and 23 h post supplementation. Also, serum cholesterol concentration was greater ($P = 0.01$) for supplemented steers as compared with NC at 23 h postsupplementation. Serum NEFA concentration 23 h postsupplementation decreased for supplemented steers ($P = 0.02$) as compared with NC and was greater for steers supplemented with fat ($P = 0.05$) as compared with those receiving supplements without fat.

DISCUSSION

Intake and Digestibility of OM, NDF, EE, and N

Increased OM intake in supplemented steers compared with NC steers suggests that the basal diet had inadequate levels of available N for optimal ruminal function (Sletmoen-Olson et al., 2000; Lawler-Neville et al. (2006). Previous studies have reported protein deficiencies with CP content of forage of 5.7% (Sletmoen-Olson et al., 2000) or even 7.7% (Lawler-Neville et al., 2006). The low ruminal ammonia concentration of the unsupplemented steers in the present study also suggests that the basal diet had inadequate levels of N for ruminal functions. The ruminal ammonia concentration suggested as necessary for optimal is 5–6 mg/dL (Miller, 1973; Satter and Slyter, 1974). Often cattle consuming low-quality forage do not obtain enough digestible protein to stimulate sufficient forage intake for growth and

Table 4. Effects of liquid supplements containing methionine hydroxy analog (MHA) and (or) fat on in situ lag time, rate, and extent of nutrient digestibility in steers consuming low-quality forage

	Supplemental treatment[1]									
	−Fat			+Fat			Contrast OSL[2]			
Item	NC	U	UM	UF	UFM	SE[3]	1	2	3	4
OM										
Lag time, h	9.24	11.20	9.82	10.02	9.71	1.15	0.40	0.42	0.83	0.61
Rate, %/h	2.38	2.58	2.35	2.48	2.45	0.23	0.71	0.55	0.99	0.64
96 h, %	42.68	47.11	46.90	46.97	46.45	1.47	0.01	0.78	0.83	0.91
NDF										
Lag time, h	11.85	12.99	11.63	11.95	11.85	1.14	0.82	0.48	0.69	0.55
Rate, %/h	2.70	2.75	2.67	2.76	2.79	0.23	0.86	0.87	0.77	0.82
96 h, %	46.48	50.35	49.81	50.85	49.73	1.32	0.01	0.50	0.86	0.81
N										
Lag time, h	11.50	13.09	11.96	11.77	11.96	1.09	0.82	0.64	0.51	0.51
Rate, %/h	3.15	3.23	3.07	3.01	3.11	0.17	0.77	0.83	0.56	0.40
96 h, %	50.60	51.94	50.89	52.19	51.01	0.79	0.25	0.14	0.79	0.93

[1]NC = negative control, U = molasses-urea, UM = U plus 1.65% methionine hydroxy analog (MHA), UF = U plus 12% yellow grease (fat), and UMF = U plus 1.65% MHA and 12% fat.

[2]Observed significance level for contrasts: 1) = supplement vs. no supplement; 2) = supplements containing MHA vs. supplements containing no MHA; 3) = supplements containing fat vs. supplements containing no fat; and 4) = interaction of MHA and fat.

[3]Standard error of least squares means.

