Plasma Concentration of Urea, Ammonia, Glutamine Around Calving, and the Relation of Hepatic Triglyceride, to Plasma Ammonia Removal and Blood Acid-Base Balance

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ABSTRACT

Two experiments were conducted to test the following two hypotheses: 1) fatty liver could hamper hepatic conversion of ammonia to urea and increase circulating ammonia or Gln% \[Gln% = \frac{Gln \times 100}{Gln + Glu}\] in cows around parturition; 2) decreased ureagenesis might cause alkalosis and in turn reduce blood Ca.

In the first experiment, 14 Holstein cows were monitored from 27 d prepartum to 35 d postpartum. There was a rise in circulating ammonia and Gln% at calving, suggesting an increase in ammonia passing to and through the liver. Stepwise regression analysis revealed the following relationship for plasma samples at 22 h and liver triglyceride at 2 d postpartum: ammonia (µM) = 32.1 + 0.89 triglyceride (% DM), Gln% = 71.2 + 0.23 triglyceride (% DM) + 1.31 urea (mM). The positive correlation between liver triglyceride and plasma ammonia and Gln% suggests that hepatic triglyceride accumulation might inhibit ureagenesis, thereby increasing ammonia concentration at the perivenous hepatocytes where Gln synthesis occurs and increasing ammonia concentration in blood leaving the liver.

In the second experiment, 28 rats were used to determine whether hepatic triglyceride accumulation, induced by choline deficiency, affects urinary ammonia N and blood pH homeostasis. There was a trend for a positive correlation between urinary ammonia N and liver triglyceride. No correlation between liver triglyceride and blood pH, bicarbonate, pCO2 or plasma Ca was found.

In conclusion, hepatic triglyceride accumulation may inhibit ureagenesis and result in increased circulating ammonia, Gln% and urinary ammonia N in vivo. Hepatic triglyceride accumulation did not affect blood pH homeostasis.

INTRODUCTION

In ruminants, ammonia is produced during the degradation of protein in the rumen and in the catabolism of amino acids in tissue. Two major pathways have evolved in the liver of mammals to detoxify ammonia: ureagenesis in the perportal region of the liver and Gln synthesis in the perivenous region of the liver (12). Ureagenesis is a low affinity system with a $K_m$ of 1 to 2 mM for ammonia, and Gln synthesis is a high affinity system with a $K_m$ of 0.2 mM for ammonia (27). These two systems work as an upstream and downstream pair, so that any ammonia escaped from ureagenesis can be sequentially removed by Gln synthetase. Circulating ammonia will increase only when both systems approach saturation. Plasma ammonia is 30 to 80 µM in ruminants (10, 14, 35). In ruminants, hyperammonemia is often associated with high nonprotein nitrogen feeding, e.g., urea feeding. Acute clinical toxicity occurs when plasma ammonia exceeds 470 µM in cattle (2). Subclinical hyperammonemia is more common and can affect intermediary metabolism (43). Plasma ammonia at three times normal physiological value was associated with hyperglycemia and hypoinsulinemia (9). A 30% increase of plasma ammonia due to excess CP intake was associated with impaired reproductive performance (17).

Fatty liver is common in cows in early lactation (34). Most of the net accumulation of triglyceride (TG) in the liver occurs by 24 h following calving, presumably in response to endocrine changes, depressed feed intake during the week prior to calving, and the dramatic elevation in plasma NEFA at parturition (4, 11, 41). Triglyceride remains elevated for at least 5 wk after calving because of sustained negative energy balance associated with lactation (41).
Triglyceride accumulation in bovine hepatocytes led to decreased ureagenesis in vitro (40, 44). Similar impairment of ureagenesis has been reported in different models of fatty liver in rats (5, 24) and in humans (18). There are very limited data in vivo on how fatty liver affects ammonia detoxification. We hypothesized that animals with fatty liver should have elevated circulating ammonia or Gln% \[ \text{Gln}\% = \frac{\text{Gln}}{\text{Gln} + \text{Glut}} \] caused by reduced ureagenesis from ammonia.

Ureagenesis is a way of removing bicarbonate and reducing blood pH (1, 13). Inhibition of urea synthesis by fatty liver might pose a threat of alkalosis to periparturient cattle even in the absence of direct ammonia toxicity. High blood pH reduces Ca mobilization from bone (3) and reduces blood Ca (33). Therefore, decreased urea synthesis might cause alkalosis and, in turn, reduce blood Ca. To date, there is no available data on the relationship between fatty liver and blood pH, bicarbonate, and Ca.

