Effects of supplementing methionine hydroxy analog on beef cow performance, milk production, reproduction, and preweaning calf performance¹

A. R. Clements,* F. A. Ireland,* T. Freitas,† H. Tucker,† and D. W. Shike*²

*Department of Animal Sciences, University of Illinois, Urbana 61801; and †Novus International, St. Charles, MO 63304

ABSTRACT: Mature Simmental × Angus cows (214 cows; 635 ± 7 kg) were utilized to determine the effects of late gestation and early postpartum supplementation of methionine hydroxy analog (MHA) on cow BW, BCS, milk production, milk composition, reproduction, and calf performance until weaning in a fall-calving, cool-season grazing system. Cows were stratified by BW, age, AI sire, and assigned to 1 of 12 pastures (17 or 18 cows·pasture⁻¹). Pastures were randomly allotted to 1 of 2 treatments: control (0.45 kg·cow⁻¹·d⁻¹ of wheat midd-based pellets, $n = 6$) or supplement including MHA (0.45 kg·cow⁻¹·d⁻¹ of wheat midd-based pellets including 10 g MHA supplied as MFP (Novus International, Inc., St. Charles, MO; $n = 6$). Treatments were fed 23 ± 7 d prepartum through 73 ± 7 d postpartum. Cow BW was collected at postcalving (27 ± 7 d postpartum), end of supplementation (73 ± 7 d postpartum), AI, pregnancy check, and end of trial (192 and 193 ± 7 d postpartum). At 73 ± 7 d postpartum, a subset of cow-calf pairs was used in a weigh-suckle-weigh procedure to determine milk production, and milk samples were taken to determine milk composition ($n = 45$ ·treatment⁻¹). Serum from blood was collected at 73 ± 7 and 83 ± 7 d postpartum to determine cow cyclic-

ity and concentrations of 2-hydroxy-4-(methylthio) butanoic acid (HMTBa) and L-Methionine. After supplementation, all cow-calf pairs were managed as a common group until weaning (193 ± 7 d of age). Cows were bred via AI at 97 ± 7 d postpartum and clean-up bulls were turned out 11 d post-AI for a 55-d breeding season. Cows fed MHA had greater ($P < 0.01$) serum concentrations of HMTBa. Cow BW and BCS were not different ($P \geq 0.10$) at any time points between treatments. There was no treatment effect ($P \geq 0.17$) on calf birth BW, calf weaning BW (193 ± 7 d of age), or calf ADG. Calculated 24-h milk production, milk composition and component production did not differ ($P \geq 0.21$). There were no differences ($P \geq 0.50$) in percentage of cows cycling, AI conception rate, and overall pregnancy rate between treatments. Post-trial nutritional modeling suggests cows experienced several nutritional deficiencies beyond methionine (Met) that limited the response to Met supplementation. Although supplementation of MHA during late gestation through estimated peak lactation increased serum HMTBa concentrations, it did not affect cow performance, cow milk production, or calf performance when fall-calving cows grazed cool-season forages.

Key words: cow-calf, fall-calving, late gestation supplementation, methionine hydroxy analog, milk production, reproduction

© 2017 American Society of Animal Science. All rights reserved. J. Anim. Sci. 2017.95:5597–5605
doi:10.2527/jas2017.1828

INTRODUCTION

Managing dam nutrition before and after calving is key to allow for ideal performance, both for optimal milk production and subsequent reproduction. Undernutrition of protein and energy in late

gestation and lactation may result in longer anestrus and decreased fertility in beef cattle (Meteer et al., 2015). Grazing beef cattle obtain the majority of these requirements from rumen microbial protein; however, further supplementation may be needed (Klopfenstein, 1996). The first limiting amino acid in grazing and lactating dairy and beef cows is methionine (Richardson and Hatfield, 1978; NRC, 1996; Waterman et al., 2007), and evidence suggests there are increased methionine requirements for gestating cattle (Bach et al., 2000).

¹Funding provided by Novus International, St. Charles, MO 63304.

²Corresponding author: dshike@illinois.edu

Received June 15, 2017.

Accepted September 18, 2017.

Methionine supplement is available in many forms; however, free amino acid sources are costly and unjustified when other rumen-protected forms are available (Kung and Rode, 1996). Protected methionine forms with physical coatings of lipid and pH sensitive polymers or analog forms bonded to hydroxyl or isopropanol groups have been effective in reducing the need for increased CP in the diet while improving N utilization in dairy cows (Chen et al., 2011). In spring-calving beef cows, Clanton and England (1980) reported increased conception rates in cows supplemented methionine hydroxy analog (MHA). Varner (1974) observed increased milk production in lactating beef cows fed MHA on range. No research has explored supplementing MHA to fall-calving cows grazing cool-season forages. The objectives of this study were to evaluate the effects of MHA supplementation pre- and postpartum on cow performance, milk production, reproduction, and calf performance. The hypothesis was that cows supplemented MHA would have similar BW and BCS as control cows but increased milk outputs and, therefore, increased calf gains, as well as improved reproductive rates.

MATERIALS AND METHODS

Cattle used in this trial were managed according to the guidelines recommended in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Consortium, 1988). All experimental procedures were approved by the University of Illinois Laboratory Animal Care Advisory Committee.