production (Hannah et al., 1991). Supplementation with urea has been shown to stimulate intake of low-quality forage (Raleigh and Wallace, 1963). Moreover, urea supplementation has been shown to stimulate similar levels of intake as casein, when substituting for 75% of the CP equivalent in a supplement for heifers fed low-quality forage (Köster et al., 1997) indicating that urea is effective in providing N to the ruminal microbes to enhance ruminal function. However, intake declines when urea supplementation is the only source of protein. In the current experiment, urea supplied 90% to 95% of the CP equivalents in the diet and stimulated intake above the NC steers. However, compared with data of Köster et al. (1997) stimulation of intake in the present study is below the level stimulated by supplementation of true protein. Although supplementing urea decreased true ruminal OM digestibility compared with NC, total digestible OM intake by animals receiving supplemental molasses-urea supplements was greater than for steers consuming hay only. A potential reason for the lower stimulation of intake for urea than that of true protein is a reduced supply of AA flowing to the small intestine. It was hypothesized that forage intake will be greater when MHA is added to a molasses-urea supplement. However, forage and total intake was greater for the supplement without MHA, intermediate for MHA, and lowest for NC. It has been suggested that MHA is not ruminally inert, and it can affect ruminal microbial population (Patterson and Kung, 1988). High levels of MHA supplementation (70 g/d) reduced total DMI of dairy cattle (Griel et al., 1968; Satter et al., 1975; Higginbotham et al., 1987). Reduced DMI has also been observed with direct infusion of MHA and Met into the rumen and the abomasum (Satter et al., 1975). Such effects of abomasal infusion and the lack of effects on OM digestibility by MHA in the present study suggest that the cause of reduced DMI was not due to changes in palatability or rumen function. Also, decreased feed intake has been reported with high levels of Met in chicks (Katz and Baker, 1975) and rats (Peng et al., 1973). It was suggested that a mechanism is initiated that causes a response from a feed intake-regulating center and that, when the proportions of amino acids in the diet deviate substantially from the proportions of the amino acid requirement, feed intake decreases and rejection of feed occurs. The reason for smaller reduction in forage intake in the present study compared with previous research includes diet quality and MHA supplementation level. Less microbial protein was synthesized with the lower-quality forage offered in the current study as compared with the diets offered to dairy cattle. Also, only 15 g/d of MHA were supplemented herein as compared with 70 g/d of the dairy cattle diets (Griel et al., 1968; Satter et al., 1975; Higginbotham et al., 1987). Therefore, the amount of Met reaching the small intestine for absorption was less in the present study, and, if the Met reaching the small intestine exceeded the amount required for an optimal amino acid balance, it exceeded at a less degree than that of the dairy cattle diets previously mentioned.

Adding fat to the molasses-urea-based supplement did not affect forage intake of steers consuming low-quality forage. In a review of literature, Hess et al. (2008) concluded that when the goal is to maximize use of forage-based diets less than 3% of DM is the optimal inclusion rate for supplemental fat. When the goal is to

Table 5. Effects of liquid supplements containing methionine hydroxy analog (MHA) and (or) fat on AA duodenal flow and serum concentration in steers consuming low-quality forage

	Supplemental treatment[1]						Contrast OSL[2]			
		−Fat		+Fat						
Item	NC	U	UM	UF	UFM	SE[3]	1	2	3	4
Duodenal flow of AA, g/d										
Total	211.8	293.1	260.7	266.7	267.6	23.0	0.02	0.45	0.64	0.43
Essential	87.4	128.7	113.1	116.3	116.8	9.4	0.01	0.38	0.61	0.36
Nonessential	124.4	164.4	147.6	150.4	150.8	13.7	0.05	0.51	0.66	0.49
Methionine	3.45	5.49	4.89	5.17	5.15	0.41	0.01	0.41	0.93	0.44
Serum concentration of AA, μM										
Total	2770	2038	2163	2315	2050	147	0.01	0.60	0.54	0.16
Essential	1271	1125	1216	1108	1058	80	0.08	0.24	0.79	0.34
Nonessential	1499	912	1054	1099	992	82	0.01	0.81	0.41	0.12
Methionine	58.3	49.8	48.0	55.5	48.5	5.1	0.13	0.65	0.50	0.58

[1]NC = negative control, U = molasses-urea, UM = U plus 1.65% methionine hydroxy analog (MHA), UF = U plus 12% yellow grease (fat), and UMF = U plus 1.65% MHA and 12% fat.

[2]Observed significance level for contrasts: 1) = supplement vs. no supplement; 2) = supplements containing MHA vs. supplements containing no MHA; 3) = supplements containing fat vs. supplements containing no fat; and 4) = interaction of MHA and fat.

[3]Standard error of least squares means.

prevent substitution of forage consumption with intake of supplemental fat the optimum fat supplementation rate is 2% of DMI or less. When fat is supplemented to increase dietary DE no more than 4% of DMI should be supplemented. The proportion of supplemental fat in the present experiment was 2.95% to 3.25% of DMI. Therefore, in agreement with previously reported information (Hess et al., 2008), fat supplementation increased dietary DE without negatively affecting forage intake.

