Supplemental Protein Plus Ruminally Protected Methionine and Lysine for Primiparous Beef Cattle Consuming Annual Rye Hay

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ABSTRACT: Primiparous beef cows \( n = 35 \) Bos taurus, average initial BW of 498 kg were allotted to treatments in a split-plot designed experiment to determine the effects of supplemental ruminally protected amino acids on cow and calf productivity and metabolic changes in the cows. Cows were fed chopped annual rye hay at 1.5% of BW. The following treatments were used: 1) .8 kg soybean hulls, 1.4 kg ground corn, .6 kg soybean meal (CON); 2) 1.4 kg ground corn, 1.4 kg soybean meal (PRO); 3) PRO plus ruminally protected methionine and lysine (supplied 5 and 10 g, respectively; PRO1); and 4) PRO with twice the level of ruminally protected amino acids in PRO1 (PRO2). Cow weight gain was not different \( (P > .26) \) among treatments and averaged 1.2 kg/d for the 45 + 6 d before parturition. After parturition, cow weight gain did not differ \( (P > .47) \) between CON and PRO treatments, but it decreased quadratically \( (P < .01) \) with increasing level of ruminally protected amino acids. Total milk yield, protein, and fat \( (4 \text{ h}) \) were greater \( (P < .05) \) for cows consuming PRO supplements than for CON, whereas CON cattle tended \( (P = .11) \) to lose less body condition. Total milk protein showed a quadratic increase \( (P < .05) \) in response to level of ruminally protected amino acids that was the inverse of the quadratic response noted for cow weight gain. Serum urea N concentration was greater \( (P = .07) \) for cattle consuming additional protein. Metabolic hormones were not affected \( (P > .18) \) by dietary treatment, but they responded \( (P < .05) \) to changes in physiological state. Supplements with additional protein supported greater \( (P = .0001) \) milk urea N concentration and output. Milk urea N concentration increased \( (P < .05) \) and milk IGF-I decreased \( (P < .05) \) as the lactation period progressed. The measurement of CON and PRO diets revealed that supplements with additional soybean meal had greater \( (P < .05) \) DM and N degradation; the extent of forage DM and NDF degradation was similar \( (P > .05) \) among treatments. Production shifted away from body weight gain to increased milk protein production when daily supplementation of ruminally protected methionine and lysine increased from 5 and 10 to 10 and 20 g, respectively. This shift in production was not reflective of changes in the metabolic regulators measured.

Key Words: Amino Acids, Supplements, Productivity, Metabolic Studies, Hormones

Introduction

Supplementing lactating beef cows with protein that escapes ruminal degradation at levels calculated to supply 130 to 270 g/d amino acids postruminally increases body weight gain, but milk production decreases (Hunter and Magner, 1988; Forcherio et al., 1995) or does not change (Wiley et al., 1991; Rusche et al., 1993). These responses have often been achieved by providing protein in excess of requirements. Repartitioning nutrients away from milk into bodily tissues has been associated with changes in metabolic hormones (Dhuyvetter and Caton, 1996). Conversely, Brockman and Laarveld (1986) maintain that pregnancy and lactation complicate metabolic regulation because fetal growth and milk production are not under complete control of circulating maternal hormones. Furthermore, it is difficult to attribute the effects of nutrient repartitioning to the supply of one or more specific amino acids.

An alternate method of supplying the animal with amino acids is to feed ruminally protected amino acids (RPAA). Increasing the supply of RPAA may influence secretion of metabolic hormones involved in

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growth and lactation. For example, amino acids, such as lysine, affect secretion of a mRNA expression of IGF-I in cultured hepatocytes (Harp et al., 1991; Pao et al., 1993). The mammary gland may sequester IGF-I from arterial supplies (Prosser et al., 1989), increasing its concentration in first colostrum of cows (Vega et al., 1991). Furthermore, the use of RPAA in production studies with lactating dairy cows increased milk protein output 5% (Robinson et al., 1995).

The objective of this experiment was to evaluate the effect of supplemental ruminally protected methionine and lysine on cow and calf productivity, metabolic changes in the cows, and supply of IGF-I to neonatal calves.

### Experimental Procedures

#### General

Thirty-eight primiparous, crossbred (Bos taurus × Bos taurus) beef cows were used to evaluate supplemental ruminally protected methionine and lysine during the last trimester of gestation through 58 d postpartum. Cows were checked for pregnancy (rectal palpation) after the spring breeding season and before the second trimester of gestation; only pregnant cows were used. Before initiation of the experimental treatments, cows were group-fed hay and a common supplement for 14 d. Approximately 45 ± 6 d prepartum, cattle were allotted randomly to treatment; BW and days to parturition were evenly distributed across treatments. The cattle were fed chopped (2.54 cm) annual rye hay (Table 1) at approximately 100% of NRC (1984) requirements for a 450-kg, nursing, 2-yr-old cow. One of four experimental supplements was top-dressed onto the forage at each feeding. Supplemental treatments (Table 2) included 1) .8 kg of soybean hulls, 1.4 kg of ground corn, .6 kg of soybean meal (CON); 2) 1.4 kg of ground corn, 1.4 kg of soybean meal (PRO); 3) PRO plus ruminally protected methionine and lysine (supplied 5 and 10 g, respectively; PRO1; Prince Agriproducts, Quincy, IL); and 4) PRO with twice the level of ruminally protected amino acids of PRO1 (PRO2; Prince Agriproducts). The CON supplement included soybean meal to meet CP requirements of a high-producing beef cow in early lactation. Supplements containing greater quantities of soybean meal were formulated at 125% of CP requirements. One-half of the daily ration was offered individually at 0600 and the other half at 1800. Initially, nine cows were allotted to treatment; one additional cow was allotted to PRO1 and one to PRO2. However, two cows