Animals and Experimental Design

Two hundred fourteen Angus $\times$ Simmental cows (mean $\pm$ SD; BW 635 ± 7 kg) from Dixon Springs Agricultural Research Station in Simpson, IL were utilized to evaluate the effects of supplementing MHA prior to and following parturition on cow performance, milk production, milk composition, conception rate, and calf performance. Cows were stratified by BW, cow age, and AI sire and assigned to 1 of 12 pasture groups (17 or 18 cows·pasture⁻¹) and randomly allotted to 1 of 2 supplemental treatments (Table 1): control (CON; 0.45 kg·cow⁻¹·d⁻¹ of wheat midd-based pellets) or 0.45 kg·cow⁻¹·d⁻¹ of wheat midd-based pellets formulated to provide 10 g of MHA supplied in the form of MFP (Novus International, Inc., St. Charles, MO). Cows in this experiment were previously bred AI with 1 of 16 Angus or Charolais sires and confirmed pregnant using ultrasound (Aloka 500 Instrument; Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array; February 10 and 12, 2014) and palpation methods (August 19, 2014). Supplementation began on

Table 1. Nutrient composition (DM basis) of wheat midd supplement fed 23 ± 7 d prepartum through 73 ± 7 d postpartum

Item	Treatment ^{1,2}	
	CON	MHA ³
DM, %	88.3	88.3
NDF, %	26.4	25.0
ADF, %	7.5	7.3
CP, %	14.1	12.1
Crude fat, %	2.8	3.6
TDN, %	86.6	86.8

¹Ad libitum access to mineral supplement: salt = 21.4%, Ca = 16.9%, Na = 8.5%, P = 7.1%, Mg = 5.9%, K = 1.1%, Cu = 997 mg·kg⁻¹, Se = 25.9 mg·kg⁻¹, vitamin A = 109 kIU·kg⁻¹, and chlortetracycline = 5.72 g·kg⁻¹.

²Wheat midd-based supplement was provided at 0.45 kg·cow⁻¹·d⁻¹. CON = contained no methionine hydroxy analog (MHA). MHA supplement provided 10 g MHA·cow⁻¹·d⁻¹.

³MHA was supplied in the form of MFP (Novus International, Inc., St. Charles, MO).

August 20, 2014, 23 ± 7 (SD) d prior to the average calving date (September 12, 2014). Thus, cows had differing lengths of supplementation time prior to and after calving. All cows grazed cool-season pastures which predominately included endophyte-infected tall fescue (*Festuca arundinacea* cv. Kentucky 31), red clover (*Trifolium pretense*), and white clover (*Trifolium repens*) and had ad libitum access to a salt and mineral block (salt = 21.4%, Ca = 16.9%, Na = 8.5%, P = 7.1%, Mg = 5.9%, K = 1.1%, Cu = 997 mg·kg⁻¹, Se = 25.9 mg·kg⁻¹, vitamin A = 109 kIU·kg⁻¹, chlortetracycline = 5.7 g·kg⁻¹) during the 96-d supplementation period ending November 24, 2014 (73 ± 7 d postpartum). Stocking density during the supplementation period was 3.7 AU (454 kg cow)/hectare for both treatments. Pasture groups were rotated under the discretion of trained University of Illinois research personnel based off of visual appraisal of forage availability. When a pasture group was rotated due to limited forage availability, a pasture group from the alternative treatment was also rotated to a new pasture. Following the conclusion of the supplementation period, on November 25, 2014 (74 ± 7 d postpartum), cow-calf pairs were commingled and managed as a common group until weaning. Cow-calf pairs grazed (stocking density = 6.4 AU/hectare) stockpiled tall fescue pastures (ADF 24.4%, NDF 50.4%, CP 11.1%, TDN 66.6%) with ad libitum access to a salt and mineral block. Cows also had access to free choice hay (ADF 35.5%, NDF 64.9%, CP 4.5%, TDN 55.6%) when forage stands were depleted. During the commingled period, all cows were supplemented (4.5 kg·d⁻¹) with a supplement consisting of 50% shelled corn (ADF 2.8%, NDF 9.6%, CP 7.8%, crude fat 3.6%, TDN 91.0% on a DM basis) and 50% distiller's dried grain and solubles (ADF 10.5%, NDF 33.1%, CP 26.3%, crude fat 8.2%, TDN 83.8% on a DM basis). The

supplementation during the commingled period began December 24, 2014 and continued until March 24, 2015. Calves were weaned March 24, 2015 (193 ± 7 d of age).

Cow Performance

At the initiation of the experiment, consecutive 2-d full BW and BCS were taken on all cows. A 1-d, midpoint full BW and BCS was taken on all the cows postcalving (27 ± 7 d postpartum). Following the end of the supplementation period and prior to commingling treatment groups, a 1-d shrunk BW and BCS was taken on the cows (73 ± 7 d postpartum). Body weight and BCS were also collected at the time of AI (97 ± 7 d postpartum) and AI pregnancy check (35 d post-AI; 132 ± 7 d postpartum). A final consecutive, 2-d full BW and BCS were taken on all cows at the end of the trial (192 and 193 ± 7 d postpartum).

Milk Production and Composition

Following the conclusion of supplementation (November 24, 2014; 73 ± 7 d postpartum) milk production and composition were evaluated on a subset of cows from each treatment group. A random subset of 8 cow-calf pairs from each treatment pasture were used in the weigh-suckle-weigh (WSW) procedure and milk composition analysis. Cows were administered 1 mL·cow⁻¹ oxytocin intramuscularly (Oxject; Henry Schein Animal Health, Dublin, OH) to stimulate milk let down and then were hand milked to obtain a 50-mL sample for milk composition analysis. Prior to sample collection, cow teats were cleaned with a clean cloth and 70% isopropyl alcohol, and the teat was hand-stripped approximately 3 times prior to collecting milk for sample analysis. Individual samples were strained through a clean 2-layer piece of cheesecloth (VWR International, Radnor, PA) to remove any foreign dirt or hair prior to filling the milk sample tube. Milk samples were stored in capped plastic tubes provided by Dairy Lab Services (Dubuque, IA) containing potassium dichromate preservative (K₂Cr₂O₇) to maintain milk quality until arrival to the lab for infrared instrumentation analysis (Ng-Kwai-Hang and Hayes, 1982). Milk sample collection began 10.5 h after supplementation and was completed 13.5 h after supplementation. Milk samples were refrigerated overnight ($< 4^{\circ}\text{C}$) and shipped to Dairy Lab Services the following morning. After milk sample collection was complete, cow-calf pairs were reunited by pasture group to nurse and to allow for the start of the 12-h milk production measure (Beal et al., 1990). Cow-calf pairs were again separated after nursing was complete. Nursing order alternated between treatment groups to reduce variation. Calves