Nitrogen Flow to the Duodenum and Microbial Efficiency

Supplementation of molasses-urea-based supplements to steers consuming low-quality roughage increased total and microbial N compared with NC steers. The increased total N flowing to the duodenum was a result of the increase of microbial N flowing to the duodenum for the molasses-urea-supplemented steers compared with the NC steers. That is evident because NANMN flowing to the duodenum was not affected by supplementation. The synthesis of microbial protein depends on OM availability for fermentation and concentration of N-containing compounds in the rumen (Hespell, 1979). The CP content of the hay used in the present study was 3.3%, and the ammonia concentration for the NC steers was 1.42 mg/dL. Also, the suggested ruminal ammonia concentration for optimal microbial synthesis is 5 to 6 mg/dL (Satter and Slyter, 1974). Moreover, total digestible OM intake by steers receiving supplemental urea was greater than for steers consuming hay only. Therefore, an increase in microbial N was expected for steers receiving molasses-urea supplements. Microbial efficiency was proportional to total digestible OM intake, therefore not affected by treatments. The prediction equations (NRC, 1996) hypothesized that the most accurate predictor of microbial synthesis most likely is carbohydrate digestion in the rumen, which agrees with the observations in the current experiment.

Molasses-urea supplements containing MHA failed to improve N flowing to duodenum. Although, NPN supplementation have been observed to improve intake and digestibility of cattle grazing low-quality forage, performance has generally been poorer than when true protein supplements were fed (Chizzotti et al., 2008; Wickersham et al., 2009). We hypothesized that the difference is likely due to a limited flow of AA duodenum with NPN compared with true protein sources of supplemental N. However, in the present study total and microbial N and NANMN were not affected when MHA was added to a molasses-urea supplement.

Supplementation of fat did not affect total N flowing to the duodenum, although the flow of microbial N decreased and of NANMN increased. As a result, microbial efficiency decreased. The reason for the effects of fat supplementation on duodenal flow on microbial N and NANMN are uncertain. Because fat supplementation did not affect OM intake, ruminal OM digestibility, or in situ OM digestibility, effects on microbial N and NANMN flow to duodenum and microbial efficiency were not expected.

In Situ Forage OM, NDF, and N, Lag Time, Rate, and Extent of Digestion

Although in situ lag time and rate of OM, NDF, and N digestibility were not affected by urea, MHA or fat supplementation, extent of OM and NDF digestion at 96 h

Table 6. Effects of liquid supplements containing methionine hydroxy analog (MHA) and (or) fat on ruminal pH, ammonia concentration, and VFA concentration in steers consuming low-quality forage

	Supplemental treatment[1]						Contrast OSL[2]			
		−Fat		+Fat						
Item	NC	U	UM	UF	UFM	SE[3]	1	2	3	4
pH	6.73	6.37	6.49	6.30	6.46	0.06	0.01	0.01	0.28	0.70
Ammonia, mg/dL [4]	1.42	13.96	15.66	14.47	13.50	2.00	0.01	0.84	0.47	0.65
Total VFA, m*M*	67.8	121.5	103.0	111.0	97.8	11.2	0.01	0.14	0.44	0.79
VFA, mol/100 mol										
Acetate [4]	73.66	73.80	73.41	71.86	72.73	0.99	0.47	0.78	0.17	0.48
Propionate	17.42	17.77	19.68	17.74	18.89	0.58	0.07	0.01	0.44	0.47
Butyrate [4]	6.05	7.02	7.30	6.58	6.78	0.45	0.07	0.55	0.27	0.93
Isobutyrate	1.60	0.58	0.71	0.40	0.70	0.25	0.01	0.25	0.67	0.70
Valerate	0.73	0.54	0.68	0.62	0.67	0.09	0.28	0.32	0.74	0.54
Isovalerate	1.30	0.49	0.60	0.67	0.77	0.16	0.01	0.47	0.22	0.96
Acetate:propionate ratio	4.22	4.17	4.16	3.72	3.84	0.16	0.13	0.74	0.02	0.64

[1]NC = negative control, U = molasses-urea, UM = U plus 1.65% methionine hydroxy analog (MHA), UF = U plus 12% yellow grease (fat), and UMF = U plus 1.65% MHA and 12% fat.

