Rumen Undegradable Protein, Rumen-Protected Choline and mRNA Expression for Enzymes in Gluconeogenesis and Ureagenesis in Periparturient Dairy Cows1

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ABSTRACT

The objective of this study was to profile mRNA expression of argininosuccinate synthetase (AS) and ornithine transcarbamylase (OTC), two enzymes that participate in the formation of urea in liver and compare these with changes in mRNA for pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) during the periparturient period in dairy cows. Forty-eight multiparous Holstein cows were fed isoenergetic prepartum diets that contained 10% RDP and either 4.0% RUP or 6.2% RUP and either 0, 6, or 12 g/d of rumen-protected choline (RPC) as CapShure (Balchem Corp., Slate Hill, NY). After calving cows received a common diet and continued RPC as per their prepartum assignments. Liver biopsies were obtained on d −28, −14, 1, 28, and 56 relative to calving, and the abundances of AS, OTC, PC, PEPCK, and 18S mRNA were determined by Northern blot analysis of total RNA. The abundance of OTC mRNA was lowest at calving and was decreased by RPC and 6.2% RUP feeding. Feeding 6.2% RUP did not alter AS, PC, or PEPCK mRNA. The expression of AS mRNA increased and PEPCK mRNA tended to increase from calving to 56 DIM. Pyruvate carboxylase mRNA increased more than twofold at calving. The data indicated adaptation to lactation for gluconeogenic enzymes that is not matched in direction and magnitude by changes in mRNA for urea cycle enzymes. Feeding additional protein, as RUP, failed to induce mRNA for key enzymes in gluconeogenesis or ureagenesis.

(Key words: ureagenesis, gene expression, transition dairy cows)

INTRODUCTION

The transition to lactation for the dairy cow is marked by decreased feed consumption, negative energy balance, mobilization of adipose tissue, and the potential for reduced protein balance (15). Decreased AA catabolism during gestation and early lactation in humans and rodents indicates a shift in metabolism to favor fetal growth, mammary protein synthesis, and maternal nitrogen gain (20, 33). Nitrogen metabolism during pregnancy is characterized by a reduction in AA catabolism, reduced rate of urea synthesis, reduced N excretion, and reduced AA catabolism during fasting or AA loading (20). Therefore, adaptations to nitrogen metabolism in periparturient dairy cows may partly counteract the effects of decreased feed intake during the transition to lactation and the possible shortfall in supply of AA.

The activities of urea cycle enzymes provide indices of protein status in ruminants (35) and nonruminants (31). Diet-dependent changes in carbamyl phosphate synthetase (29), argininosuccinate synthetase (AS; L-citrulline: L-aspartate ligase, EC 6.3.4.5) (30), and ornithine transcarbamylase (OTC; carboxymethylphosphate: L-ornithine carbamoyltransferase, EC 2.1.3.3; (29) activity are due mainly to altered abundance of their corresponding mRNA (31). The rate of urea synthesis is limited by the abundance of AS (26). Reduced basal AS activity during pregnancy (32) and a lower response to feeding high protein diets reflects more efficient protein metabolism in response to diet and pregnancy (32).

Ornithine transcarbamylase (OTC) is a mitochondrial enzyme that catalyzes the condensation of carbamylphosphate with ornithine to form citrulline. Long-term diet-induced changes in mRNA levels and activities of urea cycle enzymes are largely coordinate, but

Abbreviation key: AS = argininosuccinate synthetase, DRC = days relative to calving, MUN = milk urea nitrogen, PC = pyruvate carboxylase, PEPCK = phosphoenolpyruvate carboxykinase, PUN = plasma urea nitrogen, OTC = ornithine transcarbamylase, RPC = rumen-protected choline, TG = triacylglyceride.
differential responses to diet and hormones are sometimes observed. For example, the expression of OTC, in contrast to the other urea cycle enzymes, is not acutely responsive to cAMP or glucagon but is induced after chronic glucagon treatment (31). The expression and rate of transcription of the OTC gene are decreased in a rat model of visceral steatosis and fatty liver (42), and during biotin deficiency (22).