and cows were penned separately overnight for 12 h and, the following morning, calves were weighed to record an empty BW. Calves were then reunited with their mothers and, immediately following nursing, the calves were weighed again to record a full BW. The difference in BW from full and empty calf BW are representative of 12-h milk production of the cow. The 12-h milk production was doubled to estimate 24-h milk production.

Reproductive Performance

Blood was collected from all cows to determine serum progesterone concentrations. The first blood collection occurred at the end of the supplementation period, and the second collection occurred 10 d later (73 ± 7 d postpartum and 83 ± 7 d postpartum, respectively). Blood was collected by means of a 12-mL syringe (Monoject; Covidien, Mansfield, MA) via the jugular vein and stored in a 10-mL borosilicate glass tube (Thermo Fisher Scientific Inc., Waltham, MA) where it was allowed to clot at room temperature prior to being centrifuged at $1,300 \times g$ for 20 min at 5°C (Thermo Fisher Scientific Sorvall Centrifuge, Waltham, MA). Serum was then stored at -20°C for later analysis. Serum progesterone concentrations were analyzed by solid-phase, competitive, chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Estrous cyclicity was then determined when serum progesterone concentrations met or exceeded 1 ng·mL⁻¹ on either collection time point.

Cows were synchronized using the 7-d Co-Synch + CIDR procedure prior to breeding (87 ± 7 d postpartum; Bremer et al., 2004) and artificially inseminated to 1 of 6 AI bulls (97 ± 7 d postpartum). Ten days following AI, cows were exposed to 6 bulls for a 55-d breeding season. Conception to AI and overall pregnancy were determined at 35 d post-AI service and 96 d post-AI service, respectively. Confirmation of AI conception and overall pregnancy rates were determined by a trained technician via ultrasonography (Aloka 500 instrument; Hitachi Aloka Medical America, Inc.; 7.5 MHz general purpose transducer array). Cow fetal loss was confirmed for the cows that were confirmed pregnant at the overall pregnancy check but failed to calve the following fall-calving season. Cows that were confirmed AI pregnant at the first pregnancy check and were open or not determined 96 d pregnant at the overall pregnancy check were also recorded as an AI fetal loss.

Calf Management and Data Collection

Calf BW was collected using a hand scale within 48 h of birth. A subset of calves (8 calves per pasture group) were weighed at the end of the supplementa-

tion period during the WSW procedure (73 ± 7 d of age; 96 total calves, 26 heifers and 22 steers from CON, 19 heifers and 29 steers from MHA). All calves were weighed for a preweaning midpoint 1-d full BW (94 ± 7 d of age). At weaning, a consecutive 2-d full BW was taken on all calves (192 ± 7 d and 193 ± 7 d of age). Prior to weaning, all calves were vaccinated with Bovi-Shield Gold FP 5 VL5 (Zoetis, Florham Park, NJ), Covexin 8 (Merck Animal Health, Madison, NJ), and MpB Guard Mycoplasma Bovis Bacterin (AgriLabs, St. Joseph, MO). Calf performance was reported for all calves (173 total calves, 56 heifers and 36 steers from CON, 36 heifers and 45 steers from MHA) that were weaned, minus calves that were injured or deceased prior to 193 ± 7 d of age. Calf health was monitored by trained university farm personal. Calves were removed from the study for mortality prior to weaning (15 calves) or dam removal due to death, lameness, or chronic illness during the experiment (19 calves). Cows were also removed from trial that did not have record of calving during the experiment (6 cows). Cow and calf performance data were included in analysis up until the date the cow-calf pair were removed from the study.

Feed Sampling and Analysis

Treatment supplements were sampled 4 times over the supplementation period: at the initiation of supplementation, at the end of first supplement load, delivery of second supplement load, and at the end of supplementation. Supplemented feed provided to the cows once they were commingled were also sampled every 28 d. Forage samples were collected once monthly and composited by pasture group during the supplementation period. Forage and hay were also collected monthly and composited during the commingled period. All feed and forage samples were dried at 55°C for a minimum of 3 d and then ground using a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). All samples were then analyzed for CP (Leco TruMac; LECO Corporation, St. Joseph, MI), total ash (600°C for 2 h, Thermolyne muffle oven model: F30420C; Thermo Scientific, Waltham, MA), and ADF and NDF (using Ankom Technology Methods 5 and 6, respectively: Ankom200 Fiber Analyzer; Ankom Technology, Macedon, NY). Cow treatment samples and supplement ingredients fed during the commingled period were also analyzed for crude fat (ether extract method; Ankom Technology).

2-Hydroxy4-(Methylthio) Butanoic Acid (HMTBa) and L-Methionine Analysis

Serum from blood collected at the end of supplementation (73 ± 7 d postpartum) was used to analyze

for HMTBa (a methionine precursor and main component of MFP) and L-Methionine (L-Met). Serum was pooled by pen to determine concentration of L-Met and HMTBa ($\mu\text{g/mL}$) to confirm that cows were consuming the MHA supplement. The 12 pen samples (6 control, 6 treatment) were analyzed by a commercial lab (Seventh Wave Laboratories, LLC, Maryland Heights, MO) using LC and MS methodology. The lower limit of quantitation for HMTBa was $0.0208 \mu\text{g/mL}$. When samples were below the lower limit of quantitation, $0.0208 \mu\text{g/mL}$ was used for statistical analysis. Methionine equivalents were determined as the sum of L-Met and HMTBa concentrations.