[2]Observed significance level for contrasts: 1) = supplement vs. no supplement; 2) = supplements containing MHA vs. supplements containing no MHA; 3) = supplements containing fat vs. supplements containing no fat; and 4) = interaction of MHA and fat.

[3]Standard error of least squares means.

[4]Treatment × time interaction ($P < 0.05$).

of incubation increased with molasses-urea supplementation. This is in agreement with the increased OM intake and total digestible OM intake observed in the present study. Previous research has shown that that low-quality forage deficient in protein respond by increasing OM intake and digestibility (Wickersham et al., 2008; Sawyer et al., 2012; Cappellozza et al., 2014). That response and the increase of OM intake and total digestible OM intake observed in the present study can be explained by the increase in in situ OM and NDF digestibility after 96 h of incubation observed in the present study. The lack of effect of molasses-urea supplement on characteristics of N in situ digestibility are in agreement with its lack of effects on NANMN flow to the small intestine.

Adding MHA to the molasses-urea supplement had no effects on in situ nutrient digestibility of steers consuming low-quality forage. Therefore, the decrease of intake observed in the present study with MHA supplementation was not caused by negatively affecting microbial population or rumen function.

In agreement with in vivo results, fat supplementation did not affect the characteristics of in situ nutrient digestibility of steers consuming low-quality hay. Therefore, fat supplementation successfully increased energy density of the diet without negatively affecting the characteristics of digestion. It has been previously reported the excess fat supplementation can inhibit fibrolytic bacteria (Palmquist, 1988). Also, a lipid barrier that physically impedes enzyme penetration and adhesion to fiber particles can be formed and depress fiber digestion with fat supplementation (MacLeod and Buchanan-Smith, 1972). In the present study, fat supplementation was calculated to be between 2.95% to 3.25% (DM basis) of intake and no effects of fat supplementation on characteristics of in situ digestibility were observed.

Duodenal Flow of AA and Serum Concentrations

Duodenal flow of total, EAA, and NEAA and Met increased with molasses-urea supplementation. Such increases were caused by the increase in total N flow to the duodenum. As previously discussed, total N flow to the duodenum increased as a result of increased microbial protein synthesis observed for the molasses-urea supplementation. Duodenal flow of AA was not altered with MHA or fat supplementation, which is in close agreement with the lack of MHA and fat supplementation effects on duodenal total and microbial flow. Therefore, the potential limitation of NPN-based supplements of decreased supply of metabolizable AA flowing to the small intestine as compared with supplementation of true protein was not overcame with adding MHA to molasses-urea supplements. The flow of Met to duodenum was expected to increase with MHA supplementation. Deficiency of the EAA Met often has been reported to limit cattle growth (Richardson and Hatfield, 1978; Campbell et al., 1997; Greenwood and Titgemeyer, 2000). Cattle growth restriction might be the result of inefficient use of dietary protein for protein deposition when Met is deficient because Met is a precursor of protein synthesis. Besides protein synthesis, Met has many functions in the body that include the synthesis of polyamines, methylation of phospholipids, proteins, nucleic acids, and many other molecules (Lobley, 1992).

Table 7. Serum cholesterol and NEFA concentrations in steers consuming low-quality forage and supplemented with liquid supplements containing methionine hydroxy analog (MHA) and (or) fat

	Supplemental treatment [1]									
		−Fat		+Fat			Contrast OSL[2]			
Item	NC	U	UM	UF	UFM	SE [3]	1	2	3	4
4 h post supplementation										
Cholesterol, mg/dL	131.4	129.2	123.8	181.9	153.5	13.0	0.23	0.17	0.01	0.34
NEFA, meq/L	0.62	0.60	0.46	0.48	0.48	0.13	0.36	0.57	0.66	0.59
23 h post supplementation										
Cholesterol, mg/dL	114.2	114.7	143.3	170.4	162.7	13.4	0.01	0.34	0.01	0.11
NEFA, meq/L	0.57	0.06	0.24	0.39	0.38	0.14	0.02	0.42	0.05	0.40

[1]NC = negative control, U = molasses-urea, UM = U plus 1.65% methionine hydroxy analog (MHA), UF = U plus 12% yellow grease (fat), and UMF = U plus 1.65% MHA and 12% fat.

