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ORIGINAL ARTICLE

Effects of rumen-protected methionine on plasma amino acid concentrations during a period of weight loss for late gestating beef heifers

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Abstract This study determined changes in plasma amino acid concentration in late-gestating (beginning 58 ± 1.02 days prior to calving), primiparous, winter-grazing range heifers receiving wheat middling-based supplement without (CON) or with rumen-protected methionine (MET) to provide 15 g DL-MET each day. Plasma was collected on days −2 and 0 (start of MET supplementation just prior to individually receiving supplement at 0700 hours). Plasma was sampled again on days 40, 42 and 44 prior to supplementation at 0700 and 1100 hours (4 h after receiving daily supplement). Data were analyzed with cow as the experimental unit. Continuous variables were analyzed by the main effects of treatment, date, or time and their interaction when appropriate. Comparable BW ($P = 0.32$) and BCS ($P = 0.83$) over the 44-day metabolism trial were found between both CON- and MET-fed heifers. MET-supplemented heifers had greater ($P < 0.01$) plasma concentrations of methionine indicating that the rumen-protection technology successfully delivered methionine to the small intestine. Supplementation with rumen-protected DL-MET caused a significant supplement × date interaction for glutamine ($P = 0.03$), glycine ($P = 0.02$), methionine ($P < 0.01$), and serine ($P = 0.05$). In addition, trends for supplement × date interactions were detected for leucine ($P = 0.07$), threonine ($P = 0.09$), valine ($P = 0.08$), total amino acids (TAA; $P = 0.08$), non essential amino acids (NEAA; $P = 0.08$), branched chain amino acids (BCAA; $P = 0.08$), and glucogenic amino acids (GLUCO; $P = 0.08$). These results suggest that the BCAA (leucine and valine) were utilized more efficiently with MET supplemented heifers compared to CON supplemented heifers. Plasma AA concentrations for glutamic acid ($P < 0.01$), histidine ($P = 0.01$), tyrosine ($P < 0.01$), and EAA ($P < 0.01$), all decreased throughout the study. These results further confirm methionine is a limiting amino acid in forage fed late-gestating heifers and further suggests the limitation when grazing dormant range forages as shown by improved utilization of other plasma amino acids when supplemental methionine was provided.

Keywords Amino acids · Primiparous heifers · Rumen-protected methionine

Abbreviations

AA	Amino acids
BCAA	Branch-chain amino acids
BCS	Body condition score
BW	Body weight
CP	Crude protein
DM	Dry matter
DL-MET	DL-Methionine
EAA	Essential amino acids
GLUCO	Glucogenic amino acids
ISNDFD	In situ neutral detergent fiber disappearance
KETO	Ketogenic amino acids
LARRL	Livestock and range research laboratory
M85	Mepron® M85
NDF	Neutral detergent fiber
NEAA	Non essential amino acids
OM	Organic matter
TAA	Total amino acids

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Introduction

Late gestating heifers grazing Northern Great Plains rangelands often require supplementation to meet their nutritional requirements. However, the proper balance of amino acids (AA) still may not be met since AA requirements for pregnant beef heifers are not well defined. Supplement formulation is inexact, and some supplements are utilized less effectively. Research indicates that the first limiting AA is most likely methionine, especially when rumen microbial protein is the key supplier of AA to the small intestine (Richardson and Hatfield 1978; Rulquin and Delaby 1997; Greenwood and Titgemeyer 2000; Schingoethe 1996). Salter et al. (1979) indicate that methionine may be a limiting AA for growth and fermentation by ruminal microbes. However, studies that have supplemented the rumen with methionine have inconsistent outcomes. Clark and Petersen (1988) supplemented 15 g/day of DL-MET to cows consuming mature grass hay and measured improved DM disappearance, while others observed similar improvements in DM disappearance on maize silage-based diets (Huisman et al. 1988). In part, inconsistencies in response to MET supplementation in the rumen may be due to timing of supplementation in relation to carbohydrate availability from mature forages (Wiley et al. 1991; McCracken et al. 1993; Barton et al. 1992; Judkins et al. 1991). Cottle and Velle (1989) fed an unprotected form of methionine at a dose level of 15 g and reported that 4.3 g left the rumen intact and was available for absorption in the small intestine and thus represented approximately 70 % of the daily requirement. Bach et al. (2000) showed that preparturient multiparous Holstein cows have greater requirements for MET during late gestation and a positive net splanchnic flux of MET is achieved when a minimum of 14 g/day of metabolizable MET is delivered to the small intestine. Dietary proteins found in range forages and other feedstuffs can be extensively degraded in the rumen, incorporated into microbial protein, flow to the small intestine to be potentially used as metabolizable protein, which becomes the primary AA source for the host ruminant. Waterman et al. (2007b) concluded that pregnant beef cows exhibited improved nitrogen retention when consuming a mature forage based diet with added urea to minimize N deficiency in the rumen while infusing DL-methionine (DL-MET) directly into the abomasum.

The objectives of this study were to determine if plasma AA concentrations were altered in response to inclusion of rumen-protected, DL-MET into the diet of late gestation heifers grazing mixed grass prairie during winter and to identify if methionine is a prospective limiting AA in ruminants experiencing body weight loss. An additional objective was to determine if inclusion of rumen-protected, DL-MET would enhance ruminal disappearance of organic matter (OM) and neutral detergent fiber (NDF) and if the combined effects of each objectives would minimize body weight loss. By supplementing rumen-protected, DL-MET, we hypothesize that heifers will likely improve utilization of other non-limiting or less limiting AA.

Materials and methods

Experimental site

Research was conducted at the United States Department of Agriculture, Agricultural Research Service, Fort Keogh Livestock and Range Research Laboratory (LARRL) about 1.6 km West of Miles City, MT, USA (46°22′N 105°5′W). The LARRL encompasses approximately 22,500 ha and has an average elevation of 730 m. The terrain is defined by rolling hills and broken badlands that include small ephemeral intersecting streams which seasonally drain into permanent rivers meandering through broad, nearly level valleys. Figure 1 illustrates average weekly precipitation and temperature patterns for the period which the study was implemented. Data were obtained from Western Regional Climate Center, Reno, NV, USA (WRCC 2010). The actual high temperature was 2.22 °C, and the low temperature was −22.78 °C. Predominant grass genera at the study site include grama (*Bouteloua*), needlegrass (*Hesperostipa*), and wheatgrass (*Pascopyron*) within a mixed-grass dominated rangeland (Küchler 1964). The average annual forage standing crop at the study site is 870 ± 14 kg/ha (Grings et al. 2005). Heifers grazing dormant winter rangeland were stocked at a rate of 14.9 animal unit days (AUD)/ha, such that only 16 % of the available forage would be utilized. Quantity of mature forage availability during the experimental period was in excess of cattle needs, and the 75.9 ha pasture grazed by experimental heifers had not previously been grazed during the current year's primary growing season.