Statistical Analysis

The MIXED procedure (SAS Version 9.4; SAS Inst. Inc., Cary, NC) was utilized to analyze all variables excluding cow reproductive performance, calf morbidity, and calf mortality. Pen was experimental unit and was included as a random effect. Fixed effects included treatment and cow age in the model statements for all variables for cows and calves, excluding calf morbidity and mortality. The AI sire and calf sex was included as a fixed effect in the models for all variables pertaining to calf performance. Least squares mean function of SAS was used to separate treatment means. The GLIMMIX procedure (SAS Version 9.4; SAS Inst. Inc., Cary, NC) was utilized to analyze cow reproductive performance (cyclicality, AI conception rate, overall pregnancy rate, and fetal loss) and calf morbidity and mortality using binomial distributions. Pen was experimental unit and included as a random effect. This allowed individual Bernoulli trial data to be analyzed while maintaining pen as experimental unit. Significance was declared at $P \leq 0.05$, and trends will be discussed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Forage and Supplement Analysis and Supplement Validation

Nutrient composition of the treatment supplements is reported in Table 1; numerically, the treatment supplement and control supplement differed in CP by 2%. This difference was unexpected as the only difference in supplements was the addition of MHA. Samples were analyzed for CP prior to supplementation and CP was much more similar (control CP 12.6% and treatment CP 11.9%; Novus International, Inc., St. Charles, MO). It is possible that the differences are due to sampling or analysis error. Nutrient composition of pasture forages (Table 2) during the supplementation period was not different between treatments ($P \geq 0.64$). Intakes were not recorded in this

Table 2. Nutrient composition (DM basis) of pastures during supplementation

Item	Treatment ^{1,2}		SE	P-value
	CON	MHA ³		
DM, %	34.5	34.6	2.00	0.93
NDF, %	54.4	55.3	1.20	0.64
ADF, %	28.1	28.5	0.90	0.76
CP, %	8.9	9.5	1.10	0.72
TDN, %	63.0	62.9	1.02	0.94

¹Forage analysis during supplementation period, $n = 6$ pen/treatment.

²Wheat midd-based supplement was provided at $0.45 \text{ kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$. CON = contained no methionine hydroxy analog (MHA). MHA supplement provided $10 \text{ g MHA} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$.

³MHA was supplied in the form of MFP (Novus International, Inc., St. Charles, MO).

study, which makes it difficult to precisely determine if energy and protein were deficient. A post-trial evaluation was conducted using the NRC model (NRC, 2016) with inputs from the experiment and book values for AA of feedstuffs to assess potential energy and protein deficiencies. Using an assumed intake of 1.1% BW in dietary NDF (NRC, 2016), predicted DMI for the pregnant cow was 10.59 kg for the precalving 23 ± 7 d up to the weaning period. With this intake, there is an ME deficit of 0.23 Mcal/d and an MP deficit of 75.4 g/d per cow on average (NRC, 2016). For the period of 74 ± 7 -d postcalving up to the end of the supplementation, also assuming an intake of 1.1% BW in dietary NDF, the nutritional deficit of the lactating cow becomes even greater with an ME deficit of 2.1 Mcal/d and an MP deficit of 215 g/d per cow on average (NRC, 2016). For most of the supplementation period, cows were in an AA deficit: His (-6.45 g/d), Ile (-1.47 g/d), Leu (-8.70 g/d), Lys (-7.72 g/d), Val (-0.69 g/d). Methionine went from a deficit of 2.56 g/d to a positive balance of 0.13 g/d (NRC, 2016, computer model) with MHA supplementation.

Concentration of L-Met in serum between treatments did not differ ($P = 0.26$). Similarly, methionine equivalents did not differ ($P = 0.16$) between treatments. However, serum analysis confirmed cows on MHA treatment did consume the supplement as treatments diverged in serum concentrations of a methionine precursor, HMTBa, because serum HMTBa concentrations differed ($P < 0.01$) between treatments. Concentration of HMTBa in cows fed MHA supplement was $0.113 \text{ } \mu\text{g/mL}$ (Table 3), and the concentration of HMTBa in the serum of cows on CON supplement was below the lower limit of quantitation (reported as $0.0208 \text{ } \mu\text{g/mL}$). Not detecting differences in serum concentrations of L-Met at 73 ± 7 d postpartum does not suggest failure to convert HMTBa to L-Met as intracellular synthesis of L-Met from HMTBa is not accounted for in this measure. Previous research in lambs

Table 3. Effect of supplementing MHA on serum L-methionine and 2-hydroxy4-(methylthio) butanoic acid (HMTBa) concentrations at 73 ± 7 d postpartum

Item	Treatment ¹		SE	P-value
	CON	MHA ²		
L-methionine, $\mu\text{g/mL}$	2.15	2.51	0.207	0.26
HMTBa, $\mu\text{g/mL}$	BLQ ³	0.113	0.015	< 0.01
Methionine equivalents, ⁴ $\mu\text{g/mL}$	2.17	2.62	0.205	0.16

¹Wheat midd-based supplement was provided at $0.45 \text{ kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$. CON = contained no methionine hydroxy analog (MHA). MHA supplement provided $10 \text{ g MHA} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$.

²MHA was supplied in the form of MFP (Novus International, Inc., St. Charles, MO).

³HMTBa concentration for all CON cow groups was below lower limit of quantitation (BLQ; $0.0208 \text{ } \mu\text{g/mL}$). Data were analyzed using $0.0208 \text{ } \mu\text{g/mL}$ for CON samples.