### Table 1. Chemical composition of annual rye hay fed to primiparous beef cows supplemented with protein and ruminally protected amino acids

<table>
<thead>
<tr>
<th>Item</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>89.7</td>
</tr>
<tr>
<td>OM</td>
<td>91.6</td>
</tr>
<tr>
<td>N</td>
<td>1.8</td>
</tr>
<tr>
<td>NDF</td>
<td>82.4</td>
</tr>
<tr>
<td>ADF</td>
<td>49.1</td>
</tr>
</tbody>
</table>

### Table 2. Ingredient composition of supplements fed to primiparous beef cows consuming annual rye hay supplemented with protein and ruminally protected amino acids

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>PRO</th>
<th>PRO1</th>
<th>PRO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn</td>
<td>41.6</td>
<td>41.6</td>
<td>40.9</td>
<td>40.3</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>25.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>17.8</td>
<td>42.8</td>
<td>42.1</td>
<td>41.5</td>
</tr>
<tr>
<td>Cane molasses</td>
<td>5.1</td>
<td>5.1</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Ruminally protected methionine</td>
<td></td>
<td></td>
<td>.5</td>
<td>.9</td>
</tr>
<tr>
<td>Ruminally protected lysine</td>
<td></td>
<td></td>
<td>1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>TM salt</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Vitamin A, D, and E premix</td>
<td>.02</td>
<td>.02</td>
<td>.02</td>
<td>.02</td>
</tr>
<tr>
<td>Sodium selenite</td>
<td>.0075</td>
<td>.0075</td>
<td>.0075</td>
<td>.0075</td>
</tr>
</tbody>
</table>

^aTreatment: CON provided .8 kg of soybean hulls, 1.4 kg of ground corn, .6 kg of soybean meal daily; PRO provided 1.4 kg of ground corn, 1.4 kg of soybean meal daily; PRO1 = PRO plus 5 g of methionine and 10 g of lysine supplied postruminally; and PRO2 = PRO plus 10 g of methionine and 20 g of lysine supplied postruminally.

^bPrince Agriproducts; amino acids are protected from ruminal degradation using partially hydrogenated vegetable oil as a carrier.

^cContained 8,818,490 IU/kg of vitamin A, 1,763,698 IU/kg of vitamin D, and 2,646 IU/kg of vitamin E.
from PRO2 and one cow from the PRO1 treatment were removed from the study due to complications unrelated to the experimental treatments.

A composite hay sample was obtained via core sample (one core/bale; Forage Master, Yoder Enterprises, Sunriver, OR) and analyzed for chemical and nutrient content. Initial nutrient content of the hay was used to formulate the diets. Samples of hay and supplement were taken daily and composited. Daily composited samples were later analyzed for DM, ash, N (Leco Corp., St. Joseph, MI), NDF, and ADF (nonsequential procedures; Goering and Van Soest, 1970).

Four ruminally cannulated steers were used to determine DM and N disappearance of supplements as well as rate and extent of forage DM and NDF digestion in situ. All animals were used under the guidance of an approved University of Missouri Animal Care and Use Committee protocol. Steers were fed one of two dietary treatments (CON or PRO) in a switch-back design. Triplicate Dacron® bags containing 3 g of hay were incubated at 3, 6, 9, 12, 24, 48, and 72 h. One empty (blank) bag was included with each set of duplicate bags. After removal, bags were rinsed in cold tap water until the effluent was clear, after which bags were placed in a forced-air oven (55°C) for 48 h, then weighed. Residue remaining in the bag was analyzed for DM and N to derive in situ DM and N disappearance of supplements. Neutral detergent fiber was determined on residual forage for estimation of NDF disappearance.

Two adjoining three-sided pole barns (5 × 25 × 5 m) were available for housing the animals throughout the study. Pregnant cows were housed as a large group in the individual feeding barn until calving. Each animal was moved to a 4- × 4-m pen (jug) in a calving barn when the late stages of parturition were evident. The cow-calf pair remained in the jug until adequate dam-offspring bonding had occurred (generally 2 d). Cow-calf pairs were housed together in the extra barn for the remainder of the study. Individual feeding stanchions were only located in the barn housing preparum cows. Therefore, postpartum cows were moved for feeding and calves did not have access to their dams’ feed. Cows were placed into feeding stanchions and given equal proportions of hay and supplement twice daily (0600 and 1800; Table 3). Cattle remained in stanchions until the ration was consumed (approximately 2 h). Daily hay intake was adjusted based on weights taken on −7, 2, 16, and 30 d relative to parturition.

Beginning 45 ± 6 d prepartum, cows were weighed (unshrunk) and assessed for body condition score (BCS, 1-to-9-point scale; Wagner et al., 1988) by two experienced technicians on two consecutive days. The average of the two weights was considered the starting weight. Subsequent weights were obtained −7, 0, 2, 16, 30, and 58 d relative to parturition. Additional BCS were determined on d 0, 30, and 58 postpartum. Because BCS values did not differ (P > .70) between technician, BCS was averaged between technician. Subsequent weight change and BCS change were computed between each weigh period. Postpartum weight changes at d 16 were adjusted for conceptus weight using the weight recorded on d 2.

