Journal of Dairy Research (2009) **76** 49–58. © *Proprietors of Journal of Dairy Research 2008*
doi:10.1017/S0022029908003634 *Printed in the United Kingdom*

The effect of short-term hyperammonaemia on milk synthesis in dairy cows

Norm G Purdie[1,3], Donald R Trout[2], Scott R L Cieslar[3], Torben G Madsen[3,4], Dennis P Poppi[1] and John P Cant[3]*

[1] Schools of Animal Studies and Veterinary Science, University of Queensland, Australia
[2] Department of Clinical Studies and [3] Department of Animal and Poultry Science, University of Guelph, Canada
[4] Department of Anatomy and Physiology, The Royal Veterinary and Agricultural University, Denmark

Received 26 February 2008; accepted for publication 30 July 2008; first published online 16 October 2008

To test the hypothesis that ammonia detoxification in ruminants consumes amino acids to the detriment of milk protein production, we infused four lactating dairy cows with ammonium acetate or sodium acetate in switchback experiments. Plasma ammonia concentrations increased to 411 μM within 1 h of the start of infusion of ammonium acetate at 567 mmol/h. The rate constant for ammonia clearance from plasma was 0·054/min and the half-life was 12·9 min. Infusion at 567 mmol/h for 1 h followed by 1 h without infusion, repeated four times between am- and pm-milking, caused a decrease in feed intake. Compared with sodium acetate, continuous infusion of ammonium acetate at 360 mmol/h throughout an entire 10-h milking interval increased plasma ammonia concentrations to 193 μM and caused a 20% decrease in milk, protein and lactose production with no effect on percentage composition of milk or the yield of milk fat. Arterial concentrations of glucose and non-esterified fatty acids tended to increase; there was no effect on arterial acetate, β-hydroxybutyrate or triacylglcerol, and branched-chain amino acids, Lys and Thr decreased. Mammary plasma flow, estimated by assuming 100% uptake/output of Phe+Tyr, was significantly correlated with milk yield. Mammary uptakes of acetate tended to be reduced by hyperammonaemia, but uptakes of other energy metabolites and amino acids were not affected. Thus, while an increase in amino acid consumption during hyperammonaemia was apparent from the drop in circulating concentrations of Leu, Ile, Val, Lys and Thr, there was no evidence to support the hypothesis that milk yield is affected by the lower concentrations. An ammonia-induced depression in feed intake may have caused the decrease in milk synthesis.

Keywords: Hyperammonemia, dairy cows, milk composition, mammary uptakes.

One molecule of urea contains two atoms of N. The synthesis of urea in the mammalian urea cycle requires 1 atom of N to be supplied as ammonia and the other N atom to enter on aspartic acid. It has been suggested that this requirement for an amino acid could mean that hepatic synthesis of urea to detoxify ammonia arising from rumen fermentation consumes amino acids that might otherwise have been retained in tissue or milk protein of growing or lactating animals (Reynolds, 1992; Lobley et al. 1995). In support of the hypothesis, Mutsvangwa et al. (1999) reported a doubling of ^{15}N-incorporation from [^{15}N]methionine into urea by isolated hepatocytes exposed to increasing concentrations of [^{14}N]ammonia in vitro. However, the majority of the urea synthesized remained unenriched with ^{15}N, no matter which of many amino acids contained the ^{15}N, indicating that the urea-N from aspartate mainly arose, not directly from amino acids, but from ammonia via glutamic dehydrogenase and transamination. Thus, evidence for and against the hypothesis is equivocal.

The possibility that an ammonia load could cause a depression in protein content of milk from dairy cows has been tested by ruminal urea infusion or postruminal ammonia infusions. Administration of 240 g/d urea in two discrete meals per day, as opposed to continuous infusion into the rumen, resulted in a dry matter intake (DMI) depression at lower levels of dietary crude protein (CP) intake (Choung et al. 1990). Milk yield declined in a similar manner to the DMI depression but both protein and fat percentages were elevated at the highest doses of urea

*For correspondence; e-mail: jcant@uoguelph.ca

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

(Choung et al. 1990). Malik et al. (1999) observed a 10% drop in milk protein content and a 24% decrease in protein yield when ewes were infused intravenously with ammonium acetate. Moorby & Theobald (1999) reported no change in milk protein percentage or yield, and a 2% decline in milk casein percentage, during duodenal ammonia infusion of cows. To address the equivocal responses and to evaluate the effect of hyperammonaemia on milk composition, cows were infused intravenously for 10 h with ammonium acetate, and the concentrations and uptakes of milk precursors from circulation by the mammary glands were measured.