Fumarate, produced by the urea cycle, is metabolized in the citric acid cycle and provides a link between gluconeogenesis and ureagenesis (25). Impaired urea formation during hepatic steatosis (24, 40), increased liver triacylglyceride (TG) at calving (15), and elevated plasma ammonia (45) suggest compromised urea cycle activity during the transition to calving. Gluconeogenic substrates, pyruvate and lactate, stimulate the urea cycle (23), which may be critical to increased potential for gluconeogenesis from alanine at calving (34) but may require coordinately increased urea cycle capacity.

Triacylglyceride loading of bovine hepatocytes reduces ureagenesis (40) and decreases gluconeogenesis from propionate (3, 40), which mirrors the reduced gluconeogenesis during fatty liver in vivo (44). Hepatic lipidosis during the transition to calving (15) may compromise ureagenesis, gluconeogenesis, or both processes in liver.

Choline deficiency can rapidly induce hepatic fatty acid binding protein and fatty liver in rats within 1 d (10). Choline is a methyl donor in the synthesis of carnitine from methionine and lysine (14). Decreased hepatic fatty acid oxidation and reduced carnitine levels have been observed with choline deficiency (4). Dietary supplementation with carnitine alleviates fatty liver in the jvs mouse model of hepatic steatosis (43). Chronic hyperammonemia is partially corrected by L-carnitine feeding in Sparse-fur (spf) mutant mice with X-linked ornithine transcarbamylase deficiency (36), and carnitine and choline derivatives act to prevent ammonia toxicity in mice (28). Very little dietary choline escapes rumen degradation (8). Therefore, supplying choline in rumen-protected form during the transition to lactation may counteract the development of fatty liver and alleviate possible negative effects of hepatic lipid accumulation on the expression and, ultimately, the activities of urea cycle enzymes.

The objectives of this experiment were to determine changes in the expression of AS and OTC mRNA as indicators of urea cycle adaptation during the transition to lactation, to determine the effects of supplying additional RUP and rumen-protected choline on expression of ureagenic and gluconeogenic enzymes, and to examine the relationship between expression of urea cycle enzymes, gluconeogenic enzymes, and liver lipid content. To our knowledge, these data are the first to detail changes in mRNA expression for urea cycle enzymes in cows during the periparturient period.

MATERIALS AND METHODS

Animals and Management

Forty-eight cows entering their second or greater lactation were selected from the Purdue University Animal Science Research and Education Center. Cows were housed in individual tie stalls and were offered fresh feed once daily, had free access to water, were provided with 2 h of exercise daily and were milked twice daily at 0800 and 2000 h. Diet compositions, feeding procedures, and nutrient intake measurements and the nutrient intake and milk production data have been presented previously (16). Briefly, treatments consisted of a low RUP prepartum diet (4.0% of diet DM) and a high RUP prepartum diet (6.2% of diet DM) and three levels of RPC (0, 6, and 12 g/d) in a 2 × 3 factorial arrangement of treatments. Prepartum diets and rumen-protected choline (RPC) were fed beginning 28 d before expected calving. The RDP content was 10% of diet DM for the two diets (16). Differences in RUP were achieved through addition of AminoPLUS (Consolidated Nutrition, Decatur, IN) and RPC was supplied as Capshure choline (Balchem Corporation, Slate Hill, NY). After calving and through 120 d of lactation cows were fed a common diet and continued the RPC supplementation as per their prepartum assignments. As previously described (16), one cow was removed from each of the following treatment groups due to health problems 4.0% RUP, 0 g/d of RPC; 4.0% RUP, 12 g/d of RPC; and 6.0% RUP, 12 g/d of RPC consequently 44 cows completed the experiment. The Purdue Animal Care and Use Committee approved the animal handling and sample collection procedures.