Commencing on d 2 postpartum, milk production was estimated using a modified weigh-suckle-weigh technique (Wiley et al., 1991; Triplett et al., 1995). After the morning feeding, cows were returned to their calves. Calves were allowed to suckle approximately .5 h, after which cows were separated from calves. Each cow was injected, i.m., with 30 USP units of oxytocin to stimulate milk let-down. Injection time was recorded (Beal et al., 1990), and cows were milked using a portable milking machine. After milk flow had ceased, the machine was removed and each quarter was hand-stripped of residual milk. Cows remained separated from calves until they were milked again 4 h later. Milk from the machine and that from hand stripping was combined and weighed. After mixing, two 50-mL samples were collected. One sample was

### Table 3. Chemical and nutrient composition of diets fed to primiparous beef cows supplemented with protein and ruminally protected amino acids

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>PRO</th>
<th>PRO1</th>
<th>PRO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>88.3</td>
<td>88.4</td>
<td>88.3</td>
<td>88.3</td>
</tr>
<tr>
<td>N</td>
<td>2.0</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>NDF</td>
<td>66.6</td>
<td>62.6</td>
<td>62.4</td>
<td>62.2</td>
</tr>
<tr>
<td>ADF</td>
<td>40.1</td>
<td>36.8</td>
<td>36.6</td>
<td>36.5</td>
</tr>
</tbody>
</table>

*aTreatment: CON provided .8 kg of soybean hulls, 1.4 kg of ground corn, .6 kg of soybean meal daily; PRO provided 1.4 kg of ground corn, 1.4 kg of soybean meal daily; PRO1 = PRO plus 5 g of methionine and 10 g of lysine supplied postruminally; and PRO2 = PRO plus 10 g of methionine and 20 g of lysine supplied postruminally.*
obtained before feeding the cattle on d
scheduled milking. Additional blood samples were
approximately 12 h postpartum and at the end of each
4-h intermission between milking.

Calves were weighed during the
postpartum. Calves were weighed during the
viously described on d 5, 9, 12, 16, 30, and 58
postpartum. Laboratory Analyses

Blood samples were collected from cows and calves
approximately 12 h postpartum and at the end of each
scheduled milking. Additional blood samples were
obtained before feeding the cattle on d –45 and –14
relative to parturition. Approximately 10 mL of whole
blood was collected from a coccygeal blood vessel
(cows) or the jugular vein (calves) and allowed to clot
overnight. Clotted blood samples were centrifuged at
2,000 × g and decanted serum was stored at −20°C.

Laboratory Analyses

After thawing at 5°C, milk samples were
centrifuged at 1,120 × g for 20 min. The fat layer was
removed, and aliquots of supernatant were analyzed for
urea N and IGF-I concentrations. Serum metabolites
were determined in serum samples collected from
cows on d –41, –14, 0, 30, and 58 relative to
parturition. Colorimetric methods were used to deter-
mine milk and serum urea N (Sigma Chemical Co.,
St. Louis, MO) and NEFA (NEFA-C, Wako Chemi-
cals, Dallas, TX; Drackley et al., 1991). Serum amino
acid concentrations were determined, using stan-
dardized methods (AOAC, 1990), on cow samples
collected 14 d before and 30 d after parturition.
Specifically, a single oxidation, rapid hydrolysis pro-
cedure (Gehrke et al., 1987) was used to analyze for
concentrations of methionine and lysine.

Serum collected from cows on d –41, –14, 0, 30, and
58 relative to parturition was analyzed for insulin,
GH, and IGF-I. Insulin-like growth factor I was
determined on all calf serum samples.

Double antibody radioimmunoassay (RIA) pro-
cedures were used to determine concentrations of
insulin, growth hormone, and IGF-I. Duplicate serum
samples (300 μL) were assayed for insulin concentra-
tion following procedures outlined by Lalman (1996).
Standards and pooled aliquots of bovine serum were
linear (log/logit transformation) and parallel over a
mass of 2,000 to 10 pg/tube and a serum volume of 50
to 300 μL, respectively. Inter- and intraassay CV were
10 and 6%. Growth hormone was analyzed using the
RIA method described by Powell and Keisler (1995),
which was validated for use with bovine serum
(Lalman, 1996). Intra- and interassay CV were 5 and
10%. Concentrations of serum and milk IGF-I were
determined by methods of Vicini et al. (1991). The
RIA was validated in our laboratory (Coleman, 1996)
using bovine serum. Sensitivity of the assay was
determined to be .098 ng/mL; inter- and intraassay CV
were 7.1 and 4.5%. Concentrations of IGF-I in milk
samples collected on and after d 16 often fell below
detectable limits of the assay; therefore, only data
from d 0 to 12 are reported.

Calculations and Statistical Analyses

The 72-h incubation time was deemed to be long
enough for extent of forage degradation. Rates of DM
and NDF disappearance were calculated using non-
linear regression as described by Mertens and Loften
(1980). The nonlinear model included a coefficient for
discrete lag, which was included in the statistical
analysis. Data from the in situ digestion trial were
analyzed as a switch-back design (SAS, 1988).