Our objectives were: 1) to study the correlation between liver TG and changes in plasma ammonia, Gln%, or Ca in dairy cattle around parturition; 2) to determine the effect of fatty liver, induced by choline deficiency in rats, on urinary ammonia-N to urea-N ratio, blood pH, bicarbonate, and plasma Ca.

MATERIALS AND METHODS

Two fatty liver models were used in the following experiments. Naturally occurring fatty liver in periparturient cattle was used in the first experiment. Feed restriction is frequently used to induce fatty liver in cows. However, feed restriction induces the activities of urea cycle enzymes in rats (37). Therefore feed restriction is not an appropriate fatty liver model for studying ureagenesis. Fatty liver, induced by choline deficiency in rats, has been used to study ureagenesis capacity (24), and was used in the second experiment. Animal protocols were approved by the Animal Care and Use Committee of the College of Agricultural and Life Sciences at the University of Wisconsin-Madison.

Experiment 1

Animals and diets. Three primiparous and 11 multiparous Holstein cows were monitored from 27 d prior to expected calving (−27 d) to 35 d postpartum (35 d). Cows were fed at 0800 h once daily. From the start of the experiment through 1 d postpartum, cows were fed 2.0 kg of alfalfa hay DM containing 13.4% CP, 1.2 kg of dry-cow grain mix DM (Table 1) and given ad libitum access to alfalfa silage containing 22% CP. From 2 to 4 d postpartum, cows were fed 2.0 kg of alfalfa hay DM containing 19.6% CP and given ad libitum access to a TMR consisting of 75% alfalfa silage, 5% cottonseed, and 20% lactating-cow grain mix DM (Table 1). From 5 to 35 d postpartum, cows were fed ad libitum a TMR consisting of 45% alfalfa silage, 7% cottonseed, and 48% lactating-cow grain mix DM (Table 1). Dry matter content of forage was determined weekly in a 100°C oven and a constant forage DM:grain DM ratio was maintained. Feed offered and refused was measured daily.

Sampling and analysis. At 12, 16, and 22 h after calving and at 1130 h on −27 and 35 d relative to calving, blood samples were collected into Na-heparinized vacuum tubes from the coccygeal vessel and placed on ice. Heparin reduces red blood cell ammonia production and is a potent inhibitor of adenylic acid deaminase (16, 19). Therefore, heparin is the recommended anticoagulant for plasma ammonia determination. Within 30 min after blood was drawn, plasma was obtained by centrifugation at 850 × g for 15 min at 4°C. A portion of the plasma was immediately stored in three aliquots at −80°C before being analyzed for plasma ammonia (Sigma Diagnostics kit #171-UV, Sigma Chemical Co., St. Louis, MO), urea (Sigma Diagnostics kit #640), and Ca (Sigma Diagnostics kit #587). The remainder of the plasma was deproteinized with an equal volume of 10% (vol/vol) perchloric acid, then centrifuged at 300 × g and

![](https://example.com/table1.png)

Table 1. Ingredient and nutrient compositions of dry-cow grain mix, total mixed ration for cows at 2 to 4 d and at 5 to 35 d postpartum.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Dry-cow grain mix</th>
<th>2 to 4 d postpartum (TMR)</th>
<th>5 to 35 d postpartum (TMR)</th>
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<tbody>
<tr>
<td>Ingredient</td>
<td></td>
<td>% of DM</td>
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<tr>
<td>Alfalfa haylage</td>
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<td>Ground shelled corn</td>
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<tr>
<td>Cottonseed</td>
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<td>Vitamin mixture</td>
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<tr>
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<td>Trace mineral salt #89</td>
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<td>Tallow</td>
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<table>
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<tr>
<th>Nutrient</th>
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<th>CP</th>
<th>UIP</th>
<th>ADF</th>
<th>NDF</th>
<th>EE</th>
<th>NSC</th>
<th>Ca</th>
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<td>5.12</td>
<td>29.95</td>
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</table>

1Contains 1,500,000 IU of vitamin A/kg, 500,000 IU of vitamin D/kg, and 5,000 IU of vitamin E/kg.
2Contains Na, 36.98%; Cl, 57.00%; Mg, 0.55%; Fe, 0.35%; Zn, 0.55%; Cu, 0.14%; Co, 0.002%; I, 0.008%; Se, 0.006%.
the supernatant was neutralized with 20% KOH with Fisher universal indicator, and the supernatant was stored at −20°C for analysis of Gln and Glu (23). Glutamine and Glu are stable at neutral pH at −20°C (23). On d −27, 2, and 35, liver biopsies were obtained (41) and stored at −20°C before being analyzed for liver TG (41).