Symonds et al. (1981) reported that ammonia toxicity symptoms in dairy cows occurred at a concentration of ammonia in arterial plasma of 800 μM. Choung et al. (1990) observed depressing effects of intraruminal urea infusion on DMI and milk production at approximately 200 μM plasma ammonia. The first objective of the current experiment was to find a jugular infusion rate that would raise plasma ammonia levels to between 200 and 800 μM. Then, we measured the effect of hyperammonaemia during a milking interval on the animal and on milk production and, finally, we examined the effect of a 10-h hyperammonaemia on mammary utilization of milk precursors. To our knowledge, this is the first report of effects of an intravenous ammonia infusion on milk composition in lactating cows.

Materials and Methods

All procedures involving animals were approved by the Animal Care Committee at the University of Guelph. For each experiment, ammonium acetate was the ammonia donor and sodium acetate was the control. Infusates were prepared as 3·33 M solutions, filtered through 0·22-μm cellulose acetate filters and stored in 4-l autoclaved bottles prior to use. Actual weights of solutions infused were recorded at hourly intervals during infusion.

Experiment 1: hyperammonaemia

Four multiparous Holstein cows of body weight (BW) 669±20 kg and in the second trimester of pregnancy were housed individually in tie stalls, milked twice a day at 8.00 and 16.00, fed twice daily for ad-libitum intake with a total mixed ration (21·6% CP, 31·5% neutral detergent fibre) based on first-cut lucerne chaff, and given free access to water bowls. Cows were each fitted with indwelling jugular catheters. One catheter was for infusion and the other for blood sampling. Ammonium acetate was infused for 1 h at 567 mmol/h, which was calculated to be 50% of the maximum hepatic detoxification rate of 1·84 mmol/min per kg liver (Symonds et al. 1981). Venous blood was sampled into heparinized tubes at 10-min intervals over the 60 min of infusion and for the following 30 min. Samples were centrifuged and the plasma was removed by pipette and frozen for later analysis.

Table 1. Chemical analysis (% of dry matter unless stated otherwise) of the total mixed ration fed in Experiment 3

Dry matter, %	46·7
Crude protein	19·6
Neutral detergent fibre	28·1
Acid detergent fibre	20·3
Lignin	1·6
Fat	3·8
Ash	8·4

Experiment 2: feed intake and milk production

The four cows used in Experiment 1 were infused into one jugular catheter with ammonium acetate or sodium acetate at the same rates as in Experiment 1 for four 1-h periods, each separated by 1 h without infusion. The ammonium and sodium acetates were administered on consecutive days in a randomized sequence for each cow. The intermittent infusion protocol ensured that the total amount of ammonia entering the circulatory system was high without exceeding toxic levels.

Cows were milked twice daily. Feed was weighed into an empty manger in the morning and the infusion protocol began. At the end of 8 h, infusions were terminated, cows were milked, and feed remaining in the manger was weighed to calculate 8-h intakes.

Experiment 3: mammary arteriovenous differences

Four cows in their second lactations were fitted with catheters in a jugular vein, a subcutaneous mammary abdominal vein, and an intercostal artery acording to Mackle et al. (2000). All animals were fed with a total mixed ration based on corn and lucerne silages (Table 1) for ad libitum intake. Feed intakes were measured daily and feed was sampled every second day. Infusions into the jugular catheter commenced at the completion of the morning milking at 8.00 and continued until cows were milked at 18.00. Ammonium acetate was infused at 360 mmol/h, which was estimated to raise the plasma ammonia concentration to 200 μM for the entire 10-h interval between morning and afternoon milkings. Sodium acetate was infused, according to the same protocol, the day before or after ammonium acetate infusion at an equimolar rate. Milk was sampled from the afternoon milking to represent the effect of treatment.

Every hour after the start of the infusion, 10-ml blood samples were collected into heparinized tubes from arterial and mammary vein catheters simultaneously. Samples were centrifuged and the plasma was removed and frozen for later analysis.