The remaining data were analyzed as a split-plot in
a completely randomized design (Wilcox et al., 1990)
with treatment (type of supplement) as the main-plot
tested against animal within treatment (error a) and
the day of sampling effect and the treatment ×
sampling day interaction as the sub-plot tested
against residual error (error b). If a significant F-test
was detected (P < .05), sampling day effects and
interactions of treatment × sampling day were evalu-
ated using least significant difference procedures
(Steel and Torrie, 1980). Treatment differences were
evaluated by pooling data across days when interac-
tions were not significant (P > .10). Orthogonal
contrasts (CON vs PRO supplements; linear and
quadratic effects within PRO supplements) were used
to partition treatment sums of squares (Steel and
Torrie, 1980) and evaluate treatment differences. Two
cows from PRO2 and one cow from the PRO1
treatment were removed from the study because of
dystocia or problems unrelated to the experiment;
consequently, least squares means are reported.

Results and Discussion

Nitrogen content of the daily composited hay was .8
percentage units higher than that of core-sampled
hay; therefore, cattle were suspected to have con-
sumed more CP than originally anticipated.

In Situ Degradability

Replacing soybean hulls with soybean meal in the
supplement increased (P < .04) DM and N degradabil-
ity measured in situ (Table 4). Lower degradability
for the CON supplement may be related to higher
fiber and lower N content of the soybean hulls
compared to soybean meal. Lower 12-h DM degrada-
bility coupled with increased DM degradability from
12 to 24 h would suggest greater lag time before
initiation of supplement digestion for the CON supple-
Table 4. In situ degradability of supplements and annual rye hay fed to ruminally cannulated Holstein steers

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>PRO</td>
</tr>
<tr>
<td>Supplemental DM disappearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 h, %</td>
<td>55.6</td>
<td>75.1</td>
</tr>
<tr>
<td>24 h, %</td>
<td>83.8</td>
<td>89.4</td>
</tr>
<tr>
<td>Supplemental N disappearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 h, %</td>
<td>50.6</td>
<td>77.5</td>
</tr>
<tr>
<td>24 h, %</td>
<td>86.3</td>
<td>95.9</td>
</tr>
<tr>
<td>Forage^e DM disappearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 h, %</td>
<td>70.9</td>
<td>69.3</td>
</tr>
<tr>
<td>Disappearance rate, %/h</td>
<td>5.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Lag time, h</td>
<td>3.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Forage^e NDF disappearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 h, %</td>
<td>70.9</td>
<td>69.9</td>
</tr>
<tr>
<td>Disappearance rate, %/h</td>
<td>5.2</td>
<td>4.5</td>
</tr>
<tr>
<td>Lag time, h</td>
<td>3.9</td>
<td>2.9</td>
</tr>
</tbody>
</table>

^aPRO1 and PRO2 supplements were incubated in the rumen of steers fed the PRO supplement. ^bTreatment: CON = 25.1% soybean hulls, 41.6% ground corn, 17.8% soybean meal; PRO = 25.1% ground corn, 42.8% soybean meal; PRO1 = 40.9% ground corn, 42.1% soybean meal, .5% ruminally protected methionine, 1% ruminally protected lysine; and PRO2 = 40.3% ground corn, 41.5% soybean meal, .9% ruminally protected methionine, 1.9% ruminally protected lysine. ^cObserved significance level of contrast: 1 = CON vs PRO treatments; 2 = linear effects within PRO treatments; 3 = quadratic effects within PRO treatments. ^dIn situ degradability of forage was assumed to be the same for all PRO treatments.

Soybean meal protein is rapidly and extensively fermented in the rumen (NRC, 1996). The increase in DM degradability from 12 to 24 h for CON is within the 24 to 41% range reported for soybean hulls (Hsu et al., 1987 and Highfill et al., 1987, respectively).

Increased digestibility from protein supplementation is negligible when dietary CP is above 6 to 8% (Paterson et al., 1996). Including soybean meal instead of soybean hulls in the supplement did not affect (P > .37) in situ forage digestion. No differences in extent (72 h) and rate of in situ NDF disappearance due to supplemental protein have been reported for steers consuming fescue hay of similar quality (Judkins et al., 1991).

Cow and Calf Productivity

Dietary treatment x sampling day interactions were not detected (P > .10) for changes in cow body weight and condition when considering all of the collection dates; however, postpartum cow body weight was affected (P = .10) by a treatment x sampling day interaction (Figure 1). Cows receiving PRO were the heaviest (P < .05) 16 d postpartum. Cows supplemented with PRO1 maintained (P > .10) body weight, and CON and PRO2 cows lost (P < .01) weight from d 30 to 58. Cows consuming CON tended to lose less (P < .11) body condition (Table 5). Increasing supply of methionine and lysine postruminally did not further affect (P > .34) changes in body condition. Changes in body condition, however, exhibited numerical trends similar to postpartum body weight changes. Cows lost (P < .05) approximately one-half a body condition score between initiation of