Liver Tissue Collection and Analysis

Liver biopsy samples (500 to 1000 mg) were obtained for RNA analysis at 28 (28 ± 3) and 14 (14 ± 3) d before expected calving and 1, 28, and 56 d following calving. Samples were rinsed in saline, transferred to a tube containing guanidinium thiocyanate solution [4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.4), 0.5% sarcosyl, and 0.1 M beta-mercaptoethanol], frozen in liquid nitrogen, and stored at −80°C until RNA extraction (5). Total RNA (20 µg) was size separated by electrophoresis through a 1% agarose gel (41) and transferred to Genescreen (NEN Life Sciences, Boston, MA) membrane by capillary action (9).
cDNA Probes

The plasmid PC101, containing a 1.2-kb fragment of the human pyruvate carboxylase (PC) cDNA, was a gift from Isaiah Wexler (Dept. of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, OH). The plasmid pSK-PCK was a gift from Bruno Christ (Institute fur Biochemie, Fachbereich Medizin, Georg-August-Universitat Gottingen) and was constructed with a 1.2-kb SacI and HindIII fragment of rat phospho-enolpyruvate carboxykinase (PEPCK) provided by D. K. Granner (Vanderbilt University, Nashville, TN). The plasmid pAS4, containing a 1.5-kb fragment of the human AS cDNA, and the plasmid pOTC, containing a 1.2-kb fragment of human OTC were purchased from American Type Culture Collection (Rockville, MD).

The cDNA fragments were excised from plasmids by restriction enzyme digestion separated in a low-melting temperature agarose gel and purified with a Wizard DNA purification system (Promega, Madison, WI). Radiolabeled cDNA probes were generated with $^{32}$P[dCTP] and the Ready-to-go cDNA synthesis kit (Pharmacia, Piscataway, NY) to a specific activity of approximately $10^9$ cpm/µg of DNA.

Northern Blot Analysis

Following electrophoresis and transfer to Genescreen membrane (NEN Life Sciences, Boston, MA) RNA was cross-linked to the membrane with UV light, and the membrane was baked at 80°C for 2 h per the manufacturers recommendations. Membranes were prehybridized as previously described (9) for 12 h at 42°C. Hybridization was conducted by the addition of $^{32}$P-labeled cDNA (10$^6$ cpm/ml) to the prehybridization fluid. Membranes were hybridized for 20 h and then washed twice in 2× SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0) at room temperature for 5 min each, twice in 2× SSC, 1% SDS at 65°C for 30 min each, and twice in 0.1× SSC for 30 min at room temperature. Abundance of mRNA was visualized by autoradiography using Kodak X-Omat AR film (Eastman Kodak Co., Rochester, NY). Relative quantities of RNA were determined from digital images of the films using Kodak Digital Science 1D Image Analysis software (Eastman Kodak Co., Rochester, NY). Membranes were stripped in 0.1× SSC and 1% SDS at 100°C for 1 h and hybridized successively. Detection of PEPCK, PC, OTC, and AS mRNA required 12-, 18-, 24-, and 72-h exposures to film, respectively. Variations in gel loading and transfer of RNA were adjusted with the abundance of 18S rRNA determined within each sample. Due to a large number of samples (n = 220), multiple gels and Northern blots were necessary. To account for any differences between handling of gels or Northern blots, a 20-µg pool of bovine liver RNA was included on both sides of each row of samples within each gel. The expression of mRNA between membranes was normalized with mRNA abundance for the pooled samples.