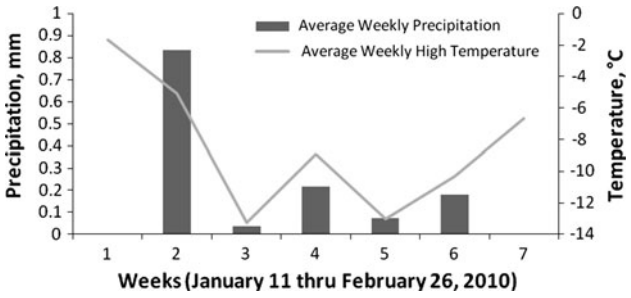

Fig. 1 Average weekly precipitation (*bars*) and high temperature (*line*) from January 11 through February 26 (weeks 1–7), 2010 for Miles City, MT. Information obtained from Western Regional Climate Center, Reno, NV, USA (WRCC 2010)

Experimental design, management and supplement

The LARRL Institutional Animal Care and Use Committee approved the procedures and use of animals for this study. The study consisted of a 44 days period from January 13 through February 26, 2010. Twenty-four late gestation heifers (BW 418 ± 6.48 kg with a BCS of 4.5 ± 0.09) of predominantly Angus (≥75 %) breeding were used to evaluate the effects of rumen-protected DL-MET (50:50 mixture of DL-MET) during late gestation.

Prior to the trial, experimental heifers were grazing native range forages along with contemporary herd mates. Heifers chosen for the present study had been previously artificially inseminated and diagnosed pregnant via rectal palpation at approximately 75 days of gestation. As heifers approached the later portion of their third gestational period (57 ± 1.02 days or a range of 49 to 66 days prior to parturition), 24 heifers were stratified by BW and randomly assigned to treatment groups (12 per experimental treatment). In addition, 4 ruminally cannulated, 2-year-old heifers, grazed alongside experimental heifers throughout the study (2 per experimental treatment). Experimental treatments were individually fed daily to provide (Table 1): (1) a 26.3 % CP wheat mid-based supplement (CON 300 g/day) and (2) CON (276.5 g/day) with 23.5 g/day of rumen-protected, DL-MET (M85; Mepron® M85, Degussa Hüls Corp., Allendale, NJ, USA 07401). Mepron® M85 is a commercial rumen-protected source of DL-MET which resists ruminal degradation by a ethylcellulose and stearic acid film coating pellets that measure 1.8 × 3 mm and contain 85 % DL-MET synthesized from DL-2-hydroxy-4-calcium methylthiobutanoic acid. Schwab (1995) reports that M85 contains 85 % DL-MET with an intestinal digestible coefficient of 90 %. The amount of rumen-protected, DL-MET targeted for this study was selected based upon previous research findings by Waterman et al. (2007b) that observed a linear response for N retention when concentrations of DL-MET were infused into the abomasum up to 15 g/day. Based on these results, M85 was supplemented at 23.5 g/day. We assumed that if M85 contained 85 % DL-MET or 19.98 g/day of DL-MET and if rumen availability was 15 %, then 16.98 g/day would be

Table 1 Grass hay nutrient composition and supplement ingredients and nutrient composition for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid-based supplement without DL-MET (CON) and with DL-MET (MET)

Item	Grass hay	Supplement	
		CON	MET
Ingredient		As fed g/hd/day (%)	
Wheat mids	–	158.6 (52.9)	145.9 (48.6)
Molasses	–	69.2 (23.1)	63.7 (21.2)
Range mineral[a]	–	57.7 (19.2)	53.1 (17.7)
Urea	–	14.4 (4.8)	13.3 (4.4)
Mepron 85	–	0.0 (0)	23.5 (7.8)
Nutrient composition[b]	% of dry matter		
Dry matter	91.5	88.7	89.4
Crude protein	11.9	26.3	29.1
Acid detergent fiber	33.8	–	–
Neutral detergent fiber	60.3	20.4	17.4
Total digestible nutrients	64.0	60.3	53.2
Sulfur	0.19	0.27	0.25
Phosphorus	0.17	2.23	2.14
Potassium	2.13	2.88	2.73
Magnesium	0.16	1.23	1.18
Calcium	0.54	2.52	2.49
Sodium	0.02	3.16	3.13
	MJ/kg		
Net energy: maintenance	5.89	2.72	2.42
Net energy: gain	3.34	1.84	1.63
	ppm		
Iron	68.0	248.6	246.9
Manganese	31.0	535.0	531.0
Copper	4.0	428.0	425.0
Zinc	18.0	856.0	850.0

[a] Range mineral contained: 11.41 % calcium, 4.10 % phosphorus, 4.10 % potassium, 0.30 % sulfur, 4.10 % magnesium, 13.49 % sodium, 2,000 ppm of copper, 4,000 ppm of zinc, 1,163 ppm of iron, 13 ppm of selenium, 2,500 ppm of manganese, 10 ppm of cobalt, 106 ppm of iodine

[b] Based on analyzed chemical composition of individual ingredients

presented to the small intestine with post-ruminal digestibility at 90 % then 15.28 g/day would be available for intestinal absorption. Mixed results have occurred when trying to describe the disappearance of M85 in both the rumen and small intestine. Other research derived values for available and post-ruminal disappearance of M85 suggest lower MET values than those used for calculations for present study (Koenig and Rode 2001; Overton et al. 1996; Berthiaume et al. 2000, 2001). Vanzant et al. (1998) report that the techniques used to estimate M85 degradability may under estimate passage due to the lack of chemotactic capabilities in the small intestine, presence of microorganisms and a decrease in mixing and compression occurring due to placement of nylon bags in the small intestine along with the effect of pore size and open surface area. Furthermore, the structural characteristics of M85 are such that the release of MET from M85 is dependent upon abrasion and physical forces for degradation. The diet consumed by heifers in this study most likely tends toward more abrasive and physical forces when compared across the spectrum of ruminant diet physical characteristics.