![Figure 1. Body weight changes of primiparous beef cows fed annual rye hay and one of four supplements: CON provided .8 kg of soybean hulls, 1.4 kg of ground corn, .6 kg of soybean meal daily; PRO provided 1.4 kg of ground corn, 1.4 kg of soybean meal daily; PRO1 = PRO plus 5 g of methionine and 10 g of lysine supplied postruminally; and PRO2 = PRO plus 10 g of methionine and 20 g of lysine supplied postruminally. Pooled SE = 3.6.](image-url)
the study to parturition and again from d 30 to 58. Each unit of BCS change has been associated with a 33-kg change in BW for primiparous cows (Lalman et al., 1997). In contrast to the present results, dam weight and BCS change were not affected by increasing levels of supplemental protein that escapes ruminal degradation (UdP) when fed to primiparous or multiparous cows grazing rye-ryegrass overseeded Coastal bermudagrass pastures (Triplett et al., 1995). Moreover, Rusche et al. (1993) reported increased daily gain without changes in body condition when protein level increased from 100 to 150% of NRC requirements; weight gain increased 55% with a 23% increase in milk fat output. Dietary protein level did not seem to influence the responses and ranged from 14.3 to 17.4% across the studies reviewed. Recently, Koch et al. (1996) reported increased milk protein for primiparous Holstein cows consuming a 17.2% CP diet supplemented with 11.7 and 14.6 g of ruminally protected methionine and lysine, respectively. In the present study, supplying 5 g of methionine and 10 g of lysine post-ruminally seemed to have redirected nutrients away from milk production and toward bodily tissues. This effect was overridden as supply of RPAA was doubled; increasing post-ruminal methionine and lysine supply supported greater milk protein and fat synthesis. This is evidenced by numerically lower milk output but higher milk fat and protein percentages for PRO2 (4.3 and 3.5%) than for PRO (4.2 and 3.4%).

Increased milk protein synthesis by cows supplemented with RPAA is understandable because of the high metabolic requirement for lysine during milk protein synthesis (King et al., 1991). Oldham (1984) suggested that supplemental dietary methionine is associated with an increase in milk fat production. This response is most likely related to methionine’s role as a methyl donor in transmethylation reactions occurring during lipid biosynthesis (Mayes, 1981). Seymour et al. (1990) demonstrated that plasma methionine concentration was depressed, while the concentrations of plasma taurine and cystathionine (metabolites of the transulfuration pathway) were increased when 11.3 g of L-methionine was infused into the abomasum of lactating dairy cows. Despite differences in dams’ milk production, growth performance for calves was not affected (P > .10) by dams’ dietary treatment (data not shown). In agreement with our results, no apparent relationship

Table 5. Effect of dietary treatment and day of sampling on body condition score and change in primiparous cows

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Contrast</th>
<th>Day</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON PRO PRO1 PRO2</td>
<td>1 2 3</td>
<td>0 30 58</td>
<td></td>
</tr>
<tr>
<td>Initial body condition score</td>
<td>6.1</td>
<td>6.1</td>
<td>6.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Body condition score change</td>
<td>−.25</td>
<td>−.37</td>
<td>−.35</td>
<td>−.42</td>
</tr>
</tbody>
</table>

*Treatment: CON provided 0.8 kg of soybean hulls, 1.4 kg of ground corn, 5.6 kg of soybean meal daily; PRO provided 1.4 kg of ground corn, 1.4 kg of soybean meal daily; PRO1 = PRO plus 5 g of methionine and 10 g of lysine supplied post-ruminally; and PRO2 = PRO plus 10 g of methionine and 20 g of lysine supplied post-ruminally.

*Observed significant level of contrast: 1 = CON vs PRO treatments; 2 = linear effects within PRO treatments; 3 = quadratic effects within PRO treatments.

Day of sampling relative to parturition.

Within a row, means lacking a common superscript differ at the P-value noted.

Milk production measurements were not affected (P > .10) by dietary treatment × sampling day interactions. Increasing supplemental protein level increased (P < .05) total milk production and milk fat and protein yield (CON = 1,395.7, 52.2, and 45.3 g/4 h; PRO = 1,756.1, 75.4, and 59.7 g/4 h; PRO1 = 1,597.9, 64.3, and 52.4 g/4 h; and PRO2 = 1,624.7, 66.4, and 57.3 g/4 h). Within PRO treatments, a quadratic response was noted (P < .03) for milk protein content and yield. The first level of amino acid addition decreased milk protein, but milk protein was similar between PRO and PRO2. Similar increases in milk yield have been reported for primiparous beef cows receiving 150% of CP requirements (Rusche et al., 1993). In a summarization of reported literature on the use of encapsulated RPAA in production studies with lactating dairy cows, Robinson et al. (1995) report a 5% increase in milk protein output and a 2.3% increase in milk fat output. Dietary protein level did not seem to influence the responses and ranged from 14.3 to 17.4% across the studies reviewed. Recently, Koch et al. (1996) reported increased milk protein for primiparous Holstein cows consuming a 17.2% CP diet supplemented with 11.7 and 14.6 g of ruminally protected methionine and lysine, respectively. In the present study, supplying 5 g of methionine and 10 g of lysine post-ruminally seemed to have redirected nutrients away from milk production and toward bodily tissues. This effect was overridden as supply of RPAA was doubled; increasing post-ruminal methionine and lysine supply supported greater milk protein and fat synthesis. This is evidenced by numerically lower milk output but higher milk fat and protein percentages for PRO2 (4.3 and 3.5%) than for PRO (4.2 and 3.4%).