⁴Sum of L-methionine and HMTBa.

has demonstrated that HMTBa is taken up across a variety of tissues (i.e., muscle, brain, liver, kidneys, GI tract), and subsequently converted to L-Met with little L-Met re-entering circulation (McCollum et al., 2000; Lobley et al., 2006; Wester et al., 2006). In the current experiment, where nutritional requirements were most likely not met, it is reasonable for the nutrient deprived tissues to absorb HMTBa, convert it to L-Met, and retain the newly synthesized L-Met for intracellular use and metabolism without releasing L-Met into circulation. Therefore, it is not expected to have greater L-Met levels in the circulation with HMTBa treatment.

Cow Performance

Cow BW and BCS data are shown in Table 4. As expected, there was no difference in BW between treatments at the initiation of supplementation ($P = 0.68$). Subsequent BW and BCS did not differ ($P \geq 0.41$) at $27 \text{ d} \pm 7 \text{ d}$ postpartum, conclusion of the supplementation period ($73 \pm 7 \text{ d}$ postpartum), at AI, or at time of AI pregnancy verification. There were also no differences ($P \geq 0.36$) observed in cow BW or BCS at weaning. No significant differences, due to supplementation of MHA, in beef cow BW and BCS were observed in other experiments over the duration of supplementation (Clanton and England, 1980; Varner, 1974; Wright et al., 1975; Thomas and Langford, 1978). In contrast, Krehbiel et al. (2001) did note there were beef cow BW change differences between the supplementation period and the grazing period among treatments; however, at the end of the experiment, there were no differences in overall cow BW change. Krehbiel et al. (2001) attributed the significance of the BW changes to compensatory gain due to increased forage quality earlier in the grazing season in Exp. 2 in comparison to forage quality in Exp. 1 in their discussion. However, Thomas and Langford

Table 4. Effects of supplementation of MHA to fall-calving beef cows on cow BW and BCS

Item	Treatment ¹		SE	P-value
	CON	MHA ²		
<i>n</i> , pen	6	6	—	—
BW, kg				
Initial ³	636	634	7.4	0.68
Postcalving ⁴	645	638	9.5	0.41
End of Supplementation ⁵	563	562	12.6	0.91
Breeding ⁶	566	559	10.7	0.61
AI Pregnancy Check ⁷	559	555	5.5	0.71
Weaning ⁸	510	500	10.4	0.36
BCS				
Initial ³	5.8	5.9	0.1	0.10
Postcalving ⁴	5.7	5.8	0.1	0.51
End of Supplementation ⁵	5.2	5.2	0.2	0.76
Breeding ⁶	4.5	4.5	0.1	0.82
AI Pregnancy Check ⁷	4.4	4.4	0.1	0.91
Weaning ⁸	3.9	3.9	0.1	0.65

¹Wheat midd-based supplement was provided at 0.45 kg·cow⁻¹·d⁻¹. CON = contained no methionine hydroxy analog (MHA). MHA supplement provided 10 g MHA·cow⁻¹·d⁻¹.

²MHA was supplied in the form of MFP (Novus International, Inc., St. Charles, MO).

³23 ± 7 d prepartum.

⁴27 ± 7 d postpartum.

⁵74 ± 7 d postpartum.

⁶97 ± 7 d postpartum.

⁷132 ± 7 d postpartum; 35 d post-AI.

⁸193 ± 7 d postpartum.

(1978), and Clanton and England (1980) reported all beef cows numerically gained weight by the conclusion of the research trial which differed from the present trial as cows continued to lose weight until weaning in our experiment. Differences in the time of year or season may have had an effect on the differences in BW gains. Cows from previous studies (Varner, 1974; Thomas and Langford, 1978; Clanton and England, 1980; Krehbiel et al. 2001) were spring calving beef cows, and spring forage quality tends to increase as the season progresses. Cows in the present study calved in the fall, and forage quality tends to decline as the season progresses. Differences in cow BW gains may have been partially attributed to seasonal conditions.

No significant differences in weight changes across previous experiments (Varner, 1974; Clanton and England, 1980) would potentially indicate that the supplemental methionine was generally not utilized for cow BW gain. Previous research with similar cow performance results would suggest that the AA, such as methionine, were being partitioned for milk synthesis in dairy cows (Chen et al., 2011). Cow BW loss would indicate supplementation was required; however, the parameters measured did not indicate that supplemen-

Table 5. Effects of supplementation of MHA to fall-calving beef cows on milk composition and component production

Item	Treatment ¹		SE	P-value
	CON	MHA ²		
<i>n</i> , pen	6	6	—	—
Milk production, ³ kg·d ⁻¹	5.94	5.76	0.48	0.76
Fat, ⁴ %	1.69	1.82	0.17	0.50
Fat, ⁴ kg·d ⁻¹	0.10	0.10	0.01	0.92
Lactose, ⁴ %	4.85	4.86	0.15	0.94
Lactose, ⁴ kg·d ⁻¹	0.29	0.28	0.03	0.68
Protein, ⁴ %	3.10	3.27	0.18	0.41
Protein, ⁴ kg·d ⁻¹	0.18	0.18	0.02	0.88
Other solids, ⁴ %	5.76	5.78	0.14	0.89
Other solids, ⁴ kg·d ⁻¹	0.34	0.33	0.03	0.69
Total solids, ⁴ %	10.50	10.85	0.20	0.21
Total solids, ⁴ kg·d ⁻¹	0.63	0.62	0.05	0.86
Milk Urea Nitrogen, ⁴ mg·dL ⁻¹	15.85	14.97	1.16	0.58

¹Wheat midd-based supplement was provided at 0.45 kg·cow⁻¹·d⁻¹. CON = contained no methionine hydroxy analog (MHA). MHA supplement provided 10 g MHA·cow⁻¹·d⁻¹.