Statistical Analysis

The data were analyzed as a randomized design using the GLM procedure of SAS (37) and cow (prepartum RUP × RPC) as the random effects term. The model accounted for days relative to calving (DRC), prepartum RUP, RPC, prepartum RUP × RPC, cow (prepartum RUP × RPC), RPC × DRC, prepartum RUP × DRC, and prepartum RUP × RPC × DRC. The effects of prepartum RUP, RPC, and prepartum RUP × RPC were analyzed using cow (prepartum RUP × RPC) as the F-statistic error term. Effects of DRC and prepartum RUP × RPC × DRC were analyzed using the residual error as the F-statistic denominator. Means differed if P < 0.05 and tended to differ if 0.05 ≤ P ≤ 0.15. The Duncan option of the means procedure of SAS was used for multiple comparisons. The relationship between liver triglyceride and expression of gluconeogenic and ureagenic enzymes was determined by calculating the ratio of mRNA to liver triglyceride at each biopsy sampling time and expressing the data as a fraction of the calculated ratio for each cow at −28 DRC. Repeated measures analysis was used within the GLM procedure of SAS to account for the effects of sequential biopsy within cows and to determine the nature of DRC effects. The values reported are LS means and standard errors.

RESULTS AND DISCUSSION

Bovine AS and OTC mRNA were detected as single transcripts of approximately 1600 and 1700 bp (Figure 1), which are similar to human AS (2) and human OTC mRNA (7), respectively. Binding of the PC and PEPCK probes used in this experiment to bovine mRNA has been demonstrated previously (12). The expression of OTC was similar on the d −28, −14, 28, and 56 relative to calving but decreased (P < 0.05) by more than 50% on the day of calving (Figure 2). There was a tendency (DRC effect; P = 0.08) for AS to increase by d 28 postcalving and to remain elevated to 56 DIM. The expression of AS did not mirror the decreases in plasma urea nitrogen (PUN) observed before calving (13) and did not follow PUN levels for 4.0 and 6.2% RUP prepartum diets in this study (16). Data for postcalving samples indicated an increase in AS during lactation that was not altered by RUP in the prepartum diet. The lack of an increase in AS when 6.2% RUP is fed points to lower sensitivity to dietary protein during late gestation. A reduction in dietary protein from 22 to 10% failed to decrease AS
activity after 7 d in rats but reduced AS by 50% after 14 d (6). Differences between protein amount and type fed in the present experiment and the duration of the feeding period may not be extreme enough to evoke changes in AS expression.

The decrease in OTC mRNA at calving is somewhat paradoxical. Reduced intake is accompanied by decreased activities of urea cycle enzymes in rodents. However, OTC activity and mRNA are induced by increased protein intake and glucocorticoids (31). Changes in expression and activity of OTC, in response to diet and glucocorticoids, are transient despite pro-

Figure 1. Northern blot analysis of total RNA from liver biopsy samples during the transition to calving from four cows. Liver biopsy samples were obtained on −28, −14, 1, 28 and 56 d relative to calving and 20 µg of total RNA was successively probed for PC, argininosuccinate synthetase (AS) and ornithine transcarbamylase (OTC) mRNA, and 18S rRNA. Autoradiographs were digitized to determine the density of each band and adjusted for variation in sample loading and transfer. Data are least squares means and standard errors for day relative to calving effects (n = 44 cows). Letters above bars for each transcript that are different indicate differences (P < 0.05) between days for each mRNA. Repeated measures analysis for days relative to calving indicates response functions: AS, linear (P < 0.05); OTC, quadratic (P < 0.05), quartic (P < 0.05); PC, linear (P = 0.07), quadratic (P < 0.05), quartic (P < 0.05); PEPCK, linear (P < 0.05), cubic (P < 0.05), and quartic (P < 0.05).