Increased milk protein synthesis by cows supplemented with RPAA is understandable because of the high metabolic requirement for lysine during milk protein synthesis (King et al., 1991). Oldham (1984) suggested that supplemental dietary methionine is associated with an increase in milk fat production. This response is most likely related to methionine’s role as a methyl donor in transmethylation reactions occurring during lipid biosynthesis (Mayes, 1981). Seymour et al. (1990) demonstrated that plasma methionine concentration was depressed, while the concentrations of plasma taurine and cystathionine (metabolites of the transulfuration pathway) were increased when 11.3 g of L-methionine was infused into the abomasum of lactating dairy cows. Despite differences in dams’ milk production, growth performance for calves was not affected (P > .10) by dams’ dietary treatment (data not shown). In agreement with our results, no apparent relationship
between milk production and gain in calves has been reported when beef cows were supplied with similar levels of amino acids using 200 or 250 g of supplemental UIP while consuming endophyte-infested tall fescue (Forcherio et al., 1995) or medium-quality grass hay (Wiley et al., 1991), respectively.

**Metabolites and Metabolic Hormones**

Dietary treatment $\times$ sampling day interactions were not detected ($P > .10$) for any metabolite or metabolic hormone. Serum urea N (SUN) increased ($P = .07$) in cows receiving additional protein, but it was not affected ($P > .77$) by additions of RPAA (CON = 11.7 mg/dL; PRO = 13.7 mg/dL; PRO1 = 13.8 mg/dL; and PRO2 = 13.4 mg/dL). No other serum metabolite or metabolic hormone was affected ($P > .18$) by dietary treatment. The increase in SUN concentrations is probably best explained by increased absorption of ruminal ammonia, resulting from greater protein fermentation in the rumen as indicated by an increase in situ N degradability. Increased quantities of absorbed ammonia enhance urea formation in the liver (Kennedy and Milligan, 1978). In this regard, Rusche et al. (1993) reported increased plasma urea N when CP intake increased from 100 to 150% of requirements, and urea N in the plasma increased to a greater degree with highly degradable protein. Although the decrease in serum methionine and lysine for PRO1 and PRO2 cattle was not of statistical relevance (CON = 1.3 and 6.3 mg/5 mL; PRO = 1.3 and 7.0 mg/5 mL; PRO1 = 1.2 and 6.7 mg/5 mL; and PRO2 = 1.2 and 6.6 mg/5 mL), the decrease may have biological implications. Depressed serum amino acid concentration without elevated SUN suggests increased removal from the serum to support metabolic activities (e.g., protein synthesis). Schwab et al. (1992) noted increased milk production and depressed plasma concentrations of essential and nonessential amino acids in lactating dairy cattle receiving duodenal infusions of methionine plus lysine. In the present study, the first level of amino acids supported body weight gain, and the next level of amino acid addition supported milk protein and fat synthesis.

Serum metabolites reflected changes in physiological state (Table 6). Serum urea N was greatest ($P < .05$) 14 d prepartum, decreased at parturition, and increased again 58 d postpartum. Serum NEFA concentrations also tended ($P < .10$) to increase 58 d postpartum. Concentrations of serum methionine and lysine were depressed ($P < .05$) on d 30 compared with 14 d prepartum. Greater SUN concentration 14 d prepartum was to be expected, provided that dietary protein exceeded requirements by approximately 130 (CON) and 160% (PRO supplements). Lower circulating amino acid concentrations during lactation suggest increased removal of these amino acids from the serum as demonstrated by Seymour et al. (1990) and Schwab et al. (1992). The changes in SUN (58 d) and NEFA concentrations reflect differences in BW and BCS, because cows seemed to have mobilized body reserves to support lactation. Serum NEFA concentrations have been shown to be negatively correlated with energy balance (Erfle et al., 1974), and increased NEFA are an indication of mobilization of body fat (Blauwiel and Kincaid, 1986).

All metabolic hormone concentrations were greatest ($P < .05$) at parturition. On d 58, concentrations of GH remained higher ($P < .05$) than prepartum values, but serum IGF-I decreased to pretreatment levels. Growth hormone has been shown to stimulate the production and secretion of IGF-I from the liver (Thissen et al., 1994), which may have increased serum IGF-I in cows at parturition. Ryan et al. (1995) reported an uncoupling of the GH/IGF-I axis during the puerperal period. This condition may result from reduced GH receptor action (Breier et al., 1988) and(or) population (Hayden, 1992), leading to decreased IGF-I and increased GH. Furthermore, uncoupling of the GH/IGF-I axis accompanied by inadequate nutrient supply may limit the expression

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### Table 6. Changes in serum metabolites and metabolic hormones relative to parturition in primiparous beef cows supplemented with protein and ruminally protected amino acids

<table>
<thead>
<tr>
<th>Item</th>
<th>Day of sampling</th>
<th>SE(^a)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-45</td>
<td>-14</td>
<td>0</td>
</tr>
<tr>
<td>Urea N, mg/dL</td>
<td>7.8(^b)</td>
<td>20.9(^c)</td>
<td>11.8(^c)</td>
</tr>
<tr>
<td>Methionine, μg/5 mL</td>
<td>—</td>
<td>1.3(^c)</td>
<td>—</td>
</tr>
<tr>
<td>Lysine, μg/5 mL</td>
<td>—</td>
<td>7.2(^c)</td>
<td>—</td>
</tr>
<tr>
<td>NEFA, mEq/dL</td>
<td>.58(^c)</td>
<td>.34(^b)</td>
<td>.45(^b)</td>
</tr>
<tr>
<td>Growth hormone, ng/mL</td>
<td>3.5(^b)</td>
<td>4.3(^c)</td>
<td>.62(^d)</td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>59.9(^b)</td>
<td>94.0(^c)</td>
<td>101.4(^d)</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>22(^b)</td>
<td>37(^c)</td>
<td>.51(^d)</td>
</tr>
<tr>
<td>Insulin:ghormone ratio</td>
<td>.10</td>
<td>.12</td>
<td>.12</td>
</tr>
</tbody>
</table>

\(^{a}\)n = 170.