Heifers were gathered daily at approximately 1000 hours, sorted into a herringbone arranged individual feeding stalls where dietary treatments were offered. Heifers consumed their daily feed allotment and were promptly released back to native range pasture. After feeding, all heifers were co-mingled and managed as a single herd. Due to snow cover restricting access to available stockpiled range forage, a 500 kg (approximately 18 kg/hd) round bale of grass hay was made available on day 14 of the study to all heifers and was repeated every 4 days until the end of the study (Table 1).

Heifer BW and BCS was measured and recorded at the initiation and termination of the study. Body condition scores (1 = emaciated to 9 = extremely obese) were assigned by two experienced technicians as described by Herd and Sprott (1986). At the termination of the study, experimental heifers were relocated to two confined pens according to previously assigned supplement treatment. To determine if consumption of methionine influenced birth weight of calves, supplements were continued to be offered as a top-dressing over a corn silage-based diet from days 45 to 54. Experimental groups remained separated through calving. At calving, calf BW was measured, and cow calf pairs were moved into a pair pen where they remained until being released back onto native rangeland.

Data collection and sample analysis

Forage characteristics

Forage rumen extrusa samples were obtained from two ruminally-cannulated heifers on days −1 and 41 of the field metabolism study. Ruminally-cannulated heifers were withheld from water and fed overnight prior to each extrusa collection. To obtain diet extrusa, ruminal contents were completely expelled and stored in 208-L plastic tubs. Ruminal walls were dried with a sponge to remove any residual moisture as described by Lesperance et al. (1960). Directly after removing ruminal contents, cannulated heifers were allowed to graze alongside herdmates for approximately 45 min. After grazing, rumen extrusa was collected, and original rumen contents were returned and cows were released back onto native range pastures. Collected extrusa samples were frozen at −20 °C, lyophilized, ground to pass a 2-mm screen, and stored until analysis for dry matter (DM), organic matter (OM) as defined in AOAC (1990), and NDF following the procedures outlined by Goering and Van Soest (1970).

To assess grazed diet quality and differential responses to supplement composition, in situ diet fermentability was measured by placing, ground extrusa samples (5 g/bag) in duplicate Dacron bags (10 cm × 20 cm; pore size = 53 ± 10 μm; Ankom Technology Corp. Fairport, NY, USA). On day 47, duplicate sealed Dacron bags containing ground extrusa samples from both collection dates (days −1 and 41, representing the initiation and terminations of the study, respectively) were placed in a 60 cm × 60 cm zippered laundry bag with an attached string. Dacron bags (12 per cow) containing ground extrusa samples plus a blank bag (1 per cow/insertion time) and were placed in the rumen of each of the 4 rumen-cannulated heifers' at specific times to allow for 96, 48, 24, and 0 h of digestion. Upon removal from the rumen, all bags were subjected to an initial cold water rinse to halt fermentation. Dacron bags representing 0 h were never subjected to the rumen, but were still rinsed in a similar manner as other digestion bags. Once all bags were removed, they were returned to the laboratory and rinsed to a common end point (depicted by consistent clear effluent) under cold tap water. Dacron bags were then frozen at −20 °C, lyophilized, and weighed. Amount of residue in the blank bag was subtracted from each sample bag collected at identical incubation times to correct for influx of forage particles and microbial cells during incubation. Residue was analyzed for DM, OM, and NDF (Goering and Van Soest 1970). In situ OM and NDF disappearance was then determined.

Serum and plasma metabolites

On days −2 and 0 (just prior to offering of supplement approximately 0900 hours), initial plasma and serum samples were collected from all heifers. On days 40, 42 and 44, final plasma and serum samples were collected from each heifer prior to supplementation ($t = 0$) at 0700 hours and again 4 h after receiving daily supplement,

approximately 1100 hours ($t = 4$). During all collection dates, plasma and serum samples were obtained via coccygeal venipuncture. Upon collection, serum and plasma [9-mL tubes for serum; 7-mL tubes with EDTA for plasma (Corvac, Sherwood Medical, St. Louis, MO)] samples were allowed to coagulate at 20 °C, and samples were then centrifuged at 1,500×g for 30 min, decanted, and stored at −20 °C until analysis.

Serum metabolite concentrations were analyzed in duplicate using commercially available diagnostic kits adapted to 96-well plates for analysis using a Bio-Tek Synergy HT microplate reader for glucose via the glucose oxidase method (intra CV = 8.09; inter CV = 13.43) (Kit TR15498, Thermo Scientific. Middletown, VA, USA 22645), blood urea nitrogen via the urease method (intra CV = 4.61; inter CV = 8.45) (Kit NC9522515, Thermo Scientific. Middletown, VA, USA 22645), and nonesterified fatty acid (NEFA; intra CV = 4.89; inter CV = 9.59; ACS-ACOD method). Serum insulin concentrations were measured in duplicate using solidphase ^{125}I-Insulin RIA (Coat-A-Count kit TKIN1, Diagnostic Products Inc., Los Angeles, CA, USA) with an interassay CV of 21.13 %, an intra-assay CV of 1.92 %, and a recovery of 99 %. Serum samples were composited according to collection time and date prior to statistical analysis. The first composite measuring serum metabolites from blood was collected on days −2 and 0 at $t = 0$, just prior to offering supplements. Two subsequent composites were made from blood collected on days 40, 42, and 44: one at $t = 0$, just prior to the offering of supplements, and another at $t = 4$, 4 h after supplement was offered.

Plasma AA were analyzed using a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd, Cambridge, UK) by a commercial laboratory (Evonik-Degussa Corp., Kennesaw, GA, USA). Determination of AA used procedures previously reported (Fontaine and Eudaimon 2000; Fontaine et al. 1998; Llames and Fontaine 1994) and samples were deproteinized using sulfosalicylic acid. Plasma concentrations for cysteine were below detectable limits using methods described above and are therefore not presented. Plasma samples were composited prior to being sent for analysis. The first composite consisted of plasma from days −2 and 0 at $t = 0$. Secondly, a composite of plasma samples was made from days 40, 42, and 44 at $t = 0$, just prior to offering of supplements, and another at $t = 4$, 4 h after supplement was offered. Composite samples were made by combining 1 mL of plasma per heifer for each specified time period.

Statistical analysis

Data were analyzed as a completely randomized design using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with cow as the experimental unit. Continuous variables were analyzed by the main effects of treatment, date, or time and their interaction when appropriate. Covariates used in the model were birth weight, weaning weight, and day to parturition. Birth weight and weaning weight are the important biological variables that indicate metabolic size. They are related to AA partitioning and potential utilization. One heifer was removed from the MET group after the trial was complete for failure to deliver a calf or loss of pregnancy between pregnancy diagnoses and expected calving. Therefore, data from this heifer were not included in any of the statistical analysis. Results were considered as a tendency between $P \leq 0.10$ and $P > 0.05$ and significant if $P \leq 0.05$.

