Supplemental methionine and urea for gestating beef cows consuming low quality forage diets[1]

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ABSTRACT: A study was conducted to evaluate Met requirements of late-gestation beef cows consuming low quality forages on the premise that inadequate supply of metabolizable AA may limit protein accretion during pregnancy. Five ruminally cannulated, multiparous late-gestation beef cows (490 ± 27 kg), of predominantly Angus (≥75%) with Hereford and Simmental breeding, were used in a 5 × 5 Latin square experiment to evaluate the effects of postruminal DL-Met supplementation on N retention, serum metabolites, and plasma AA concentrations during the third trimester of pregnancy. The basal diet was fed individually, and weights of refusals were recorded for N intake determination. Treatments consisted of no urea, urea (0.053 ± 0.002 g/kg of BW daily), urea + 5 g of Met/d, urea + 10 g of Met/d, and urea + 15 g of Met/d. Cows were adapted to the experimental diet 30 d before the beginning of the study, with periods lasting for 14 d; 4 d to allow for clearance of the previous treatment effects, 4 d for adaptation to the treatments, and 6 d for total fecal and urine collection. Blood samples were collected every 4 h on d 13 of each period for analysis of serum metabolites and plasma AA. Inclusion of urea increased DM and OM intakes (urea vs. no urea; $P = 0.05$), but no further improvement in intake was observed with inclusion of Met. Serum urea concentrations increased with inclusion of urea ($P = 0.03$) and responded quadratically ($P = 0.06$) when Met was added, with the lowest concentration observed in the urea + 5 g of Met/d treatment. More N was retained with the inclusion of urea ($P = 0.04$), and N retention increased linearly ($P = 0.07$) with inclusion of Met. Plasma Met concentration increased linearly ($P < 0.01$) with inclusion of Met. These data suggest that Met was a limiting AA and that supplementation of a combination of urea and 5 g/d of rumen-protected Met to low quality, forage diets will improve N retention and promote protein accretion during late pregnancy.

Key words: beef cow, gestation, methionine, nitrogen retention, plasma amino acid

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INTRODUCTION

In the southwestern United States, range livestock graze senescent forages nearly 40 wk/yr, and the nutritional quality of the range forage during that time is less than optimal to meet livestock production demands. Certain limiting nutrients, such as energy, ruminally available N, and AA may contribute to the less than desired animal performance (Titgemeyer and Löest, 2001).

Metabolizable protein may limit protein accretion in gestating beef cows that consume senescent (<7% CP) forages (Patterson et al., 2003). The combination of ruminally undegradable protein and microbial protein contributing to MP may not eliminate a specific AA deficiency if the ruminally undegradable protein is deficient in the first limiting AA. Therefore, providing a postruminal supply of a specific limiting AA may help improve the efficiency of N usage for fetal development and maternal metabolism.

The first limiting AA is often Met, especially when rumen microbial protein is the predominant source of AA supplied to the small intestine (Richardson and Hatfield, 1978; Rulquin and Delaby, 1997; Greenwood and Titgemeyer, 2000). Bach et al. (2000) showed that preparturient, multiparous Holstein cows have increased requirements for Met during late gestation and that a minimum of 14 g/d of metabolizable Met is required to achieve a positive net splanchnic flux of Met.

The objectives for this study were to evaluate N retention and plasma AA concentrations in response to ruminally supplemented urea and postruminally delivered DL-Met in late gestation beef cows consuming a diet similar to southwestern senescent forages. The hypoth-

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Table 1. Feed ingredient and diet composition for experimental diets fed to beef cows

Item	
Ingredient, % of DM	
Wheat straw	67.0
Alfalfa hay	32.6
Urea	0.4
Nutrient	
Wheat straw	
OM, % of DM	87.7
CP, % of OM	1.9
NDF, % of OM	78.7
Alfalfa hay	
OM, % of DM	82.1
CP, % of OM	17.0
NDF, % of OM	43.2

esis was that postruminal supplementation of Met would improve protein accretion of gestating beef cows.