²MHA was supplied in the form of MFP (Novus International, Inc., St. Charles, MO).

³Milk production determined via weigh-suckle-weigh technique at 73 ± 7 d postpartum on a subset of cows (45 cows per treatment).

⁴Milk composition conducted on a subset of cows at 73 ± 7 d postpartum (45 cows per treatment).

tation of MHA offered any increased performance. We speculate that energy was also limiting due to the continued decrease in cow BW, and if supplemental energy was provided in addition to the supplemental treatments we may have observed a different response.

Milk Production and Composition

Cow milk production and composition analyses are shown in Table 5. Milk production did not differ between treatments ($P = 0.76$). Milk production was approximately 1 kg·d⁻¹ lower than previously reported for this herd (Shoup et al., 2015). We speculate that energy intake may have limited milk production as well. There were no differences in any milk component parameters measured and, in turn, no differences in 24-h milk component production ($P \geq 0.21$). Results of this study differ from previous experiments. Varner (1974) reported increased milk production for beef cows receiving MHA at 5 g·cow⁻¹·d⁻¹ and 15 g·cow⁻¹·d⁻¹ compared to the control cows receiving no MHA. In addition, Varner (1974) noted a significant difference in 4% fat corrected milk between control cows and cows receiving MHA at 15 g·cow⁻¹·d⁻¹; cows receiving MHA had increased 4% fat corrected milk. Thomas and Langford (1978) and Krehbiel et al. (2001) reported no differences in milk production and milk composition between control

treatments receiving no MHA and treatments with MHA included in the supplement. Estimated average milk production was numerically similar to the results of this study. There were no differences ($P \geq 0.68$) in milk component production per day. In dairy cattle, supplementing a rumen-protected methionine increased milk production-related traits compared to control diets potentially by meeting essential AA requirements delivered in MP (Osorio et al., 2013). Additionally, a meta-analysis concluded that dairy cattle fed MHA have increased milk fat concentration and milk yield compared to dairy cows not receiving MHA (Zanton et al., 2014). Differences in results across previous research and results from this study could be attributed to differences in cattle breeds, meeting of nutrient requirements, addition of hay included with the forage source, and the inclusion rate of MHA in the supplement.

Calf Performance

Calf performance data from birth through weaning are shown in Table 6. There was no difference ($P = 0.95$) in calf birth BW between treatments. Varner (1974) reported similar results to the current study; calf birth BW did not differ among treatments when adjusted on a male calf basis. The fetus has an increased requirement for AA (Bell, 1995); however, previous literature suggests that increased demands do not necessarily correspond to increased protein deposition in muscle mass of the fetus due to no significant difference in birthweight (Battaglia, 1992; Bell, 1995). In summary, it was not surprising there were no differences in calf birth BW between treatments in this study.

Foreshadowed by the milk production and composition analysis, there were no differences in calf performance measures. The subset of calves weighed during the WSW (45·treatment⁻¹) did not differ in BW ($P = 0.74$) nor did the midpoint BW ($P = 0.80$) of all calves taken at 94 ± 7 d of age. Calf BW, taken at 193 ± 7 d, were again not different ($P = 0.85$) among treatments. Health status of calves in terms of morbidity and mortality did not differ ($P \geq 0.61$). Calculated calf ADG (Table 6) were not different ($P \geq 0.17$) over the course of experiment. In studies where calf performance differences were observed, there were, at minimum, substantial numeric differences in milk component analysis, particularly milk butterfat percentages. Thus, authors attributed the calf performance differences to improved cow milk composition (Varner, 1974; Thomas and Langford, 1978). Increased butterfat production per day has been positively associated with increased calf ADG among crossbred beef cows (Belcher and Frahm, 1979; Chenette and Frahm, 1981). Crossbred beef calves receiving more nutritionally dense milk are able to grow at

Table 6. Effects of supplementation of MHA to fall-calving beef cows on calf performance and calf ADG

Item	Treatment ¹		SE	P-value
	CON	MHA ²		
n, pen	6	6	—	—
Calf birth BW, kg	35	35	1.2	0.95
Calf BW at WSW, ³ kg	104	105	4.6	0.74
Calf interim BW, ⁴ kg	114	115	4.1	0.80
Calf weaning BW, ⁵ kg	149	149	4.9	0.85
Morbidity, %	27.2	27.8	1.1	0.61
Mortality, %	28.5	28.7	1.2	0.89
Calf ADG				
Birth to WSW	0.95	0.98	0.06	0.58
WSW to interim BW	0.69	0.69	0.06	0.90
Interim BW to weaning	0.33	0.31	0.03	0.48
WSW to weaning	0.42	0.46	0.04	0.17
Birth to weaning	0.59	0.59	0.02	0.86

¹Wheat midd-based supplement was provided at $0.45 \text{ kg} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$. CON = contained no methionine hydroxy analog (MHA). MHA supplement provided $10 \text{ g MHA} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$.

²MHA was supplied in the form of MFP (Novus International, Inc., St. Charles, MO).

³Collected at weigh-suckle-weigh (WSW 73 ± 7 d of age; 45 per dam treatment).

⁴ 94 ± 7 d of age.

⁵ 193 ± 7 d of age.

an increased rate compared to those who do not receive the same quality milk when nursing the dam even if quantity is greater (Brown and Brown, 2002). Similarly, Clanton and England (1980) observed no differences in calf gains or weaning BW among the calves born to dams supplemented MHA, especially when cows are nutritionally stressed. Unlike the results of this study and the Clanton and England (1980) study, Thomas and Langford's (1978) study reported calf weaning BW was improved among treatment groups of beef cows supplemented with MHA pre- or postcalving. Varner (1974) also reported increased ADG and weaning BW among beef calves when cows were supplemented MHA at a level of $15 \text{ g} \cdot \text{cow}^{-1} \cdot \text{d}^{-1}$ compared to cows that received the control treatment with no MHA included.