Results and discussion

Forage characteristics

Forage diet quality assessed from extrusa at the initiation and termination was 5.2 and 5.3 ± 0.33 % CP (OM basis), respectively, and the concentration of NDF was 78.2 and 66.8 ± 1.15 % (OM, basis), respectively. In situ OM disappearance tended to be greater at 24 h of incubation for cows receiving the MET treatment ($P = 0.09$; Fig. 2); however, after 24 h there was no evidence of disappearance differences due to supplement composition. Overall rate of disappearance was not influenced by dietary supplement ($P = 0.32$; 3.5 vs. 2.8 ± 0.45 %, respectively for CON and MET).

Although the DL-MET supplement fed in this study was from a rumen-protected source, some of the MET is still available to the rumen microbes. Early studies by Gil et al. (1973) suggest that sulfur containing amino acids or methionine hydroxyl analog can improve microbial growth

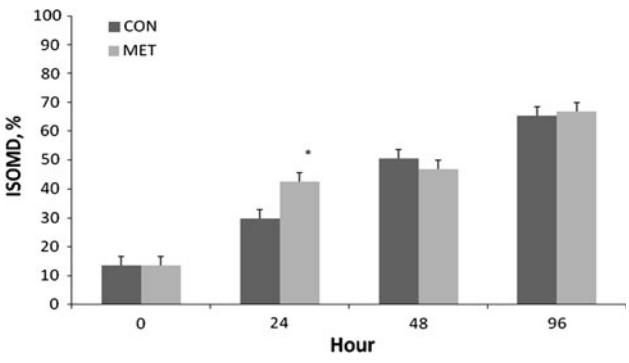

Fig. 2 In situ organic matter disappearance (ISOMD) from rumen extrusa samples collected from cows grazing dormant winter rangeland and given a wheat mid based supplement with and without DL-MET. A tendency * ($P = 0.09$) was measured for ISOMD at 24 h of incubation

over supplements that are absent in this AA. Improved microbial growth should lead to better DM, OM and NDF disappearance. Lodman et al. (1990) found supplements containing soybean meal to have greater NDF disappearance than supplements with DL-MET, whereas Clark and Petersen (1988) observed improved DM disappearance and no differences in NDF disappearance in methionine-supplemented cows grazing winter range compared with cows supplemented with soybean meal. Nonetheless, these researchers noted that all supplemented cows had increased in situ DM and NDF digestion rates compared with non-supplemented cows in the same study. Greater forage DM disappearance was reported by Huisman et al. (1988) as a result of supplementing DL-MET in either liquid or ruminally protected forms (20 % ruminally degraded). Conversely, Lodman et al. (1990) noted only numerical increases in the extent and rate of DM and NDF digestion as a result of methionine and urea supplementation. Wiley et al. (1991) suggested that the timing of methionine addition may have a differential effect on NDF digestion depending on the timing relative to forage consumption and availability of carbohydrates in the rumen.

Rumen microbes utilize limiting AA (DL-MET) to promote growth which in turn could allow for greater utilization of mature forages (Salter et al. 1979). In the present study, equal disappearance at the termination of incubation was expected, since extent is a function of the chemical and physical properties of the mature forages which would not be changed by a more active microflora and higher concentrations of DL-MET in rumen liquor.

Heifer performance

Heifer BW ($P = 0.32$) and BCS ($P = 0.83$) were similar throughout the study (44-day supplemental period), regardless of supplemental treatment (Table 2). However, heifer BW tended to decline ($P = 0.07$) from initiation to termination of the supplementation period. No differences ($P = 0.66$) were observed for calf birth weight between treatments (35.9 and 35.1 ± 1.24 kg, respectively, for calves born from CON and MET treated heifers). Even though fetal growth is most rapid during late gestation increasing N (AA) accretion by the gravid uterus (i.e., placenta and fetus), as shown by equation 4–10 in the nutrient requirements for beef cattle (NRC 2000), no differences were observed in BW or BCS for CON and MET heifers. Therefore, BW losses occurring in late gestation would indicate catabolism of maternal tissues to partially support nutrient needs for fetal development. Accretion of the fetal mass concealed true dam weight loss and the severity of BW loss was greater than measured.

Protein accretion in the ruminant is an energy-dependent process. The ruminant relies on dietary feedstuffs to provide energy and N (protein) to support rumen microbial growth, which in turn, supplies both energy (fermentation by-products) and protein (microbial origin) to the host ruminant. The AA requirements for the host ruminant are primarily met by this supply of microbial protein and to a lesser extent that portion deriving from ruminally undegradable protein from dietary feedstuffs. Conversely, if energy is limiting in the host animal, protein deposition becomes secondary and microbial protein and ruminally undegradable protein along with catabolism of muscle tissue are diverted to meet energy requirements. Once the ruminants energy requirements are satisfied, protein deposition can increase; however, protein deposition may be restricted if there are limiting AA. For instance, when only a single AA is limiting, then protein deposition increases in response to supplementing that AA until the requirement is met, at which point no further increases are observed (Campbell et al. 1997a; Froidmont et al. 2000). Titgemeyer and Loest (2001) suggest that grazing cattle most likely will not respond to supplemental AA when energy is limiting or when animals are in a weight loss nutritional environment.

Serum metabolites

To evaluate the nutritional status of late gestating first-calf heifer's, serum metabolites were measured at the initiation and termination of the 44-day trial. No differences were measured for serum glucose ($P = 0.22$), insulin ($P = 0.12$), urea N ($P = 0.16$), or NEFA ($P = 0.10$; Table 3). However, serum glucose and insulin concentrations decreased ($P < 0.01$) from initiation to termination of the 44-day trial, whereas serum urea N and NEFA concentrations increased ($P < 0.01$), which is consistent with what was observed for BW measurements and indicates the

Table 2 Heifer body weight (BW), and body condition score (BCS) for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid-based supplement without DL-MET (CON) and with DL-MET (MET) throughout a 44-day supplementation period

Item	Supplement		SEM	*P* value	Date		SEM	*P* value
	CON	MET			On trial	Off trial		
BW (kg)	416.6	425.1	5.66	0.32	453.2	388.6	17.52	0.07
BCS	4.45	4.49	0.11	0.83	4.62	4.33	0.34	0.67