Figure 2. Effect of time relative to calving on phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate carboxylase (PC) mRNA abundance. Liver biopsy samples were obtained on −28, −14, 1, 28 and 56 d relative to calving and 20 µg of total RNA was successively probed for PC, argininosuccinate synthetase (AS) and ornithine transcarbamylase (OTC) mRNA, and 18S rRNA. Autoradiographs were digitized to determine the density of each band and adjusted for variation in sample loading and transfer. Data are least squares means and standard errors for day relative to calving effects (n = 44 cows). Letters above bars for each transcript that are different indicate differences (P < 0.05) between days for each mRNA. Repeated measures analysis for days relative to calving indicates response functions: AS, linear (P < 0.05); OTC, quadratic (P < 0.05), quartic (P < 0.05); PC, linear (P = 0.07), quadratic (P < 0.05), quartic (P < 0.05); PEPCK, linear (P < 0.05), cubic (P < 0.05), and quartic (P < 0.05).
lack of sensitivity of the urea cycle to prepartum protein intake, or overriding signals at calving to reduce OTC expression despite increased protein intake. Decreased ammonia detoxification has been observed with hepatocytes from rats that have been induced to develop fatty liver (24) and during lipid loading of bovine hepatocytes (40). Decreased feed intake and milk production have been noted when RUP and RDP are increased during the last 28 d of gestation in dairy cows (13, 16), indicating a physiological response to additional dietary protein during the transition to calving. Postcalving increases in AS mRNA indicate changes in hepatic metabolism that are sensitive to increased dietary protein in the common TMR or a delayed response to the onset of lactation, or both. The values of milk urea nitrogen (MUN) of 17 to 18 mg/dl in milk samples from cows used in the present experiment (16) indicate protein intake in excess of requirements (19), which corresponds with the postcalving increase in AS mRNA.

Decreased OTC expression has been observed during biotin deficiency and is linked to elevated plasma ornithine and glutamine concentrations but too little change in other urea cycle intermediates, AS activity or AS mRNA (22). It is generally thought that tissue biotin requirements for dairy cows are met by ruminal biotin synthesis; however, biotin supply may be limited as intake declines during the transition to calving. Recent data suggest beneficial effects of supplemental biotin for dairy cows (11, 46). Plasma and liver analysis to determine biotin status were not conducted in the present study. Therefore, establishing a direct link between biotin status and OTC expression in transition dairy cows awaits further investigation.

Pyruvate carboxylase mRNA increased \( (P < 0.05) \) during the transition to calving (Figure 2), but there were no effects of dietary treatment. There was a dramatic increase in PC expression at calving, which is similar to previous observations from our laboratory (12) and suggests increased capacity for lactate and AA metabolism by the liver during this period. There was little consistent change in PEPCK mRNA during the preparum and postpartum periods, although there was a DRC effect that was characterized by a slight increase at \(-14\) d, followed by a decrease on the day of calving and postcalving increase by 28 DRC that persisted to 56 DRC (Figure 2). A trend \( (P = 0.07) \) for a RPC \( \times \) DRC effect on PEPCK mRNA that is characterized by a slight increase at \(-14\) d relative to calving for cows receiving