Reproductive Performance

Cow cyclicity at the end of supplementation and other reproduction performance measures are shown in Table 7. Supplement treatments had no effect ($P \geq 0.67$) on cyclicity or AI conception. Prior to synchronization and breeding, 74% of control treatment cows were cycling versus 77% of MHA treatment cows ($P = 0.69$). Artificial insemination conception rate was not different ($P = 0.67$) between treatments. Clanton and England (1980) reported contrasting results in Exp. 3 of their study. With increasing concentrations of MHA included

Table 7. Effects of MHA supplementation to fall-calving beef cows on cow reproductive performance

Item	Treatment ¹		P-value
	CON	MHA ²	
Cycling, %	74	77	0.69
AI conception, ³ %	51	47	0.67
Overall pregnancy, ⁴ %	78	73	0.50
AI fetal loss, ⁵ %	2.2	9.3	0.68
Fetal loss, ⁶ %	1.3	5.9	0.66

¹Wheat midd-based supplement was provided at 0.45 kg·cow⁻¹·d⁻¹. CON = contained no methionine hydroxy analog (MHA). MHA supplement provided 10 g MHA·cow⁻¹·d⁻¹.

²MHA was supplied in the form of MFP (Novus International, Inc., St. Charles, MO).

³Cows confirmed pregnant via ultrasound at 35 d post-AI.

⁴Cows confirmed pregnant via ultrasound and palpation at 33 d post-bull removal.

⁵AI fetal loss represented cows confirmed AI pregnant that were not pregnant at overall pregnancy check.

⁶Fetal loss represented cows confirmed pregnant that did not have a calf the following calving season.

in the supplement, there was a significant increase in percent cycling. Clanton and England (1980) also reported a 12% or greater increase in AI conception rate of beef cows fed 8 or 10 g·cow⁻¹·d⁻¹ in comparison to those receiving lower concentrations of MHA. In the Clanton and England (1980) discussion, authors mention that cows were more nutritionally stressed in Exp. 3 compared to Exp. 1 and Exp. 2 where no significant differences in reproductive performance were observed among MHA treatments. Cows in Exp. 3 continued to lose weight during lactation postcalving across all MHA inclusion treatment levels. In contrast, cows in Exp. 1 and 2 had small numeric gains during the same period postcalving (Clanton and England, 1980). Experimental conditions of Exp. 3 are somewhat similar to that of the current study, where animals are under several nutritional deficiencies and had a treatment group where MHA was supplemented at 10 g·cow⁻¹·d⁻¹, yet failure to see benefits in reproductive performance may be limited by the severity of nutritional deficiency. Comparisons with other studies (Varner, 1974; Thomas and Langford, 1978; Krehbiel et al., 2001) are not possible because they did not report AI conception rate.

Cow BCS at time of breeding averaged 4.8 ± 0.16 for cows that received CON and 4.5 ± 0.11 for cows that received MHA. Ideally, a BCS ≥ 5 has been generally accepted as an adequate threshold for optimum beef cow reproductive performance (Meteer et al., 2015). Results of BCS at time of breeding would indicate that cows on both treatments were nutritionally challenged. There were no differences ($P = 0.50$) in overall pregnancy rate at time of weaning between the CON and MHA (78% and 73%, respectively). Varner

(1974), Thomas and Langford (1978), and Krehbiel et al. (2001) also reported no differences in overall pregnancy rate between treatments. However, these studies reported overall pregnancy rates numerically greater than results of the present study. Results of the present study would suggest that the opportunity for reproductive improvement did exist since pregnancy rates were lower than previous research and industry averages most likely due to the nutritional deficiency cows were experiencing. Cow AI fetal loss and fetal loss were not different ($P \geq 0.66$) between treatments.

Supplementing MHA pre- and postpartum to fall-calving, nutritionally-deficient cows grazing cool season forages resulted in no effects on cow performance, milk production and composition, or cow reproductive performance. Since milk production and composition was not affected by MHA supplementation, it was not surprising that calf performance did not differ either. These data suggest that pre- and postpartum supplementation of MHA may not provide additional performance to fall-calving cows grazing cool season pastures when energy and/or protein deficiency is present, nor improve performance of calves through normal weaning.

LITERATURE CITED

- Bach, A., G. B. Huntington, and M. D. Stern. 2000. Response of nitrogen metabolism preparturient dairy cows to methionine supplementation. *J. Anim. Sci.* 78:742–749. doi:10.2527/2000.783742x
- Battaglia, F. C. 1992. New Concepts in Fetal and Placental Amino Acid Metabolism. *J. Anim. Sci.* 70:3258–3263. doi:10.2527/1992.70103258x
- Beal, W. E., D. R. Notter, and R. M. Akers. 1990. Techniques for estimation of milk yield in beef cows and relationships of milk yield to calf weight gain and postpartum reproduction. *J. Anim. Sci.* 68(4):937–943. doi:10.2527/1990.684937x
- Belcher, C. G., and R. R. Frahm. 1979. Productivity of two-year-old crossbred cows producing three-breed cross calves. *J. Anim. Sci.* 49(5):1195–1206. doi:10.2527/jas1979.4951195x
- Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.* 73:2804–2819. doi:10.2527/1995.7392804x
- Bremer, V. R., S. M. Damiana, F. A. Ireland, D. B. Faulkner, and D. J. Kesler. 2004. Optimizing the interval from PGF to timed AI in the CoSynch+CIDR and 7-11 estrus synchronization protocols for postpartum beef cows. *J. Anim. Sci.* 82(Suppl. 2):106.
- Brown, M. A., and A. H. Brown, Jr. 2002. Relationship of milk yield and quality to preweaning gain of calves from Angus, Brahman, and reciprocal-cross cows on different forage systems. *J. Anim. Sci.* 80:2522–2527. doi:10.2527/2002.80102522x
- Chen, Z. H., G. A. Broderick, N. D. Luchini, B. K. Sloan, and E. Devillard. 2011. Effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows. *J. Dairy Sci.* 94:1978–1988. doi:10.3168/jds.2010-3578
- Chenette, C. G., and R. R. Frahm. 1981. Yield and composition of milk from various two-breed cross cows. *J. Anim. Sci.* 52(3):483–492. doi:10.2527/jas1981.523483x