[2]Observed significance level for contrasts: 1) = supplement vs. no supplement; 2) = supplements containing MHA vs. supplements containing no MHA; 3) = supplements containing fat vs. supplements containing no fat; and 4) = interaction of MHA and fat.

[3]Standard error of least squares means.

Serum concentrations of total AA and NEAA decreased and EAA tended to decrease with molasses-urea supplementation. The decrease of serum AA probably reflect an improvement of AA utilization for tissue protein accretion. Serum Met concentration did not increase likely because Met requirements were not met (Bergen, 1979). Serum AA were not affected by MHA or fat.

Ruminal pH, Ruminal Ammonia N, Total VFA, and Molar Proportions

Ruminal VFA production increased with molasses-urea supplementation. The acid load increased with increasing ruminal VFA concentration, and as a result, ruminal pH decreased. Production of VFA increased in response to the increase in digestible OM intake with molasses-urea supplement. Ruminal ammonia concentration required for optimal microbial synthesis and/or ruminal digestibility is 5 to 6 mg/dL (Satter and Slyter, 1974). Control diets presented a ruminal ammonia concentration of 1.42 mg/dL, which makes it evident that ammonia was limiting microbial digestion in rumen. Molasses-urea supplementation successfully increased ruminal ammonia concentration. Also, ruminal pH increased for supplements with MHA as compared with those without MHA, which was due to the lower digestible OM intake observed for supplements containing MHA as compared with those without it.

Serum Cholesterol and NEFA Concentration

Serum cholesterol and NEFA were not affected 4 h postsupplementation; however, serum cholesterol increased and NEFA decreased 23 h after molasses-urea supplementation. It has been reported that serum cholesterol increases in response to fat consumption (Talavera et al., 1985). Therefore, lipid absorption might be responsible for the increase in plasma cholesterol 23 h after molasses-urea supplementation. The improved microbial synthesis observed with the molasses-urea supplementation probably improved the lipid concentration reaching the small intestine for absorption 23 h after supplementation. However, the reason for the lack of effect on serum cholesterol concentration 4 h after molasses-urea supplementation. With respect to plasma NEFA concentration, the decrease observed with molasses-urea supplementation reflect an improvement of nutritional status because plasma NEFA are released from adipose tissue by lipolysis of triglycerides in response to hormone sensitive lipase when the animal nutrition status decreases, and therefore energy mobilization is required (Annison, 1960).

Fat supplementation increased plasma cholesterol concentration at 4 and 23 h after supplementation reflecting an increase in fat absorbed. Also, plasma NEFA concentration increased 23 h after fat supplementation. Increased plasma NEFA concentrations are indicative of lipid mobilization of cows in negative energy balance (Richards et al., 1989; Staples et al., 1990; Bossis et al., 1999). However, in the current experiment, it is assumed that cows supplemented with fat were in greater energy balance because fat supplementation improved caloric intake without negative effects on DMI or digestion. Greater plasma NEFA concentrations had been reported in beef (Vizcarra et al., 1998; Lake et al., 2006) and dairy cows (Busato et al., 2002) maintained in optimal BCS during early lactation compared with cows in suboptimal BCS. It was hypothesized that the greater circulating NEFA reflected greater nutrient mobilization and energy availability for milk production. The reason for the increased concentration of NEFA in fat-supplemented cows in the present study is not certain. It may be indicative of increased absorbed FFA that were not esterified to triglycerides after absorption at the small intestine.

In summary, forage intake and characteristics of digestion were improved with urea on molasses-based liquid supplement. However, the inclusion of urea and MHA on molasses-based liquid supplements for cattle consuming low-quality forage had no additive effects on forage intake and characteristics of digestion. While adding fat to molasses-urea liquid supplement successfully improved caloric intake without negatively affecting forage intake and characteristics of digestion of cattle consuming low-quality forage.

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