Table 3 Composite serum metabolite concentrations for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid-based supplement without DL-MET (CON) and with DL-MET (MET) for glucose, insulin, serum urea nitrogen (SUN), and nonesterified fatty acids (NEFA) prior to initiation of supplementation (On trial) and at the termination of the 44-day supplementation period (Off trial)

Item	Supplement		SEM	*P* value	Date		SEM	*P* value
	CON	MET			On trial	Off trial		
Glucose (mM)	3.53	3.65	0.07	0.22	3.80	3.39	0.05	<0.01
Insulin (ng/mL)	0.15	0.22	0.03	0.12	0.24	0.12	0.02	<0.01
SUN (mM)	1.31	1.53	0.10	0.16	1.00	1.84	0.11	<0.01
NEFA (μmol/L)	1,004	836	67.3	0.10	844	996	48.8	<0.01

Table 4 Composite serum metabolite concentrations for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid based supplement without DL-MET (CON) and with DL-MET (MET) for glucose, insulin, serum urea nitrogen (SUN), and nonesterified fatty acids (NEFA) prior to receiving daily supplementation (0 h) and again 4 h after supplementation at the termination of the 44-day supplementation period

Item	Supplement		SEM	*P* value	Time		SEM	*P* value
	CON	MET			0 h	4 h		
Glucose (mM)	3.30	3.49	0.07	0.10	3.39	3.41	0.05	0.65
Insulin (ng/mL)	0.10	0.14	0.12	0.14	0.12	0.12	0.01	0.86
SUN (mM)	2.05	1.98	0.16	0.75	1.84	2.20	0.13	0.02
NEFA (μmol/L)	1,016	910	71.9	0.33	997	929	51.2	0.12

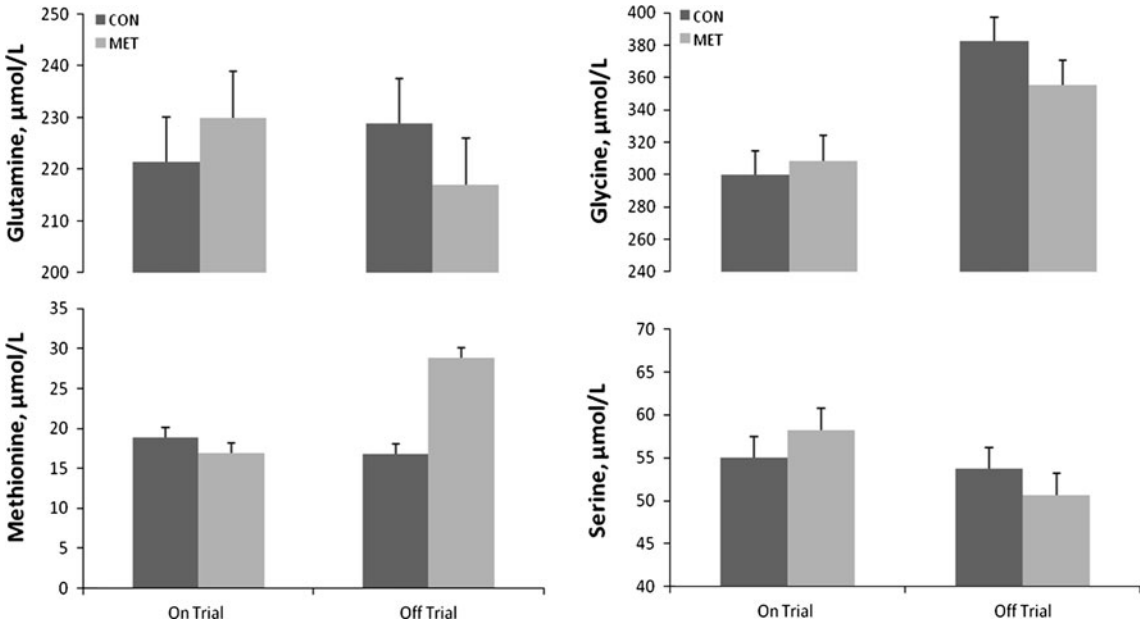

Fig. 3 Composite plasma AA concentrations for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid based supplement without DL-MET (CON) and with DL-MET (MET) prior to initiation of supplementation (On trial) and at the termination of the 44-day supplementation period (Off trial). Dietary supplement × time on trial interactions were significant for glutamine ($P = 0.03$), glycine ($P = 0.02$), methionine ($P < 0.01$), and serine ($P = 0.05$)

catabolism of maternal tissues in support of the gravid uterus.

Serum concentrations at the termination of the period at the two serum sampling times (prior to and 4 h after feeding) tended to differ ($P = 0.10$) for serum glucose and was greater for MET supplemented heifers (Table 4). Normal range for glucose concentrations is between 2.5 and 4.2 mM (Kaneko 1989) and concentrations were

similar to those previously reported (Waterman et al. 2007a, b); however, no differences were measured between cows consuming supplements with or without MET for serum insulin ($P = 0.14$), urea N ($P = 0.75$), or NEFA ($P = 0.33$; Table 4). Similarly, 4-h after supplementation no differences were measured for serum glucose ($P = 0.65$), insulin ($P = 0.86$), or NEFA ($P = 0.12$). There was no interaction between treatment and sampling time ($P > 0.05$), which suggests that both treatment groups were equal in N supply. Similarly, NEFA concentrations were elevated indicating that heifers were mobilizing adipose tissue in support of energy requirments. Yet, NEFA concentrations were not influenced by treatment ($P = 0.33$) or sampling time ($P = 0.12$). In the ovine, as gestation advances an increase in serum NEFA and reduction in serum insulin had been observed (Regnault et al. 2004). Also, in the ovine, the utilization of NEFA during pregnancy may be supportive in conserving other glucogenic precursors by the mobilization of lipid reserves to the liver (Freetly and Ferrell 2000). Furthermore, elevated serum NEFA concentrations and low-insulin concentrations during peripaturient period have been associated with metabolic disorders (Cameron et al. 1998; Drackley 1999).