Statistical analysis. Data were analyzed as repeated measures using the PROC MIXED procedure of SAS (36). Stepwise regression analysis was performed within each time using the PROC REG procedure in SAS (21). Stepwise regression analysis was performed whether a cow was an outlier. A dummy variable was treated as the cow was declared as an outlier if rest of the cows. The dummy variable was treated as the cow was declared as an outlier if rest of the cows.

Experiment 2

Animals. Twenty-eight rats were used in the experiment. They were male white albinos, wistar strain, 4 to 5 wk old and weighing 140 to 150 g (Harlan Sprague Dawley, Indianapolis, IN). Rats were housed individually in screen-bottom cages in a room with a 12-h light, 12-h dark light-schedule and an average room temperature 21 ± 1°C.

Diets. All ingredients were purchased from Harlan Teklad (Madison, WI) except that peanut flour was obtained from Seabrook Enterprises (Edenton, NC). Two choline deficient basal diets were formulated to contain 23.4% CP. Basal diets contained either hot ethanol-extracted peanut flower (PF) or soy protein isolate (SPI). The diet containing PF was formulated according to Chahl and Kratzing (7). To extract phospholipid, PF was placed in dacron bag and extracted with 60 to 70°C ethanol for 3 d, ethanol was refreshed every day. Diets based on SPI were used to induce fatty liver in rats (24). However, liver TG in response to a range of choline in the diet was not evaluated. Unlike PF, SPI is commercially available. If liver TG is correlated to supplemental choline intake in the SPI containing diet in rats, then SPI containing diet can be used in the future experiment.

Basal diets consisted of 6% casein (fat and vitamin-free), 15% beef fat, 5% maize oil, 4% mineral mixture, and 1% vitamin mixture. The PF diet contained 30% PF and 39% sucrose. The SPI diet contained 20% SPI and 49% sucrose. The vitamin mixture contained: thiamin hydrochloride, 50 mg; riboflavin, 25 mg; pyridoxine, 20 mg; Ca pantothenate, 90 mg; nicotinamide, 100 mg; folic acid, 5 mg; biotin, 5 mg; vitamin B12, 75 µg; 2-methyl-1,4-naphthoquinone, 10 mg; p-aminobenzoic acid, 1 g; vitamin E acetate (500 U/g), 250 mg; inositol, 5 g; vitamin A palmitate (500,000 U/g), 1 g; vitamin D3 (500,000 U/g), 0.1 g; made up to 100 g with fine sucrose. The mineral mixture, Hubbell-Mendel-Wakeman, was purchased from Harlan Teklad (Madison, WI). A 1-kg mineral mixture contained: CaCO3, 543 g; MgCO3, 25 g; MgSO4, 16 g; NaCl, 69 g; KCl, 112 g; KH2PO4, 212 g; FePO4·4H2O, 20.5 g; KI, 0.08 g; MnSO4, 0.35 g; NaF, 1 g; AlK(SO4)2·12H2O, 0.17 g and CuSO4, 0.9 g.

All other diets contained variable concentrations of choline by adding different amounts of choline bitartrate to the basal diet. Choline bitartrate was chosen over choline chloride because choline bitartrate does not have a direct effect on blood pH, while choline chloride will acidify the blood. Food and water were offered ad libitum.

Treatments. There were seven concentrations of supplemental choline (0, 0.015%, 0.03%, 0.045%, 0.06%, 0.075%, and 0.09% of diet DM) for each protein source. Seven choline concentrations and two sources of protein were arranged as 2 × 7 factorial. Twenty-eight rats were divided into two blocks and 14 rats within each block were randomly assigned to one of the 14 diets.