\(^{b,c,d}\)Within a row, means lacking a common superscript differ at the P-value noted.
of growth hormone's anabolic effects (Buonomo and Baile, 1991). Oda et al. (1989) reported similar changes in GH and insulin concentrations around parturition in dairy cows fed a basal diet of orchardgrass hay. As a cow prepares for calving, her metabolic hormone status changes to reduce nutrient uptake by maternal tissues in order to redirect nutrients to the fetus, and toward milk production during lactation (Hart, 1983). Growth hormone may facilitate these processes by inducing insulin resistance (Sechen et al., 1990). Similar insulin:GH ratios noted in the present study support the conclusion of Brockman and Laarveld (1986), who indicated that lactation and pregnancy complicate metabolic regulation because milk production and fetal growth are not under complete control of circulating maternal hormones. It is not possible to attribute production responses to a single metabolic regulator, rather, the combined effects of elevated GH, decreased IGF-I, and perhaps resistance to insulin action seem to have been equally important in governing the effects observed during early lactation in the present study.

Dietary treatment × sampling day interactions were not evident (P > .10) for milk urea N (MUN). An interaction between supplemental treatment and sampling day was detected (P < .05) for milk IGF-I; however, the nature of the interaction did not preclude evaluation of main effects (changes in magnitude of differences but not direction). Additional protein increased (P = .0001) MUN, and there was a tendency (P = .08) for a linear increase in MUN concentration within supplemental protein groups (Table 7). Greater total milk yield for PRO, however, negated differences in total milk urea output within PRO-supplemented cattle. Because urea equilibrates within bodily fluids, MUN should be similar to SUN as an indicator of urea nitrogen status (Roseler et al., 1993). In a review of the literature, Harris (1996) found MUN values to be 83 to 98% of the urea N concentrations in the blood. Considering postpartum SUN concentrations only, mean concentrations of MUN were 89% of SUN for PRO, 87% for PRO1, 98% for PRO2, and 59% for CON. Sample preparation was identical among treatments, excluding the possibility of treatment differences arising from microbial activity as suggested by Harris (1996). Alternatively, the discrepancies between MUN and SUN concentrations are more likely related to protein intake and ruminal N degradability (Tables 3 and 4).

Calculating the MUN:SUN ratio from data of Roseler et al. (1993) illustrates that MUN levels were 68% of SUN concentrations for dairy cows fed diets at 80% of crude protein requirements, but they ranged from 78 to 86% for cows fed at 100 to 120% of CP requirements. Values obtained from both tests are influenced by meal time. Gustafsson and Palmquist (1993) showed that the maximum plasma urea N concentration is approximately 4 to 6 h after feeding, and peak MUN concentrations are delayed by approximately 1 to 2 h. Although protein degradation rates were not evaluated directly, the extensive ruminal N degradation of PRO supplements at 12 h coupled with the close relationship observed between SUN and MUN suggest that SUN and MUN were close to peak concentrations for the PRO treatments. The lower N degradability for CON at the 12-h incubation time would support the lower SUN and MUN concentrations observed. The time of sampling relative to feeding may have been appropriate to detect peak SUN concentrations but was perhaps too early to obtain maximum concentrations of MUN for CON cows.

There was a slight tendency (P = .14) for greater colostral IGF-I concentrations for PRO treatments, but IGF-I concentration and yield in the milk were not affected (P > .20) by dietary treatment. Other investigators have confirmed the presence of IGF-I in bovine colostrum (Ronge and Blum, 1988; Oda et al., 1989). The mean values and pooled standard error for colostral IGF-I concentration observed in the present

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Table 7. Effect of dietary treatment on milk urea N and IGF-I collected from primiparous beef cows

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Contrast&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SE&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>PRO</td>
<td>PRO1</td>
</tr>
<tr>
<td>Urea N, mg/dL</td>
<td>6.7</td>
<td>11.0</td>
<td>11.6</td>
</tr>
<tr>
<td>Urea output, mg/4 h</td>
<td>95.2</td>
<td>197.6</td>
<td>181.8</td>
</tr>
<tr>
<td>Colostrum IGF-I, ng/mL</td>
<td>195.0</td>
<td>247.7</td>
<td>249.4</td>
</tr>
<tr>
<td>Milk IGF-I&lt;sup&gt;d&lt;/sup&gt;, ng/mL</td>
<td>15.1</td>
<td>19.7</td>
<td>12.8</td>
</tr>
<tr>
<td>Milk IGF-I, µg/4 h</td>
<td>20.4</td>
<td>35.8</td>
<td>18.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Treatment: CON provided 8 kg of soybean hulls, 1.4 kg of ground corn, 6 kg of soybean meal daily; PRO provided 1.4 kg of ground corn, 1.4 kg of soybean meal daily; PRO1 = PRO plus 5 g of methionine and 10 g of lysine supplied postruminally; and PRO2 = PRO plus 10 g of methionine and 20 g of lysine supplied postruminally.