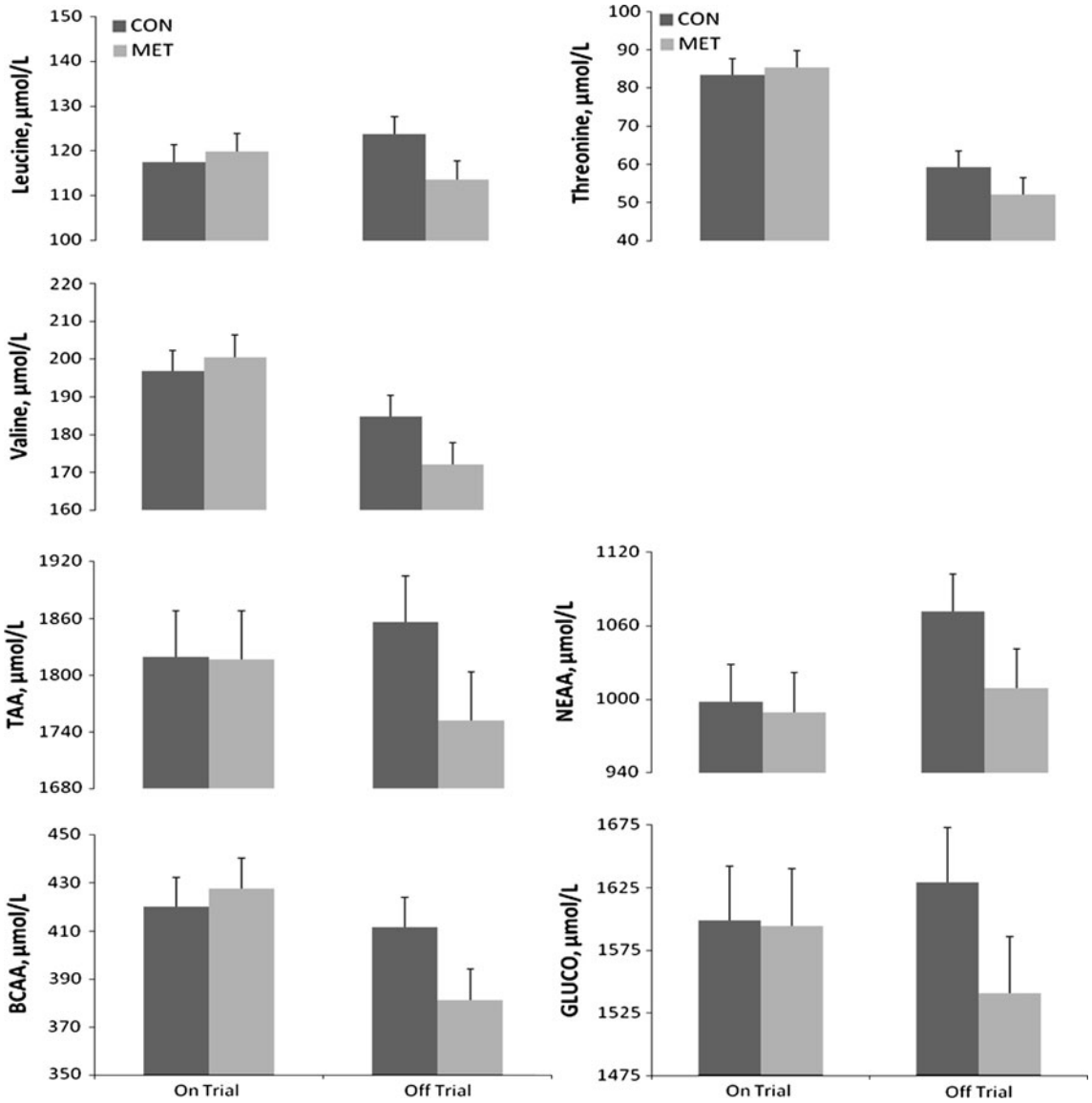

Fig. 4 Composite plasma AA concentrations for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid based supplement without DL-MET (CON) and with DL-MET (MET) prior to initiation of supplementation (On trial) and at the termination of the 44-day supplementation period (Off trial). Dietary supplement × date on trial interactions tended to be significant for leucine ($P = 0.07$), threonine ($P = 0.09$), valine ($P = 0.08$), total amino acids (TAA; $P = 0.08$), non essential amino acids (NEAA; $P = 0.08$), branched chain amino acids (BCAA; $P = 0.08$), and glucogenic amino acids (GLUCO; $P = 0.08$)

Plasma amino acids

The elevations in plasma methionine concentration in MET supplemented heifers following 44 days of supplementation indicate that the bypass technology successfully presented DL-MET to the small intestine for absorption (Fig. 3). When adequate energy is available, protein deposition is linearly related to the supply of the most limiting AA until another factor becomes more limiting (Titgemeyer and Loest 2001). Although as discussed previously, there are inconsistent results showing the availability of rumen protected methionine to the small intestine for absorption; however, our study showed that methionine did get absorbed and influenced the use of other AA. Others have also reported elevated concentrations of plasma methionine with rumen protected sources (Blum et al. 1999; Südekum et al. 2004; Bach and Stern 2000; Overton et al. 1996; Soder and Holden 1999; Pisulewski et al. 1996).

The greatest plasma AA concentration increase was methionine ($P < 0.01$). The accumulation of methionine can be partially explained by the use of DL form of methionine. Although both isomers are absorbed from the small intestine at the same rate, Campbell et al. (1996) concluded that D-MET may be metabolized more slowly than the naturally occurring L-MET. In this instance, D-MET will accumulate (due to slow metabolism) and can result in less efficient utilization (Campbell et al. 1997b).

Supplementation with rumen-protected DL-MET caused a significant supplement × date interaction for glutamine ($P = 0.03$), glycine ($P = 0.02$), methionine ($P < 0.01$), and serine ($P = 0.05$; Fig. 3). Glutamine concentrations from the initiation to termination of the study increased for CON supplemented heifers and declined for MET supplemented heifers, whereas glycine concentrations increased for both CON and MET supplemented heifers but more for MET supplemented heifers. Methionine concentrations decreased in heifers receiving CON compared to MET supplemented heifers throughout the study and serine concentrations decreased for both CON and MET supplemented heifers but more for MET supplemented heifers.