MATERIALS AND METHODS

The New Mexico State University Institutional Animal Care and Use Committee approved the procedures and use of animals for this study. Five ruminally cannulated, multiparous, late-gestation beef cows (84 ± 10 d prepartum; 490 ± 27 kg of initial BW), that were predominantly Angus (≥75%) with Hereford and Simmental breeding, were used in a metabolism study to evaluate the effects of postruminal infusion of DL-Met on N balance and plasma AA concentrations. Cows were placed in individual pens (8 × 38 m) with ad libitum access to water, mineralized salt (11.5% Ca; 8% P; 4% Mg; 4% K; 2,000 ppm Cu; 4,000 ppm Zn; 2,500 ppm Mn; 13 ppm Se; and 264,550 IU/kg of vitamin A), and the basal diet (Table 1). The basal diet was fed once daily at 0600, after removal and weighing of the orts.

To stabilize intake, cows were adapted to their basal diet for 30 d before the initiation of the experiment. Cows were then randomly assigned to treatment within a 5 × 5 Latin square. Cows were diagnosed for pregnancy via rectal palpation and determined to be approaching their last third of gestation. Experimental periods encompassed 14 d: 4 d to allow for clearance of the previous treatment effects, 4 d for adaptation to the treatments, and 6 d for total fecal and urine collection.

Experimental treatments consisted of no urea (**NU**), urea (**U**; 0.053 ± 0.002 g/kg of BW daily), U + 5 g of Met/d (**5MU**), U + 10 g of Met/d (**10MU**), and U + 15 g of Met/d (**15MU**). Urea was administered into the rumen via the rumen cannula in 2 gelatin capsules (#Su07, Torpac Inc., Fairfield, NJ) daily at 0800 (2 h after the basal diet was offered) to establish a rumen environment adequate in ruminally degradable protein. The first urea-containing gelatin capsule was placed under the forage mat at the interface with the liquid fraction of the ruminal contents, whereas the second urea gelatin capsule was placed on top to the forage mat to encourage slower release of urea into the rumen. We assumed that microbial efficiency would be 100 g of microbial CP/kg of TDN (Lardy et al., 2004), and urea inclusion increased CP concentration of the diet from 6.9 to 7.9% CP (OM basis).

Abomasal infusions for each treatment were prepared by dissolving DL-Met (99% DL-Met, Degussa-Hüls, Theodore, AL) into 500 mL of water containing 1 mL of 12 *M* HCl and mixed on a warm stir plate for 12 h before infusion. A 250-mL aliquot of water (NU and U) or of a solution containing Met (5MU, 10MU, and 15MU) was infused into the abomasum twice daily at 0600 (immediately after feeding) and at 1800. Solutions were infused into the abomasum by placing flexible plastic tubing (Tygon; 4.8 mm i.d. × 7.9 mm o.d.) through the rumen cannula and circumventing the rumen and omasum via the reticulo-omasal orifice for direct delivery into the abomasum. A rubber flange (11-cm diam.) was attached to the abomasal end of the tubing to ensure that the infusion line would remain secure in the abomasum (Spires et al., 1975; Löest et al., 2001).

On d 9, cows were fitted with fecal bags containing plastic liners for complete collection of feces and urine. On removal of the fecal bags (daily at 3 h after feeding during the collection period), excreta (i.e., feces and urine) were immediately transferred into 40-L plastic containers, at which time the excreta weight was recorded by subtracting the container weight from the overall weight. Homogenous slurries then created by mixing the feces and urine together for 3 min using a large paint paddle attached to an electric drill. After mixing, 5% aliquots were collected, composited by period, and frozen (–20°C). Frozen excreta samples were thawed and mixed thoroughly using a handheld mixer before analysis for Kjeldahl N (AOAC, 1990).

Serum and plasma samples were collected via coccygeal venipuncture [7-mL tubes for serum; 7-mL tubes with EDTA for plasma (Corvac, Sherwood Medical, St. Louis, MO)] every 4 h for 24 h on d 13 of each period beginning 2 h after treatment infusion into the abomasum. Serum samples were allowed to coagulate for 30 min at 20 to 22°C, and plasma samples were inverted for complete mixture with EDTA and placed on ice before being transported to the laboratory for centrifugation at 1,500 × *g* for 25 min.