Analytical procedures

Milk samples were analysed by infrared spectroscopy for protein, fat and lactose. Plasma samples were analysed spectrophotometrically for glucose (kit 510-A; Sigma

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

Chemical Co., Oakville, Ontario), triglycerides (Sigma kit 336), non-esterified fatty acids (NEFA C kit; Wako Chemicals GmbH, Neuss, Germany), acetate (Boehringer Mannheim kit; R-Biopharm GmbH, Darmstadt, Germany), β-hydroxybutyrate (Cant et al. 1993), urea (Sigma kit 640-B) and ammonia (Sigma kit 171-C).

Concentrations of amino acids in plasma were analysed by the method of MacKenzie (1987). Briefly, 0·5 ml sample with 0·3 ml 2·5 mM-norleucine was deproteinized with 1·2 ml 12% (w/v) TCA and centrifuged at 2000 ***g*** for 20 min. The supernatant was run through a cation-exchange column, vacuum-dried and converted to N-heptafluorobutyryl n-propyl ester derivatives according to Culea & Hachey (1995). Derivatives were separated on a model 6890 GC linked to a 5973N quadrupole MS (Agilent Technologies, Mississauga, Canada) and quantified using norleucine as the internal standard.

Mammary uptakes of plasma metabolites during the final hour of infusion were calculated as the product of arteriovenous concentration difference and plasma flow rate. Mammary plasma flow (MPF) was estimated from the quotient of arteriovenous concentration difference of Phe+Tyr and uptake of Phe+Tyr from plasma, assuming that 96·5% of Phe+Tyr output in milk protein is equal to Phe+Tyr uptake from plasma (Cant et al. 1993). Mammary extraction of a plasma metabolite, calculated as the quotient of its arteriovenous difference and arterial concentration, is a consequence of both transmembrane transport and plasma flow. As an alternative to calculating extraction, then, the product of the first-order rate constant for capillary uptake (k) and the total volume of perfused capillaries ($Vol_{cap}{\cdot}N_{cap}$) was calculated as $k{\cdot}Vol_{cap}{\cdot}N_{cap} = -\ln(1 - \text{extraction}) \times MPF$, according to Cant & McBride (1995).

Statistical analyses

For Experiment 2, variance in each observation (Y_{ijk}) was analysed by using the following model, with the general linear models procedure of SAS Institute Inc. (2000):

$$Y_{ijk} = \mu \ldots + cow_i + per_j + trt_k + \varepsilon_{ijk}$$

where

$\mu \ldots$ = mean,
cow_i = ith cow effect (1 = 1 to 4)
per_j = jth period effect (j = 1 to 2),
trt_k = kth treatment effect (k = 1 to 2), and
ε_{ijklm} = error.

For Experiment 3, treatment effects were detected by an analysis of covariance (Neter et al. 1985). For all observations except plasma ammonia and urea, the categorical treatment effect in the above model was replaced by the continuous effect of plasma ammonia concentration. In place of the cow effect, observations of DMI and milk production and composition the day before infusions commenced served as covariates for those variables during infusions. Likewise, observations of plasma metabolite concentrations at 8·00, immediately prior to the onset of infusions, served as covariates for metabolite concentrations during the final hour of infusion. Variances in MPF and amino acid concentrations were analysed with a categorical cow effect. Significance was declared at $P \leqslant 0{\cdot}05$ and tendencies were declared at $0{\cdot}05 < P \leqslant 0{\cdot}15$.

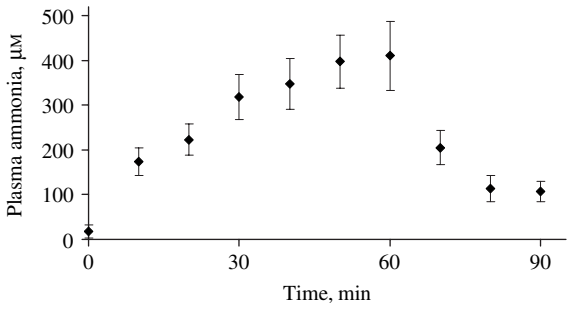


Fig. 1. Mean (± SE) jugular plasma ammonia concentrations measured during and after 1 h of infusion into the contralateral jugular vein of ammonium acetate at 567 mmol/h (n=4; Experiment 1).