Glycine is the most abundant AA in maternal blood (Bell and Ehrhardt 2000). Excess AA from the diet, in this case DL-MET, must be catabolized. Glycine is required for the catabolism of excess DL-MET. Even small increases in methionine can reduce the glycine supply during gestation

Table 5 Composite plasma AA concentrations for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid based supplement without DL-MET (CON) and with DL-MET (MET) prior to initiation of supplementation (On trial) and at the termination of the 44-day supplementation period (Off trial)

Item[a]	Supplement		SEM	*P* value	Date		SEM	*P* value
	CON (μmol/L)	MET (μmol/L)			On trial (μmol/L)	Off trial (μmol/L)		
Alanine	221.3	205.8	7.24	0.16	214.5	212.7	5.19	0.69
Arginine	53.8	54.2	2.43	0.91	53.9	54.0	1.90	0.94
Asparagine	21.3	20.0	1.18	0.45	20.3	21.0	0.89	0.48
Aspartic acid	10.2	10.2	0.46	0.94	10.2	10.2	0.39	0.93
Glutamic acid	64.1	59.2	3.08	0.28	65.9	57.4	2.25	<0.01
Histidine	47.0	44.7	1.53	0.33	46.4	45.3	1.10	0.22
Isoleucine	104.4	101.3	3.03	0.49	106.5	99.3	2.37	0.01
Leucine	120.6	116.7	3.37	0.44	118.6	118.7	2.73	0.99
Lysine	103.3	100.2	3.30	0.52	102.7	100.5	2.70	0.46
Phenylalanine	50.1	47.8	1.43	0.30	49.4	48.5	1.04	0.37
Proline	97.3	94.8	3.01	0.56	96.6	95.5	2.43	0.73
Threonine	71.4	68.7	4.00	0.65	84.4	55.7	2.89	<0.01
Tyrosine	43.9	42.4	1.28	0.44	45.8	40.6	1.00	<0.01
Valine	190.8	186.3	4.77	0.53	198.7	178.5	3.84	<0.01
TAA	1,838	1,784	47.3	0.45	1,818	1,804	33.6	0.61
EAA	803	785	20.6	0.56	824	764	16.0	<0.01
NEAA	1,035	999	30.3	0.43	994	1,040	21.0	<0.01
BCAA	416	404	10.6	0.47	424	396	8.6	0.01
KETO	422	409	10.7	0.39	423	408	8.7	0.15
GLUCO	1,614	1,568	42.4	0.46	1,597	1,585	29.9	0.62

[a] *TAA* total amino acids, *EAA* essential amino acids, *NEAA* non essential amino acids, *BCAA* branched chain amino acids, *KETO* ketogenic amino acids, *GLUCO* glucogenic amino acids

(Rees et al. 2006). As depicted in Fig. 3, glycine is found at a lower concentration in the MET treatment group following 44 days of supplementation showing that MET supplemented heifers have a greater supply of DL-MET to breakdown. The placenta converts serine, mostly taken up from maternal blood, to glycine (Chung et al. 1998) and explains the decrease in Ser concentrations in the MET treatment group compared to the CON heifers.

Since methionine supply limitations were eliminated with supplementation, other AA could be used more efficiently for needs such as protein-rich fetal tissues. McNeill et al. (1997), using traditional N balance and comparative slaughter techniques, estimated the utilization of apparently digested CP in late gestating ditocous ewes, which resulted in a value of 0.7 for apparent N efficiency of small intestine absorption and subsequent tissue accretion when ewes were fed to required energy and protein levels during late-gestation. Calculations from Bell and Ehrhardt (1998) indicate that 80 % of apparently digested CP can be used in the gravid uterus. The remainder is used to support metabolism and net deposition of AA in the developing mammary glands and visceral organs.

Since no differences were found for calf birth weights ($P = 0.66$), it suggests that either MET supply did not limit fetal growth or MET was unable to flow into the placenta. Placental growth is most rapid during mid gestation (Ehrhardt and Bell 1995). Moderate undernutrition of ewes during early to mid pregnancy has caused conflicting results, both positive (Faichney and White 1987; McCrabb et al. 1992) and negative (McCrabb et al. 1992; Clarke et al. 1998) effects on placental size. This reported variation can be explained partially by dam body condition. Fatter ewes responded to undernutrition by increasing placental size, whereas the opposite occurred in lean ewes (McCrabb et al. 1992). These results suggest that if maternal energy stores are unavailable, the dam will use a majority of dietary energy and AA for placental growth. However, no placental data were collected in the present study.

With DL-MET supplementation, utilization of nearly all other AA should increase until another factor becomes limiting. Figure 4 indicates strong trends for supplement × date interactions for leucine ($P = 0.07$), threonine ($P = 0.09$), valine ($P = 0.08$), total amino acids (TAA; $P = 0.08$), non essential amino acids (NEAA; $P = 0.08$), branched chain amino acids (BCAA; $P = 0.08$), and glucogenic amino acids (GLUCO; $P = 0.08$). These results suggest that two of the BCAA (leucine, and valine) were utilized more efficiently with MET supplemented heifers compared to CON supplemented heifers.

Quantity and composition of AA delivered to the fetus are highly dependent on placental metabolism (Bell and Ehrhardt 2000). Liechty et al. (1991) and Chung et al. (1998) indicate that net consumption of glutamate, serine, and BCAA by uteroplacental tissues indicates both catabolism or transamination of these AA. In the uteroplacenta, the placenta is the main source of ammonia production, which is derived from BCAA (Jóźwik et al. 1999). In the placenta, BCAA are transaminated to glutamate and the placenta takes up fetal glutamate (Vaughn et al. 1995; Chung et al. 1998). Klimek et al. (1993) indicate that in vitro placental mitochondria incubation with glutamate promotes NADPH production, which is used in the synthetic process for progesterone. Thus, BCAA transamination to glutamate may promote progesterone synthesis in the mitochondria, and the major role of BCAA at the placenta is to provide glutamate, rather than using BCAA as an energy substrate (Jóźwik et al. 1999).