The experiment consisted of an adjustment period and a treatment period. Adjustment period was 6 d for one block and 9 d for the other block. Treatment period was 21 d for both blocks. During the adjustment period, all rats were fed the diet containing 0.09% choline and 30% PF. At 17 d after treatments began, rats were transferred to metabolism cages and allowed to adapt to the cages for 3 d. At 20 d after treatment began, 24 h urine samples were collected into 0.4 ml 5 M HCl to be acidified and stored in −20°C before being analyzed for urea N and ammonia N (Sigma Diagnostics kit #640). Urine volume was measured for each rat, and 24-h urinary urea N (ammonia N) excreted was calculated as urine volume (24 h) × urinary urea N (ammonia N) concentration. At the end of adjustment period and treatment period, all rats were weighed. Body weight gain during 21-d treatment period was calculated and feed intake during this period was recorded. Feed efficiency was calculated as BW gain (g)/feed intake (g) × 100. Supplemental choline intake for each rat during 21-d treatment period was calculated. At the end of the experiment, while rats were awake, tail arterial blood was collected into 1-ml Na-heparinized syringe and immediately capped and stored on ice. Blood gas and pH
were analyzed (Radiometer ABL500, Radiometer American Inc., Westlake, OH) within 30 min after blood was drawn. Rats were then anesthetized in a CO₂ chamber. Blood samples were collected into Na-heparinized tubes by cardiac puncture and immediately stored on ice. Within 30 min after blood was drawn, plasma was obtained and immediately stored in three aliquots at −80°C before being analyzed for plasma ammonia, urea, and Ca. After all blood samples were taken, rats were sacrificed and liver was harvested. Liver samples were rinsed in saline three times and placed in liquid nitrogen to freeze rapidly, then stored at −20°C. Liver TG was analyzed in triplicate for each liver sample.

Statistical analysis. Experiment 2 was a completely randomized block design with a 2 × 7 factorial arrangement of treatments within each block. Analysis of variance on feed intake, BW gain, feed efficiency, and liver TG was performed by the PROC GLM procedure of SAS (36). The model used was: block, protein source, supplemental choline, and protein source × supplemental choline. Supplemental choline was treated as a continuous variable and protein source was treated as a categorical variable. Residual was used to test all treatment effects. In addition, within each protein source, liver TG was regressed against supplemental choline using the PROC REG procedure of SAS (36).

To determine the relation of liver TG and other variables, regression analysis was performed by the PROC REG procedure of SAS (36). The independent variables were liver TG as a continuous variable and protein source as a categorical variable. The dependent variable was blood pH, bicarbonate, pCO₂, plasma ammonia N, urea N, ammonia N to urea N ratio, Ca, urinary ammonia N, or ammonia N to urea N ratio.

Two rats died during the experiment. Both rats were from block 1 and consumed PF-containing diet with 0.075 or 0.09% supplemental choline. We were unable to collect tail arterial blood from four rats. All four rats consumed diets containing PF. The treatments for three of the four rats were 0.03, 0.045, and 0.06% supplemental choline in block 1, and the treatment for one of the four rats was 0.03% supplemental choline in block 2. We were unable to collect heart chamber blood from a rat in block 1 that consumed diet with PF and 0.03% supplemental choline. Consequently, there were only 22 data points for blood gas and pH measurement, and 25 data points for the other measurements.

RESULTS AND DISCUSSION

Experiment 1

Dry matter intake. There was a time effect on DMI (P < 0.0001). The DMI was 11.6 kg/d at −27 d, 9.0 kg/d at 2 d, and 21.1 kg/d at 35 d, a pattern normally observed around calving (4). The CP intake was 2.25 kg/d at −27 d, 1.88 kg/d at 2 d, and 4.23 kg/d at 35 d.

Animal performance. Milk production was 35.6 kg/d during the first 35 d of lactation, 21.4 kg/d at 2 d, and 43.1 kg/d at 35 d. Milk protein was 2.8% and milk fat was 3.68% on d 35. Body weight was 702 kg at d −27, 667 kg at d 2, and 640 kg at d 35. Cows with the above d-35 milk production and BW require 4.05 kg CP/d (30), which is similar to the CP intake at d 35 in the present trial (4.23 kg CP/d). Milk was not sampled on d 2 for milk fat; however, assuming milk fat was 3.0%, then the CP requirement is 2.1 kg/d (30), which is higher than the CP intake at d 2 in this trial (1.88 kg CP/d). According to NRC (30), CP requirement at −27 d is 1.36 kg/d, which is lower than the CP intake at −27 d in the present study (2.25 kg CP/d).