Serum samples were analyzed for the following metabolites: serum urea via the urease method (Sigma Procedure No. 315, Sigma Diagnostics, St. Louis, MO), glucose via the glucose oxidase method (Sigma Procedure No. 66-UV, Sigma Diagnostics), and NEFA (NEFA-C, Wako Chemicals US Inc., Richmond, VA) using commercially available diagnostic kits adapted to 96-well plates for using a BioTek Synergy HT microplate reader. Insulin was measured using commercially available reagents for RIA as outlined by Reimers et al. (1982), with an intraassay CV of 8.5% and 100% recovery. Serum concentrations of IGF-I were determined by RIA (Berrie et al., 1995), with an intraassay

CV of 8%. The RIA analyses were conducted in the New Mexico State University endocrinology laboratory overseen by D. M. Hallford. Plasma samples at each sampling time were analyzed for AA by capillary GLC after procedures described by Chen et al. (2002). Arginine, an essential AA, was undetectable in this study using the GLC procedures.

Nitrogen excretion was not measured for 1 cow during periods 4 and 5 due to observed signs of parturition in period 4 (the fecal bag was removed) and subsequent calving during period 5. Nevertheless, rumen and abomasal treatment administration continued, and serum and plasma samples were collected from this cow.

Statistical Analysis

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC), with the effects of animal, period, treatment, time, and treatment × time (when appropriate), with animal and period in the RANDOM statement and time in the REPEATED statement. Animal (period × treatment) was the subject, and compound symmetry was the covariance structure. No treatment × time interactions were noted ($P = 0.12$ to 0.96); therefore only main effects for treatment are presented. Single degree of freedom, preplanned contrasts were used to determine the effects of additional urea and of incremental concentrations of abomasally infused Met when urea was provided.

RESULTS AND DISCUSSION

Absorption of the supplemental Met from the small intestine was not measured in our study, but Campbell et al. (1996) concluded that D- and L-Met are absorbed and used equally in support of N retention, and this was the basis on which DL-Met was used.

Dry matter and OM intakes (Table 2) were increased ($P = 0.05$) with the dosing of U, but no subsequent increases were observed with the inclusion of Met ($P \geq 0.41$). By design, dietary and total intake N increased ($P < 0.01$) in response to dosing the urea to the rumen (Table 2). However, postruminal infusion of Met did not supply sufficient N to alter dietary and total N intake ($P > 0.61$). Fecal plus urinary N excretion was not affected ($P = 0.29$) by urea supplementation but decreased linearly ($P = 0.06$) in response to Met infusion. These data indicated that a bolus dose of urea alone did not influence N excretion, but inclusion of Met can influence the amount of N excreted (Table 2).

Nitrogen retention was improved with the addition of urea ($P = 0.04$; NU vs. U) and increased linearly ($P = 0.07$) with the addition of Met for gestating cows consuming a low- quality forage diet (Table 2). The addition of urea improved N retention and demonstrates the importance of satisfying ruminal N requirements before postruminal effects can be considered (Titgemeyer and Loest, 2001). Providing readily available N (i.e., urea) to the rumen environment during periods when N is a limiting factor will enhance the synthesis of microbial protein. Nitrogen retention was improved by 33, 53, 55, and 62% for cows receiving U, 5MU, 10MU, and 15MU, respectively, compared with NU cows. The greatest improvement in N retention in response to Met was observed with the initial 5 g/d increment of Met, with only small benefits from greater amounts of infused Met. These results demonstrated that the requirement for Met in late-gestating beef cows was not satisfied with urea supplementation alone but likely was met with postruminal infusion of 5 g of Met/d. The present results concur with previous research demonstrating a reduction in N excreta when the postruminal supply of a limiting AA is increased (Richardson and Hatfield, 1978; Titgemeyer and Merchen, 1990; Campbell et al., 1997).

Serrato-Corona et al. (1997) demonstrated that acidification of the fecal-urine material did not change N content but may alter collection bag integrity, so we did not acidify excreta during the collection period. However, without acidification of fecal-urine material, the possibility of ammonia loss is apparent and will likely result in an overestimation of N balance, although treatment differences should remain legitimate.