### Table 1. Effect of day relative to calving, RUP and RPC on abundance of AS and OTC mRNA abundance.

<table>
<thead>
<tr>
<th>Day relative to calving</th>
<th>(-28)</th>
<th>(-14)</th>
<th>1</th>
<th>28</th>
<th>56</th>
<th>SE(^1)</th>
<th>Mean(^2)</th>
<th>SE(^3)</th>
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<tbody>
<tr>
<td><strong>Argininosuccinate synthetase</strong>(^4)</td>
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<tr>
<td>4.0% RUP</td>
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<tr>
<td>0 g/d of choline</td>
<td>0.96</td>
<td>1.48</td>
<td>1.13</td>
<td>1.19</td>
<td>1.28</td>
<td>0.19</td>
<td>1.20</td>
<td>0.09</td>
</tr>
<tr>
<td>6 g/d of choline</td>
<td>1.21</td>
<td>0.91</td>
<td>1.13</td>
<td>1.45</td>
<td>1.50</td>
<td>0.19</td>
<td>1.24</td>
<td>0.09</td>
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<tr>
<td>12 g/d of choline</td>
<td>1.08</td>
<td>0.86</td>
<td>1.01</td>
<td>1.03</td>
<td>1.21</td>
<td>0.19</td>
<td>1.04</td>
<td>0.09</td>
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<tr>
<td>Mean(^5)</td>
<td>1.08</td>
<td>1.08</td>
<td>1.09</td>
<td>1.22</td>
<td>1.32</td>
<td>0.11</td>
<td>1.16</td>
<td>0.09</td>
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<td>6.2% RUP</td>
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<td>0 g/d of choline</td>
<td>0.86</td>
<td>0.81</td>
<td>0.69</td>
<td>0.82</td>
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<td>0.18</td>
<td>0.84</td>
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<td>6 g/d of choline</td>
<td>0.75</td>
<td>1.27</td>
<td>1.02</td>
<td>1.71</td>
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<td>0.18</td>
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<td>1.16</td>
<td>0.89</td>
<td>1.50</td>
<td>1.32</td>
<td>1.29</td>
<td>0.19</td>
<td>1.23</td>
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<tr>
<td>Mean(^6)</td>
<td>0.92</td>
<td>0.99</td>
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<td>1.14</td>
<td>0.11</td>
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<td>0.09</td>
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<td><strong>Ornithine transcarbamylase</strong>(^7)</td>
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<tr>
<td>4.0% RUP</td>
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<tr>
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<td>2.27</td>
<td>4.29</td>
<td>0.72</td>
<td>1.57</td>
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<td>0.85</td>
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<td>0.46</td>
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<tr>
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<td>2.21</td>
<td>0.57</td>
<td>1.31</td>
<td>1.60</td>
<td>0.27</td>
<td>1.49</td>
<td>0.13</td>
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<tr>
<td>6.2% RUP</td>
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<tr>
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<td>1.51</td>
<td>0.55</td>
<td>1.50</td>
<td>1.47</td>
<td>0.44</td>
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<tr>
<td>6 g/d of choline</td>
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<td>0.56</td>
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<td>1.12</td>
<td>0.43</td>
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<tr>
<td>12 g/d of choline</td>
<td>1.48</td>
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<td>0.70</td>
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<td>1.27</td>
<td>0.46</td>
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<tr>
<td>Mean(^6)</td>
<td>1.38</td>
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<td>0.60</td>
<td>1.28</td>
<td>1.29</td>
<td>0.25</td>
<td>1.17</td>
<td>0.12</td>
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\(^1\)Pooled standard error for RPC \( \times \) RUP \( \times \) DIM.
\(^2\)Means values for RPC levels within RUP group.
\(^3\)Pooled standard error for RPC \( \times \) RUP effect.
\(^4\)Treatment effects: DIM, \( P = 0.08 \).
\(^5\)Means values for 4.0% RUP treatments.
\(^6\)Means values for 6.2% RUP treatments.
\(^7\)Treatment effects: DIM \( P < 0.05 \), RPC, \( P < 0.05 \), RUP = 0.08, RPC \( \times \) RUP = 0.07.
6 g/d of RPC contributed to the overall DRC effect. Causes of the effect of RPC on PEPCK during the prepartum period are not apparent. Analysis of treatments lacking RPC revealed no change in PEPCK mRNA during the prepartum period and a postpartum increase in PEPCK, which agrees with previous data (12).

Feeding RPC decreased OTC mRNA but did not alter PC, PEPCK, or AS mRNA abundance (Table 1). Production responses to RPC were not observed, although there was a reduction in TG accumulation in cows that were slightly overconditioned (BCS > 3.75 at 28 d before calving) (16). Using initial BCS as a blocking variable in analysis of the present data did not indicate any different effects of RPC on mRNA for gluconeogenic or ureagenic enzymes, and there was no interaction effect (prepartum RUP × RPC) for any of the transcripts measured.