Plasma ammonia. There is lack of data on plasma ammonia in cows around calving. We observed that plasma ammonia increased at 12 and 16 h then decreased at 22 h after calving and by 35 d returned to the level at −27 d (Table 2). The plasma ammonia N to urea N ratio followed the same pattern as plasma ammonia, which suggests that the capacity of ureagenesis may have reached its limit immediately after calving. Plasma ammonia at −27 d was within the range of most values described in literature, 30 to 80 µM (10, 14, 35). At 16 h after calving, plasma ammonia concentration ranged from 52 to 89 µM and nearly doubled compared to −27 d. This magnitude of increase of plasma ammonia is too small to cause acute toxicity, and its potential effect on subsequent reproduction performance is largely unknown. A doubling of plasma ammonia caused by additional urea feeding was associated with decreased embryo viability in ewes (25). A 30% increase of plasma ammonia caused by excess CP intake was associated with impaired reproductive performance in dairy cows (17, 43). However, the effect of a transient increase of plasma ammonia 2 mo before insemination on the subsequent reproduction performance has not been studied.

Plasma Gln and Gln%. Plasma Gln decreased at 16 h after calving and continued to decrease until 35 d (Table 2). This result is in agreement with that reported by Meijer et al. (28). In contrast, Verbeke et al. (42) observed that plasma Gln concentration was higher at 6 to 20 h after calving than that at 4 d prepartum.

Plasma Gln% was greatest at 12 h, decreased at 16 h and by 35 d was lower than at −27 d (Table 2). The rise in ammonia and Gln% at calving suggests an increase in ammonia passing to and through the liver.

Plasma urea. The concentration of plasma urea found in our study (Table 2) is in agreement with those reported in the literature (20, 26, 29). There were no time effects on plasma urea concentration, as observed
by Oltner and Berglund (32). In contrast, Blum et al. (6) found that plasma urea decreased during the first week of lactation, then increased and remained steady during the remaining lactation. However, in the above study, plasma urea was determined every 2 wk (6). Our results showed that plasma urea is not as sensitive as plasma ammonia and Gln% to measure N metabolism.

**Plasma Ca.** Plasma Ca decreased at 12, 16, and 22 h and by 35 d returned to the same level as −27 d (Table 2). Most cows experienced some degree of hypocalcemia at 12 to 16 h after calving, as observed in other studies (15, 33). Typically, cows became paréctic when the concentration of serum Ca is below 4 mg/dl (39). In this study, one cow had 3.4 mg of plasma Ca/dl at 22 h without showing clinical signs of milk fever and was identified as an outlier for plasma Ca, ammonia, ammonia:urea ratio, and Gln. Therefore, all the data from this cow were excluded from the analysis. For this cow, liver TG and DMI at 2 d were 12.3% of DM and 11.6 kg/d, and plasma ammonia, urea, and Gln% at 22 h were 104.4 µM, 6.67 mM and 92.8% respectively.

**Liver TG.** Liver TG peaked at 2 d and by 35 d was still higher than that at −27 d (Table 2). These results are in agreement with those reported by Vazquez-Anon et al. (41). The concentration of liver TG at 2 d ranged from 2.4 to 38.8% of DM.

**Regression analysis.** No significant relationship was found at −27 d. No relationship was found between liver TG at 2 d and plasma ammonia or Gln% at 12 h or 16 h. The following regression equations were obtained for plasma samples at 22 h and liver TG or DMI at 2 d (units as in Table 2): ammonia = 32.1 + 0.89 urea (R² = 0.45, P < 0.05), Gln% = 71.2 + 0.23 TG + 1.31 urea (R² = 0.71, P < 0.01), Ca = 11.97 - 0.31 DMI - 0.04 ammonia (R² = 0.86, P < 0.001). The positive correlation between liver TG and plasma ammonia or Gln% suggests that hepatic TG accumulation might decrease the conversion of ammonia to urea and result in increased conversion of ammonia and Glu to Gln, and in more ammonia escaping intact from the liver. This is consistent with our observation that TG inhibited ureagenesis in vitro (40, 44). The absence of correlation between plasma Ca and liver TG suggests that hepatic TG accumulation does not affect blood pH or Ca. It also suggests that the negative correlation between plasma Ca and ammonia is not related to liver TG. The negative correlation between plasma Ca and ammonia does not prove a cause and effect relationship. The mechanism for this relationship is unknown. At 35 d, plasma Ca was negatively correlated with plasma urea. The regression equations are: Ca = 10.91 - 0.22 urea (R² = 0.66, P < 0.01), urea = 31.19 + 0.13 DMI - 2.69 Ca (R² = 0.82, P < 0.001).