When lactating or gestating cows are in a positive N balance (i.e., consuming more N than excreting), one would conclude that proteinaceous tissue is being deposited as maternal or fetal tissue. With late gestating beef cows, maternal anabolism is not likely to occur, especially when cows consume senescent southwestern forages, so we hypothesize that the bulk of protein accretion was within the gravid uterus. Battaglia (1992) suggested that the umbilical uptake of essential AA solely reflects the exogenous maternal supply to the fetus, whereas nonessential AA supply may be derived from transplacental transport or production within the placenta. Furthermore, most AA enter fetal circulation in excess of their net rates of excretion (Lemons and Schreiner, 1983; Bell et al., 1989; Marconi et al., 1989). The NRC (2000) equation 4–10 reveals that the demand for N (AA) accretion by the gravid uterus increases exponentially during the last trimester of pregnancy. This demonstrates the potential for maternal partitioning of essential dietary AA toward the needs of the gravid uterus (i.e., placenta and fetus).

To describe the overall nutritional status of late-gestating beef cows, certain serum metabolites were monitored. A common serum metabolite measured to describe N status and potential recycling of N to the rumen for microbial protein synthesis is serum urea, with optimal concentrations ranging from 3.6 to 4.3 m*M* (Hammond et al., 1993; Stateler et al., 1995). Serum urea concentrations in our study ranged from 3.6 to 4.7 m*M*, with the greatest change associated with the inclusion of urea ($P = 0.03$; NU vs. U) into the rumen (Table 3).

An increase in serum urea when urea was administered into the rumen may demonstrate increased absorption of ruminal ammonia. This may suggest that

Table 2. Effects of ruminal urea and postruminal DL-Met on N retention of gestating, multiparous beef cows

	Treatment[1]						Contrast, *P*-value			
Item	NU	U	5MU	10MU	15MU	SEM	NU vs. U	Linear	Quadratic	Cubic
No. of cows	4	5	5	5	4	—	—	—	—	—
DMI, kg/d	5.8	6.1	6.1	6.0	6.1	0.4	0.05	0.66	0.34	0.66
OM intake, kg/d	5.4	5.6	5.5	5.5	5.6	0.3	0.05	0.64	0.34	0.64
Nitrogen, g/d										
Dietary	58.3	72.1	70.5	71.1	70.3	3.4	<0.01	0.37	0.68	0.45
Infused	0.0	0.0	0.5	0.9	1.4	—	—	—	—	—
Total intake	58.3	72.1	70.9	72.0	71.7	3.4	<0.01	0.98	0.68	0.45
Fecal plus urinary	27.5	30.5	22.8	23.3	21.3	5.0	0.48	0.06	0.32	0.39
Retained	31.4	41.6	48.1	48.8	50.9	6.5	0.04	0.07	0.49	0.59
Efficiency,[2] %	51.3	57.7	67.4	67.2	69.5	7.3	0.25	0.06	0.32	0.44

[1]Treatments: no urea (NU), urea (U; 0.053 ± 0.002 g/kg of BW daily), U + 5 g of Met/d (5MU), U + 10 g of Met/d (10MU), and U + 15 g of Met/d (15MU).
[2]N Efficiency = (Retained/total intake) × 100.

not only was more N available for microbial protein synthesis, but some ammonia may have been absorbed from the rumen and converted to urea in the liver. In addition, an increase in serum urea when urea was administered may be due to increased absorption of microbial N from the small intestine. No differences in fecal plus urinary N excretion were observed between NU and U ($P = 0.48$), but N retention improved ($P = 0.04$) for U vs. NU indicating that the provision of N to the rumen improved microbial production and postruminal supply of microbial protein or availability of energy from enhanced ruminal fermentation. Typically, decreases in serum urea concentration in response to supply of a limiting AA (e.g., Met) would suggest a decrease in AA deamination by the liver due to increased uptake of all AA by peripheral tissues (Campbell et al., 1997). Although our serum urea concentrations and N retention data support this concept, plasma AA data (Table 4) failed to reveal any decreases in other AA that would have been expected for this claim to be true. Additionally, a quadratic effect ($P = 0.06$) for serum urea was observed indicating that postruminal inclusion of Met lowered serum urea concentrations. These findings agree with the observed changes in N retention, which showed the greatest improvement with inclusion of urea and 5 g of Met/d.