Experimental models of fatty liver have lower ureagenic rates (40), lower activities of urea cycle enzymes (42), reduced mRNA expression of ureagenic enzymes (43), and, in some cases, reduced gluconeogenesis (3). To determine the relationship between gene expression and liver lipid content for the bovine we examined the ratios of ureagenic and gluconeogenic enzyme mRNA to liver TG during the transition to calving (Table 2). The ratios were calculated from the abundance of transcripts and liver TG content (µg of TG/100 µg of DNA) and are expressed as a fraction of the values for each cow 28 d before expected calving. The liver TG data for this experiment have been presented previously (16). There were no RPC, RUP, or interaction effects on these ratios. The ratio AS mRNA:TG decreased from −14 d prepartum to +1 d relative to calving. Changes in this ratio are primarily due to increased liver TG. Likewise, the ratio of OTC mRNA:TG followed a similar pattern, but because OTC decreased at calving, the OTC mRNA:TG ratio was reduced even more at calving. The ratio of PEPCK mRNA to TG was decreased (P < 0.05) at d 1 postpartum and then increased (P < 0.05) by d 56 postpartum. Changes in PEPCK mRNA:TG are also primarily the result of differences in liver TG. There is a 70% numerical decrease in the ratio of AS mRNA:TG, which indicates a potential limitation on ammonia clearance that may ultimately cause reduced gluconeogenic capacity (40). There was a DRC effect to decrease (P < 0.05) AS mRNA:TG at calving, which is primarily due to an increase in PC mRNA. The lack of change in AS at calving may limit gluconeogenesis from AA despite increased PC mRNA if there is inadequate disposal of N released through AA catabolism.

CONCLUSIONS

No main effects of protein and choline feeding on expression of the urea cycle enzymes AS and OTC were observed. A DRC effect on PEPCK and PC expression confirm that these enzymes respond to the onset of lactation. The lack of a protein effect on AS expression during the prepartum period, is particularly interesting in light of the responsiveness of the activity and mRNA for these enzymes to dietary protein intake in other species (32, 38, 39) and coordination of activity and mRNA expression (30). The differences in protein level in this study were perhaps not large enough to evoke changes in mRNA expression of urea cycle enzymes. The increases in enzyme ratio to TG were due to the increases in liver TG during this transition period. These data suggest the limitations in adaptation of the urea cycle, during the transition to lactation, may precipitate ammonia toxicity, especially if protein is overfed during late pregnancy.

REFERENCES


Table 2: Ratio of ornithine transcarbamylase (OTC), argininosuccinate synthetase (AS), pyruvate carboxylase (PC), and phosphoenolpyruvate carboxykinase (PEPCK) mRNA (arbitrary units) to liver triacylglyceride (mg of triacylglyceride (TG)/100 µg of DNA) during the transition to calving. The values are expressed relative to the ratio for each cow at −28 d relative to calving.

<table>
<thead>
<tr>
<th>Ratio</th>
<th>−28</th>
<th>−14</th>
<th>1</th>
<th>28</th>
<th>56</th>
<th>SE±</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS:TG2,3</td>
<td>1.00ab</td>
<td>1.68a</td>
<td>0.29b</td>
<td>0.16b</td>
<td>0.85ab</td>
<td>0.31</td>
</tr>
<tr>
<td>OTC:TG2,3</td>
<td>1.00b</td>
<td>1.47a</td>
<td>0.10c</td>
<td>0.15c</td>
<td>0.74b</td>
<td>0.17</td>
</tr>
<tr>
<td>PC:TG2</td>
<td>1.00b</td>
<td>1.67b</td>
<td>0.75ac</td>
<td>0.21c</td>
<td>0.99b</td>
<td>0.21</td>
</tr>
<tr>
<td>PEPCK:TG2,3</td>
<td>1.00b</td>
<td>1.26b</td>
<td>0.23b</td>
<td>0.18b</td>
<td>0.94a</td>
<td>0.13</td>
</tr>
</tbody>
</table>

a,b,c Means within rows with different superscripts differ (P < 0.05).
1 Pooled standard error for −28, −14, +1, +28, and +56 d relative to calving.
2 Quadratic DRC effect (P < 0.05).
3 Cubic DRC effect (P < 0.05).