### Experiment 2

**Feed intake and BW gain.** Supplemental choline level in the diet did not affect feed intake, BW gain, or feed efficiency. The least square mean is 395 g/21 d for feed intake, 134 g/21 d for BW gain and 33.8% for feed efficiency. Protein source did not affect feed intake, BW gain, or feed efficiency. There was no interaction between protein source and supplemental choline level.

**Liver TG.** As intended, a range of liver TG (2 to 45% of DM) was created in rats by adding different amounts of supplemental choline to basal diets. Both basal diets (without choline supplement) caused fatty liver. Increased choline intake resulted in decreased liver TG (P < 0.0001). Rats that consumed diets with PF had lower liver TG than rats that consumed diets with SPI (9.8 vs. 19.7% of DM, P < 0.001). There was an interaction between supplemental choline level and protein source (P < 0.01). Because of the interaction, regression analysis was performed separately for rats that consumed SPI and PF. The equations were TG% = −388.3% supplemental choline + 36.3 (R² = 0.66, P < 0.001) for

### Table 2. Plasma ammonia, urea, Ca, Gln, Gln%, and liver triglyceride (TG) at different times relative to calving.

<table>
<thead>
<tr>
<th>Time relative to calving</th>
<th>Ammonia (µM)</th>
<th>Ammonia-N/ Urea (%)</th>
<th>Gln (mM)</th>
<th>Gln%</th>
<th>Urea (mM)</th>
<th>Ca (mg/dl)</th>
<th>Liver TG (% of DM)</th>
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</thead>
<tbody>
<tr>
<td>−27 d</td>
<td>33.4 ± 5.0</td>
<td>0.29 ± 0.04</td>
<td>0.38 ± 0.02</td>
<td>88.8 ± 0.9</td>
<td>5.96 ± 0.32</td>
<td>6.87 ± 0.27</td>
<td>2.58 ± 2.57</td>
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<tr>
<td>+12 h</td>
<td>61.1 ± 5.0</td>
<td>0.50 ± 0.04</td>
<td>0.38 ± 0.02</td>
<td>92.0 ± 0.9</td>
<td>6.34 ± 0.32</td>
<td>7.93 ± 0.22</td>
<td>2.58 ± 2.57</td>
</tr>
<tr>
<td>+16 h</td>
<td>64.8 ± 5.4</td>
<td>0.55 ± 0.05</td>
<td>0.33 ± 0.02</td>
<td>90.1 ± 1.0</td>
<td>6.08 ± 0.32</td>
<td>7.35 ± 0.23</td>
<td>2.58 ± 2.57</td>
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<tr>
<td>+22 h</td>
<td>44.2 ± 5.5</td>
<td>0.40 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>88.9 ± 1.0</td>
<td>5.78 ± 0.33</td>
<td>7.70 ± 0.23</td>
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<td>+2 d</td>
<td>5.40 ± 0.55</td>
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<td>0.29 ± 0.02</td>
<td>84.8 ± 1.0</td>
<td>5.68 ± 0.35</td>
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<td>+35 d</td>
<td>28.1 ± 5.4</td>
<td>0.25 ± 0.05</td>
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<td>5.68 ± 0.35</td>
<td>8.49 ± 0.23</td>
<td>7.89 ± 2.81</td>
</tr>
</tbody>
</table>

- Gln% = Gln * 100/(Gln + Glu).
- Data represent least squares means and standard error of mean. One hypocalcemia cow was excluded from the analysis.
- Ammonia-N/Urea-N (%) = Ammonia (µM)/(Urea (mM) * 2 * 1000) * 100.
- Not significant at P < 0.05.
rats consumed SPI, and TG% = −131.1% supplemental choline + 14.0 ($R^2 = 0.28, P = 0.08$) for rats consumed PF.