Glucose concentrations averaged 3.2 m*M* with no significant differences ($P > 0.54$) among treatments (Table 3). Kaneko (1989) states normal glucose concentrations range from 2.5 to 4.2 m*M*. The lower glucose concentrations in this range most likely represent cows consuming diets similar to the diet fed in the current study. Furthermore, glucose concentrations in our study are similar to concentrations observed for cows grazing senescent native range forages (Appeddu-Richards, 1999; Sawyer, 2000; Obeidat et al., 2002).

An additional measure used to evaluate nutrient status was serum NEFA concentrations. Although, no differences ($P \geq 0.42$) were observed in NEFA concentrations in response to U or Met inclusion, elevated NEFA concentrations associated with low insulin concentrations during the periparturient period have been associated with metabolic disorders (Cameron et al., 1998; Drackley, 1999). Furthermore, Regnault et al. (2004) indicates that maternal insulin concentrations fall and NEFA concentrations increase as gestation advances in the ovine.

Insulin-like growth factor-I concentrations were not influenced by the inclusion of urea ($P = 0.33$), but responded in a quadratic fashion in response to abomasal infusion of Met ($P = 0.01$). The quadratic nature of the IGF-I response resulted from an initial 26% decrease

Table 3. Effects of ruminal urea and postruminal DL-Met on serum urea, glucose, NEFA, insulin, and IGF-I of gestating, multiparous beef cows

	Treatment[1]						Contrast, *P*-value			
Item	NU	U	5MU	10MU	15MU	SEM	NU vs. U	Linear	Quadratic	Cubic
No. of cows	5	5	5	5	5	—	—	—	—	—
Serum urea, m*M*	3.6	4.7	3.8	4.0	4.3	0.5	0.03	0.59	0.06	0.52
Glucose, m*M*	3.1	3.2	3.2	3.1	3.2	0.2	0.54	0.97	0.56	0.93
NEFA, μmol/L	422	400	457	392	398	64	0.78	0.76	0.63	0.42
Insulin, ng/mL	1.9	1.7	1.6	1.7	1.9	0.3	0.42	0.30	0.31	0.85
IGF-I, ng/mL	58.1	50.6	37.5	46.3	63.6	7.1	0.33	0.05	0.01	0.55

[1]Treatments: no urea (NU), urea (U; 0.053 ± 0.002 g/kg of BW daily), U + 5 g of Met/d (5MU), U + 10 g of Met/d (10MU), and U + 15 g of Met/d (15MU).

Table 4. Effects of ruminal urea and postruminal DL-Met on plasma AA concentrations of gestating, multiparous beef cows

	Treatment[1]						Contrast, *P*-value			
Item	NU	U	5MU	10MU	15MU	SEM	NU vs. U	Linear	Quadratic	Cubic
No. of cows	5	5	5	5	5	—	—	—	—	—
Amino acid, μM										
Ala	247.2	237.5	234.1	233.2	226.9	13.7	0.33	0.29	0.83	0.80
Asn	101.8	78.1	78.3	82.1	82.1	17.9	0.19	0.77	0.99	0.89
Asp	9.5	9.4	10.1	8.6	10.2	1.2	0.95	0.76	0.60	0.18
Cys	13.6	13.3	14.3	13.5	14.0	0.9	0.73	0.60	0.65	0.27
Glu	86.8	87.7	89.9	82.8	85.5	10.0	0.91	0.61	0.97	0.48
Gln	154.2	151.2	151.9	139.3	153.5	15.3	0.82	0.89	0.48	0.35
Gly	231.9	214.3	209.0	211.2	209.2	17.4	0.14	0.71	0.84	0.75
His	97.3	107.0	112.1	112.2	103.8	11.3	0.17	0.66	0.17	0.87
OH-Pro	26.2	24.7	26.4	24.7	23.8	2.0	0.48	0.48	0.35	0.51
Ile	132.0	128.6	127.1	131.0	116.2	7.0	0.56	0.07	0.11	0.18
Leu	123.4	124.6	121.4	124.3	117.7	8.9	0.88	0.48	0.76	0.54
Lys	131.8	134.5	139.8	127.7	124.0	14.0	0.76	0.12	0.46	0.34
Met	32.7	30.3	43.2	63.0	93.2	4.9	0.71	<0.01	0.06	0.86
Orn	87.6	90.6	93.2	87.5	91.1	6.0	0.63	0.82	0.91	0.38
Phe	55.4	56.4	57.3	56.5	56.4	4.6	0.79	0.94	0.87	0.84
Pro	87.2	87.0	83.3	89.6	80.1	5.5	0.98	0.42	0.46	0.15
Ser	58.8	53.8	55.8	50.8	59.4	4.6	0.31	0.37	0.21	0.33
Thr	100.9	64.6	99.7	106.3	97.9	9.7	0.33	0.40	0.14	0.40
Trp	38.2	39.4	36.8	40.7	37.7	4.5	0.54	0.85	0.88	0.05
Tyr	46.9	49.7	48.8	49.9	49.3	2.3	0.32	0.99	0.94	0.69
Val	221.3	218.7	210.6	223.3	215.3	13.4	0.82	0.94	0.99	0.26
Essential[2]	928	934	948	985	966	64	0.89	0.35	0.61	0.57
Nonessential[3]	1,151	1,097	1,093	1,073	1,085	45	0.30	0.72	0.82	0.72
Glucogenic[4]	2,547	2,500	2,521	2,533	2,501	130	0.66	0.97	0.72	0.91
Ketogenic[5]	257	259	261	252	247	26	0.86	0.34	0.74	0.73