Results

Experiment 1

The single hour of infusion of ammonium acetate was conducted to find a jugular infusion rate that would elevate plasma ammonia to a concentration between 200 and 800 μM. The increase in concentration of ammonia was rapid, reaching a mean of 411 μM within 1 h of the start of infusion at 567 mmol/h (Fig. 1). Animals appeared normal throughout. Ammonia concentrations dropped rapidly within 30 min of cessation of the infusion to around 107 μM. Concentrations of ammonia above baseline from 0 to 90 min, $[A]_t$, were used in Excel® Solver to estimate the plateau ammonia concentration above baseline, $[A]_{max}$, and rate constant for first-order clearance from plasma, k, by fitting the equations: $[A]_{max}\ (1 - e^{-kt})$ when $t \leqslant 60$, and $[A]_{60}e^{-k(t-60)})$ when $t > 60$. The rate constant, k, was 0·054/min for a half-life of 12·9 min. $[A]_{max}$ was estimated to be 377 μM. The volume of ammonia distribution was calculated from the quotient of the infusion rate of 567 mmol/h and $k[A]_{max}$ to be 465 l, or 69·5% of BW. This percentage agrees closely with the total body water content of lactating dairy cows (Brown et al. 1989) and reflects rapid distribution of ammonia throughout. Clearance was calculated as 60 k × volume to be 1504 l/h.

Experiment 2

Because a single hour of ammonia infusion at 567 mmol/h appeared safe for the cows, the procedure was repeated four times during a milking interval with a 1-h washout between each infusion hour. There was a significant

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

Table 2. Average feed intake, plasma ammonia concentration, and yield of milk from four cows (Experiment 2) after four 1-h periods of infusion into the jugular vein of 567 mmol/h ammonium acetate or sodium acetate, each followed by a 1-h washout

	Sodium acetate	Ammonium acetate	SEM	P†
Feed intake, kg/8 h	4·7	1·9	0·3	0·015
Milk yield, kg/8 h	9·2	8·4	0·5	0·411
Plasma ammonia, μM	31	298	58	0·082

† Probability of no effect of ammonium compared with sodium acetate

decline in feed intake during the interval studied but no significant decline in milk production compared with infusion of sodium acetate in the same fashion (Table 2). Plasma ammonia concentration in one cow rose to twice that of the other cows on the ammonia infusion, and that cow also consumed no feed during the 8-h experiment. The linear regression between plasma ammonia concentration and feed intake was significant (P=0·014) and exhibited a coefficient of determination of 0·66 (Fig. 2).

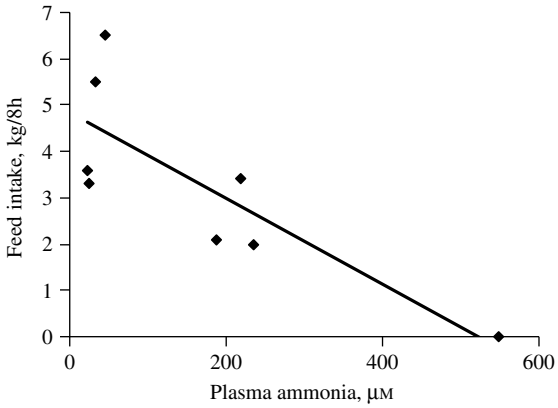


Fig. 2. Relationship between feed intake and plasma ammonia concentrations in four cows (Experiment 2) infused with 567 mmol/h ammonium acetate and sodium acetate on consecutive days in four 1-h intervals, each followed by a 1-h washout; linear regression (solid line) is $y=-0{\cdot}0092x+4{\cdot}8$ (P=0·014, r^2=0·66).

Experiment 3

Effects of hyperammonaemia on uptake and utilization of milk precursors by the mammary glands were studied with a continuous infusion of ammonium acetate throughout the entire interval between am- and pm-milking. The infusion rate was 60% of that used in Experiments 1 and 2 in an attempt to minimize the feed intake depression that could confound the ability to test the hypothesis that hepatic amino acid utilization affects milk synthesis. The hyperammonaemia caused a 20% decrease in milk, protein and lactose production with no effect on percentage composition of milk or the yield of milk fat (Table 3).

Arterial and venous concentrations of ammonia and urea were both elevated by the ammonia infusion. The increase in plasma ammonia concentration, predicted from the infusion rate of 360 mmol/h and the clearance rate of 1504 l/h estimated in Experiment 1, was 239 μM and the observed increase was 152 μM. The difference suggests that clearance was faster in Experiment 3 than in Experiment 1. Arterial concentrations of glucose and NEFA tended to increase (Table 4). There were no differences in arterial concentrations of acetate, β-hydroxybutyrate or triacylglycerol.