**Correlation among variables.** Blood, plasma, and urinary variables are summarized in Table 3. There was no correlation between liver TG and plasma ammonia, which differs from what was observed in experiment 1. In experiment 1, cows were in negative energy balance due to the combination of initiation of milk production and depression of feed intake after calving. Cows mobilize protein and fat from body reserve immediately after calving (8). Up to 15 kg of protein may be mobilized in the first 2 mo of lactation (30). This rapid onset of protein mobilization may precede the adaptation of urea cycle enzymes that require 4 to 7 d in rats (37). Therefore, cows at calving may experience larger ammonia stress than do growing rats. If liver TG accumulation inhibits ureagenesis and leads to increased circulating ammonia concentration, we will more likely detect it in cows at calving than in rats. Liver TG was not correlated with urinary ammonia N to urea N ratio or urea N. There was a trend for urinary ammonia N to be positively correlated to liver TG ($P = 0.08$). The regression equation was: $UAN$ (mg/d) = $1.63 + 0.64 X1 + 0.018 T$ ($\%$ of DM) ($R^2 = 0.42, P < 0.01$), where $UAN$ is urinary ammonia N and X1 is a dummy variable to represent protein source. Urinary ammonia can be derived from plasma Gln% (38). This observation coincides with the positive correlation between liver TG and plasma Gln% in experiment 1. There was no correlation between liver TG and blood pH, bicarbonate, or pCO$_2$. Similar to experiment 1, liver TG and plasma Ca were unrelated. Although pH affected urea production in vitro (22) and urinary urea excretion decreased with acidosis in vivo (31), no direct evidence shows that decreased ureagenesis raises blood pH. If liver TG accumulation inhibited ureagenesis in present experiments, as shown previously in bovine hepatocytes in vitro (40, 44) and in rats in vivo (5, 24), then the inhibition was too subtle to influence either blood urea concentration or blood acid-base balance.

**Conclusion**

We tested two hypotheses in this study. 1) Immediately after parturition cows should have increased circulating ammonia or Gln% because of decreased ureagenesis caused by decreased liver TG accumulation. 2) Decreased ureagenesis caused by hepatic TG accumulation may alkalinize blood and lead to decreased circulating Ca.

We observed that right after cows calved circulating ammonia, ammonia to urea ratio, and Gln% increased, accompanied by an increase in blood pH. Liver TG at 2 d postpartum was positively correlated to plasma ammonia or Gln% at 22 h postpartum, which suggests that liver TG accumulation may reduce the conversion of ammonia to urea and result in increased conversion of ammonia to Gln and in more ammonia escaping intact from the liver. Therefore, hepatic TG accumulation right after calving accentuated the already increased circulating ammonia and Gln% due to parturition.

Liver TG and plasma Ca were not related in experiments 1 and 2, which indicates that the negative correlation between plasma ammonia and Ca in experiment 1 is not due to liver TG accumulation. The absence of correlation between liver TG and blood pH or bicarbonate in experiment 2 suggests that decreased ureagenesis by liver TG accumulation may be too subtle to influence blood pH or plasma Ca.

**Acknowledgments**

The authors are grateful to Scott Hubbard-Vanstelle for his help on collecting arterial blood in experiment 2.

**References**


**Table 3. Summary of blood, plasma, and urinary variables in rats.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pH</td>
<td>7.41</td>
<td>0.04</td>
<td>22</td>
</tr>
<tr>
<td>Blood bicarbonate (mM)</td>
<td>20.1</td>
<td>2.86</td>
<td>22</td>
</tr>
<tr>
<td>Blood pCO$_2$(mm Hg)</td>
<td>33.1</td>
<td>3.4</td>
<td>22</td>
</tr>
<tr>
<td>Plasma ammonia (µM)</td>
<td>145.4</td>
<td>27.8</td>
<td>25</td>
</tr>
<tr>
<td>Plasma urea (mM)</td>
<td>5.43</td>
<td>2.28</td>
<td>25</td>
</tr>
<tr>
<td>Plasma Ca (mg/dl)</td>
<td>10.2</td>
<td>0.9</td>
<td>25</td>
</tr>
<tr>
<td>Urinary ammonia-N (mg/d)</td>
<td>2.25</td>
<td>0.73</td>
<td>25</td>
</tr>
<tr>
<td>Urinary urea-N (mg/d)</td>
<td>249.2</td>
<td>48.3</td>
<td>25</td>
</tr>
</tbody>
</table>
IV. Effects of unprocessed or extrusion-cooked mixtures of grain and urea, biuret, or dicyanamide and liquid supplements on rumen and blood changes associated with toxicity. J. Anim. Sci. 45:1397–1408.