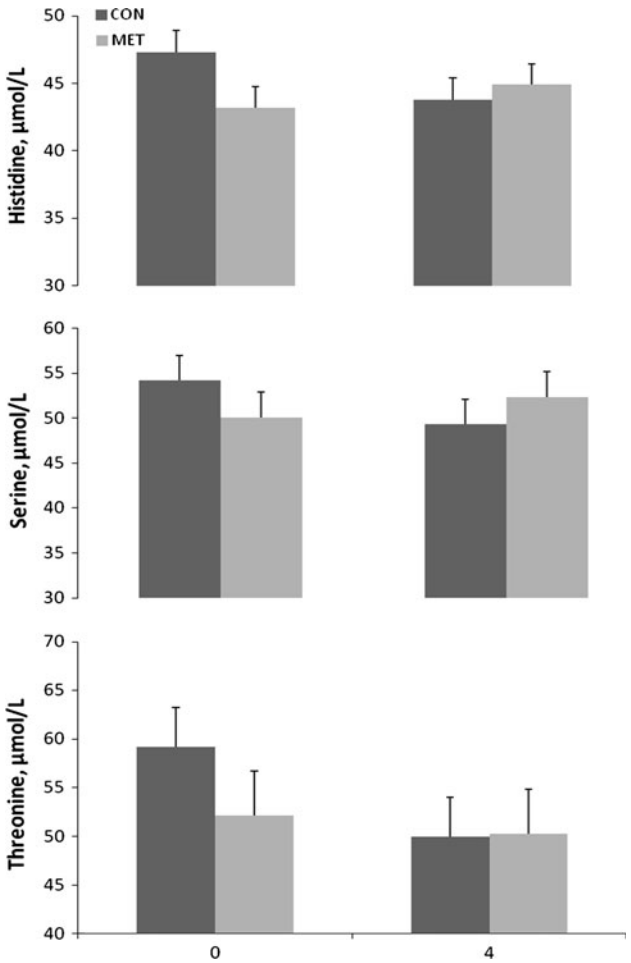

Fig. 5 Composite plasma AA concentrations for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid-based supplement without DL-MET (CON) and with DL-MET (MET) prior to receiving daily supplementation (0 h) and again 4 h after supplementation at the termination of the 44-day supplementation period. Dietary supplement × time tended to be significant for histidine ($P = 0.07$), serine ($P = 0.09$), and threonine ($P = 0.08$)

The supplement × date interactions measured in Fig. 4 also indicate a better utilization efficiency for TAA, NEAA, and GLUCO amino acids with the inclusion of DL-MET as concentrations declined over the study. Waterman et al. (2007b) observed greater N retention in late gestating beef cows with incremental inclusion of DL-MET into the abomasum. Although N retention was not measured in the present study, these data would suggest that similar improvements in N retention would have been observed in the present study based on plasma AA concentrations.

Plasma AA concentrations for glutamic acid ($P < 0.01$), histidine ($P = 0.01$), tyrosine ($P < 0.01$), and EAA ($P < 0.01$), decreased over the 44-day supplementation period (Table 5). The decrease in histidine and EAA during late gestation may indicate that other EAA may be limiting in heifers experiencing body weight loss when consuming dormant range forage. Thus, in addition to methionine, other EAA need to be evaluated as part of improving overall AA utilization in beef cows grazing senescent range forages.

Plasma AA concentrations evaluated during the last week of supplementation, both before (0 h) and after (4 h) supplementation, indicated a trend for a supplement × time interactions for histidine ($P = 0.07$), serine ($P = 0.09$), and threonine ($P = 0.08$; Fig. 5). Both histidine and serine increased from 0 to 4 h in relation to supplementation for MET supplemented heifers; whereas, CON supplemented heifers declined in AA concentrations. Threonine on the other hand remained unchanged for MET supplemented heifers, while CON heifers measured a decrease from 0 to 4 h in relation to supplementation. Plasma methionine concentrations were greater ($P < 0.01$) overall for MET supplemented heifers, regardless of when plasma was collected during the last week of the study (Table 6). Furthermore, there was a consistent decrease in plasma AA from 0 h to 4 h following supplementation indicating that within 4 h of supplementation nutrients provided in both supplements aided in the removal or utilization of other AA.

Table 6 Composite plasma AA concentrations for late gestation first calf heifers grazing dormant winter rangeland and given a wheat mid-based supplement without DL-MET (CON) and with DL-MET (MET) prior to receiving daily supplementation (0 h) and again 4 h after supplementation at the termination of the 44-day supplementation period

Item[a]	Treatment		SEM	*P* value	Time		SEM	*P* value
	CON (μmol/L)	MET (μmol/L)			0 h (μmol/L)	4 h (μmol/L)		
Alanine	207.9	206.0	5.61	0.82	212.6	201.2	5.02	0.11
Arginine	53.7	53.6	2.52	0.98	54.0	53.3	1.97	0.73
Asparagine	20.5	20.6	0.79	0.96	21.0	20.1	0.68	0.29
Aspartic acid	10.5	9.6	0.59	0.29	9.7	10.4	0.50	0.32
Glutamic acid	57.5	53.1	2.81	0.30	57.4	53.2	2.13	0.07
Glutamine	221.4	221.0	5.86	0.96	223.0	219.5	5.18	0.63
Glycine	375.5	366.2	13.84	0.65	369.0	372.7	10.83	0.76
Histidine	45.5	44.0	1.30	0.44	45.2	44.3	1.08	0.51
Isoleucine	97.3	93.9	2.65	0.38	99.3	92.0	2.39	0.04
Leucine	116.7	111.2	3.50	0.30	118.6	109.2	2.92	0.02
Lysine	96.6	95.1	2.80	0.71	100.5	91.2	2.52	0.01
Methionine	16.5	26.8	1.23	<0.01	22.8	20.6	0.93	0.03
Phenylalanine	46.6	46.2	1.12	0.82	48.5	44.3	1.04	0.01
Proline	78.9	79.1	2.37	0.94	95.6	62.4	1.94	<0.01
Serine	51.8	51.2	2.51	0.86	52.1	50.8	1.91	0.51
Threonine	54.6	51.2	4.03	0.58	55.7	50.1	2.80	0.01
Tyrosine	40.1	39.3	0.92	0.53	40.6	38.8	0.85	0.14
Valine	175.3	168.5	3.90	0.25	178.4	165.4	3.67	0.02
TAA	1,167	1,737	32.63	0.53	1,804	1,699	34.38	0.07
EAA	742.9	729.8	15.97	0.58	763.6	709.1	15.85	0.03
NEAA	1,024	1,007	20.31	0.57	1,040	990.3	20.56	0.13
BCAA	389.3	373.6	9.37	0.27	396.3	366.6	8.63	0.02
KETO	397.3	385.6	8.83	0.37	407.5	375.4	8.62	0.02
GLUCO	1,554	1,530	28.59	0.58	1,585	1,499	30.18	0.09

[a] *TAA* total amino acids, *EAA* essential amino acids, *NEAA* non essential amino acids, *BCAA* branched chain amino acids, *KETO* ketogenic amino acids, *GLUCO* glucogenic amino acids

Conclusion

First calf heifers grazing dormant rangelands in late gestation had improved AA utilization when rumen protected methionine was offered. Furthermore, this research indicates that methionine was a limiting AA in late gestating heifers experiencing body weight loss. The benefit of supplying additional methionine in a form that is rumen protected such as Mepron® to the rumen appears to be dependent upon passage rate, since detectable difference were emerging after only 24 h. This benefit can encourage greater utilization of the dormant forage as observed with improved OM disappearance at 24 h of in situ incubation. The results of this study did not show any improvements in heifer BW, BCS or calf birth weight when MET was fed. Future work is needed to elucidate the potential of other co-limiting amino acids and their interaction with heifers in late gestation and when experiencing body weight loss.

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