- Clanton, D. C., and M. E. England. 1980. Methionine hydroxy analog in supplements for lactating beef cows. *J. Anim. Sci.* 51(3):539–543. doi:10.2527/jas1980.513539x
- Consortium. 1988. Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching. Consortium for Developing a Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching, Am. Soc. Anim. Sci. Assoc. Headquarters, Savoy, IL.
- Klopfenstein, T. 1996. Need for escape protein by grazing cattle. *Anim. Feed Sci. Technol.* 60:191–199. doi:10.1016/0377-8401(96)00977-7
- Krehbiel, C. R., G. D. Pulsipher, M. K. Petersen, J. E. Sawyer, and E. E. Parker. 2001. Use of urea, ruminally protected methionine, and fat alone or in combination in liquid supplements to enhance nutrient status and milk production in beef cows grazing native range. Final Rep. Novus Int., St. Charles, MO. p. 1–24.
- Kung, L., and L. M. Rode. 1996. Amino acid metabolism in ruminants. *Anim. Feed Sci. Technol.* 59:167–172. doi:10.1016/0377-8401(95)00897-7
- Lobley, G. E., T. J. Wester, A. G. Calder, D. S. Parker, J. J. Dibner, and M. Vázquez-Añón. 2006. Absorption of 2-hydroxy-4-methylthiobutyrate (HMTBA) and conversion to Met in lambs. *J. Dairy Sci.* 89:1072–1080. doi:10.3168/jds.S0022-0302(06)72175-0
- McCollum, M. Q., M. Vázquez-Anon, J. J. Dibner, and K. E. Webb. 2000. Absorption of 2-hydroxy-4-(methylthio) butanoic acid by isolated sheep ruminal and omasal epithelia. *J. Anim. Sci.* 78:1078–1083. doi:10.2527/2000.7841078x
- Meteer, W. C., D. W. Shike, and F. C. Cardoso. 2015. Prepartum and postpartum nutritional management to optimize fertility in beef cattle. *Acta Sci. Vet.* 43:1286.
- Ng-Kwai-Hang, K. F., and J. F. Hayes. 1982. Effects of potassium dichromate and sample storage time on fat and protein by milko-scan and on protein and casein by modified pro-milk Mk II method. *J. Dairy Sci.* 65:1895–1899. doi:10.3168/jds.S0022-0302(82)82435-1
- NRC. 1996. Nutrient requirement for beef cattle. 7th ed. Natl. Acad. Press, Washington, DC.
- NRC. 2016. Nutrient requirement for beef cattle. 8th ed. Natl. Acad. Press, Washington, DC.
- Osorio, J. S., P. Ji, J. K. Drackley, D. Luchini, and J. J. Loor. 2013. Supplementing Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. *J. Dairy Sci.* 96:6248–6263. doi:10.3168/jds.2012-5790
- Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. *J. Anim. Sci.* 46:740–745. doi:10.2527/jas1978.463740x
- Shoup, L. M., A. C. Kloth, T. B. Wilson, D. Gonzalez-Pena, F. A. Ireland, S. Rodriguwez-Zas, T. L. Felix, and D. W. Shike. 2015. Prepartum supplement level and age at weaning: I. Effects on pre- and postpartum beef cow performance and calf performance through weaning. *J. Anim. Sci.* 93:4926–4935. doi:10.2527/jas.2014-8564
- Thomas, O. O., and W. J. Langford. 1978. Methionine hydroxy analog in Beef Cow Supplements given pre- and post calving. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 29:454–455.
- Varner, L. W. 1974. Supplementary diets of beef cows with methionine hydroxy analog. *Montana Agric. Exp. Sta. Res. Rep.* 52:34.
- Waterman, R. C., C. A. Löest, W. D. Bryant, and M. K. Petersen. 2007. Supplemental methionine and urea for gestating beef cows consuming low quality forage diets. *J. Anim. Sci.* 85(3):731–736. doi:10.2527/jas.2006-425
- Wester, T. J., M. Vázquez-Añón, J. Dibner, D. S. Parker, A. G. Calder, and G. E. Lobley. 2006. Hepatic metabolism of 2-hydroxy-4-thiomethylbutyrate (HMTBA) in growing lambs. *J. Dairy Sci.* 89:1062–1071. doi:10.3168/jds.S0022-0302(06)72174-9
- Wright, J. G., W. E. Sharp, M. Compton, and R. Totusek. 1975. Supplemental value of MHA for range cows. *Oklahoma Agric. Exp. Stn. Anim. Sci.* 1975 Rep. Oklahoma State Univ., Oklahoma City. p. 22–24.
- Zanton, G. I., G. R. Bowman, M. Vázquez-Añón, and L. M. Rode. 2014. Meta-analysis of lactation performance in dairy cows receiving supplemental dietary methionine sources or postpartum infusion of methionine. *J. Dairy Sci.* 97:7085–7101. doi:10.3168/jds.2014-8220