<sup>b</sup>Observed significance level of contrast: 1 = CON vs PRO treatments; 2 = linear effects within PRO treatments; 3 = quadratic effects with PRO treatments.

<sup>c</sup>n = 42.

<sup>d</sup>IGF-I concentration determined from samples collected on d 2 to 12 (n = 24).
Table 8. Changes in milk urea N and IGF-I secretion by primiparous beef cows supplemented with protein and ruminally protected amino acids during early lactation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Day of Lactation</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea N, mg/dL</td>
<td>2</td>
<td>9.5c</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.6c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10.1c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>10.3c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>11.2d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>12.3d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>12.3d</td>
<td></td>
</tr>
<tr>
<td>Urea output, mg/dL</td>
<td>2</td>
<td>145.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>165.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>156.0</td>
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<td></td>
<td>12</td>
<td>171.2</td>
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<td>16</td>
<td>170.0</td>
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<td>183.2</td>
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<tr>
<td></td>
<td>58</td>
<td>187.6</td>
<td></td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>2</td>
<td>40.7d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8.4c</td>
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</tr>
<tr>
<td></td>
<td>9</td>
<td>7.7c</td>
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<td>12</td>
<td>3.1c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IGF-I, µg/4 h</td>
<td>2</td>
<td>61.8d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>16.9c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>8.3c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5.2c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

*IGF-I concentration determined from samples collected on d 2 to 12 (n = 136); SE = 1.6 (ng/mL) and 2.3 (µg/4 h) if d-2 observations are deleted from the statistical analyses.

**n = 238.

Within a row, means lacking a common superscript differ at the P value noted.

- Study are consistent with results reported by Vega et al. (1991) using Holstein cows (233.8 ± 26.9). The importance of increased colostral IGF-I concentration is difficult to assess. Insulin-like growth factor I present in colostrum or milk can be absorbed and may have systemic effects on the neonate (Baumrucker and Blum, 1993; Baumrucker et al., 1994). Baumrucker et al. (1992) indicated that dietary IGF-I was absorbed by calves fed milk fortified with [125I]IGF-I. Feeding colostrum or mature cow’s milk to neonatal calves revealed that colostrum-fed calves had higher blood IGF-I concentrations (Gruetter and Blum, 1991). In our study, IGF-I levels in newborn (approximately 12 h after birth) calves (average 119.5 ± 10.4) were not affected (P > .20) by IGF-I concentration in the colostrum. Our finding agrees with results of Skaar et al. (1994), who reported that total blood IGF-I concentration was not affected by dietary IGF-I during the first 4 d of feeding calves colostrum, milk replacer, or milk replacer plus recombinant human IGF-I.

- Milk urea N and IGF-I concentrations as well as IGF-I secreted in the milk were influenced (P < .05) by day of sampling (Table 8). Milk content and secretion of IGF-I were greatest (P < .05) on d 2, but large variation within d 2 of sampling prevented detecting statistical differences between d 5 through 12. When d 2 was excluded from the analyses, milk IGF-I concentration and output declined (P < .05; SE = 1.6 and 2.3, respectively) from d 5 to 12. Milk urea N concentrations increased (P < .05) between d 16 to 30 and again from d 30 to 58. Vega et al. (1991) demonstrated that high concentrations of IGF-I in the first colostrum of the cow are established during the latter stages of the prepartum period. Others (Ronge and Blum, 1988; Prosser et al., 1989) have shown that IGF-I concentrations in milk are associated with blood concentrations and may follow immunoglobulin G1 secretion (Hadsell et al., 1993). Higher IGF-I in the milk on d 2 may be residual from the colostrum before the onset of lactation. Rapid declines in milk IGF-I have also been reported in dairy cows during the first (Oda et al., 1989) and second (Vega et al., 1991) week following parturition. The decline in milk IGF-I concentrations occurring after the colostral phase cannot be solely attributed to a dilution effect with the increase in milk production because total milk volume increases at a greater rate than the decline in milk IGF-I concentrations (Cambell and Baumrucker, 1989). Decreased milk IGF-I concentrations with the onset of lactation may also reflect changes in the mammary epithelium and its subsequent secretion of the IGF binding proteins (Baumrucker and Blum, 1993). Only on d 30 and 58 were MUN levels within the range (11 to 17 mg/dL) considered to be necessary for optimal nutrient utilization (Harris, 1996). Recently, Butler et al. (1996) demonstrated that urea N concentrations greater than 19 mg/dL in plasma and milk were associated with decreased pregnancy rate in dairy cows. The current study was terminated before reproductive traits could be evaluated.

- Implications

Production shifted away from body weight gain to increased milk protein production when daily supplementation of ruminally protected methionine and lysine increased from 5 and 10 to 10 and 20 g, respectively. Changes in physiological state had a more profound impact on serum metabolites and metabolic regulators than dietary treatment. Increased insulin-like growth factor I in the colostrum of cows fed protein above requirements was not sufficient to influence neonatal serum insulin-like growth factor I or calf growth performance. Supplying 5 g of methionine and 10 g of lysine postruminally in addition to supplemental protein above requirements seems to be an effective method to maintain body weight of primiparous beef cows during the early stages of lactation.

- Literature Cited