[1]Treatments: no urea (NU), urea (U; 0.053 ± 0.002 g/kg BW daily), U + 5 g of Met/d (5MU), U + 10 g of Met/d (10MU), and U + 15 g of Met/d (15MU).
[2]Essential AA = His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val.
[3]Nonessential AA = Ala, Asn, Asp, Cys, Gln, Glu, Gly, OH-Pro, Pro, Orn, Ser, and Tyr.
[4]Glucogenic AA = Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, OH-Pro, Met, Pro, Ser, and Val.
[5]Ketogentic AA = Leu and Lys.

in IGF-I concentrations with abomasal infusion of 5 g of Met/d followed by substantial incremental increases in IGF-I with the additional increments of Met. Hess et al. (1998) also observed incremental increases in IGF-I prior to partition for prepartum cows receiving protein supplements with and without rumen-protected Met.

Changes in plasma AA concentrations in response to changes in diet concentrations are often used to define individual AA requirements and may be used to interpret or identify other limiting AA. Interestingly, plasma Ile concentration declined linearly ($P < 0.07$) with incremental increases of Met. However, it is unclear if the decline in Ile implies the Ile was a limiting AA or just being utilized more efficiently once Met was prevalent. Furthermore, there was a tendency ($P = 0.12$) for Lys to decline linearly as Met was supplemented. Plasma concentrations of total essential, nonessential, glucogenic, and ketogenic AA were not affected by treatment ($P > 0.30$; Table 4).

Plasma Met concentrations were not influenced by inclusion of urea ($P = 0.84$). However, plasma Met concentration increased linearly ($P < 0.01$) in response to Met supplementation (Table 4). Bergen (1979) indicated that plasma AA concentrations will remain low or static when below the requirement but will accumulate once the requirement is reached. However, this is likely only true for naturally occurring L-AA that are metabolized rapidly. The D-form of Met used in this study may have been metabolized at a slower rate (Campbell et al., 1997), which may be the cause of the observed increase in plasma concentrations of Met when 5 g/d of DL-Met was infused.

IMPLICATIONS

Supplementation of limiting nutrients during periods of nutritional stress, such as in late gestation when fetal development is occurring at an exponential rate, may minimize maternal catabolism by providing the developing fetus with essential nutrients directly from the diet. Using nitrogen retention as a basis for describing methionine requirements, we conclude that supplemental methionine requirements are 5 grams per day or less for late gestation beef cows consuming poor quality forages. Gestating beef cows consuming low-quality forages supplemented with urea were able to retain more nitrogen when methionine was supplemented. A combination of urea and 5 grams per day of rumen-protected

methionine mixed into range protein supplements may improve animal performance to levels similar to those achieved with traditional natural protein sources.

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