The 18% reduction in average MPF was not statistically significant (Table 4), although it was significantly (P=0·046) related to the level of milk production over the 10-h infusion (Fig. 3). Extractions of metabolites were not affected by the hyperammonaemia but the $k{\cdot}Vol_{cap}{\cdot}N_{cap}$ product for acetate removal by the mammary glands tended to be 19% lower with ammonia infusion. Where the change was of the same magnitude as for MPF, the drop in $k{\cdot}Vol_{cap}{\cdot}N_{cap}$ probably represents changes in capillary perfusion volume $Vol_{cap}{\cdot}N_{cap}$ and not in the efficiency of uptake, k. Mammary uptakes of acetate tended to be reduced by hyperammonaemia. Uptakes of other metabolites were not affected. On a net basis, ammonia and urea were released by the mammary glands although the rates were not significantly different from zero.

Arterial concentrations of all three branched-chain amino acids (BCAA) were reduced during hyperammonaemia (Table 5). There was also a tendency for Lys and Thr concentrations to decrease, which resulted in a decrease in the summed concentration of all essential amino acids. Uptakes of amino acids were not affected (Table 6). Percentage extraction of Leu tended to increase (Table 7).

Discussion

Ammonia toxicity arises when portal ammonia is not removed by the liver and is allowed to accumulate in the systemic circulation, primarily affecting function of the central nervous system through shifts in amino acid metabolism (Bachmann et al. 2004). Although ruminant animals, as a consequence of pregastric microbial fermentation, absorb ammonia at rates that approach or even exceed the capacity of the liver to remove it (Symonds et al. 1981), the effects of hyperammonaemia on milk synthesis in lactating ruminants have not been well characterized. When lactating ewes were infused abomasally with 46 mmol/h ammonium bicarbonate, milk production declined by 15%, protein yield by 24%, and milk fat percentage increased by 14% (Malik et al. 1999). Plasma ammonia concentrations were not measured but if ammonia were cleared at 0·054/min from a volume of distribution of 69·5% of BW as in Experiment 1, then arterial concentration of ammonia would have been $46 \div (60{\cdot}0{\cdot}054{\cdot}0{\cdot}695{\cdot}BW) = 0{\cdot}307$ mM, or close to 10-times normal. Moorby & Theobald (1999) infused 200 mmol/h ammonium acetate into the duodenum of lactating cows

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

Table 3. Average feed intake and yield and composition of milk from four cows (Experiment 3) after 10 h of infusion into the jugular vein of 360 mmol/h ammonium acetate or sodium acetate

	Sodium acetate	Ammonium acetate	SEM	*P*†
Feed intake, kg/d	18·7	18·1	0·2	0·239
Milk yield, kg/10h	10·8	8·8	0·6	0·038
Milk component percentage				
Protein	3·63	3·51	0·09	0·418
Fat	4·90	5·14	0·23	0·702
Lactose	4·94	4·90	0·05	0·922
Milk component yield, g/10h				
Protein	391	301	24	0·032
Fat	528	444	54	0·173
Lactose	531	427	26	0·026

† Probability of no continuous effect of arterial plasma ammonia concentration

and observed only a 2% decrease in milk casein percentage, a 4% increase in milk fat percentage, and no change in yields of milk or its components. Again, plasma ammonia concentrations were not measured. To use the data from Experiment 1 to estimate the level of hyperammonaemia reached in the work of Moorby & Theobald (1999) we must take into account the different site of ammonia infusion. The jugular infusion given by Malik et al. (1999) and by ourselves bypasses the normal route of ammonia entry from the digestive tract into circulation, which is via the portal vein from which the liver can extract a large proportion. First-pass extraction of portal ammonia by the porcine liver was estimated as 73% by fitting dilution curves of [^{13}N]ammonia following pulse doses (Keiding et al. 2001). Keiding et al. (2001) also estimated from the arteriovenous concentration difference measurements of Lobley et al. (1995) and Symonds et al. (1981) that first-pass extraction in sheep and lactating dairy cows was approximately 75%. Assuming a clearance of 1504 l/h as in Experiment 1 and 75% extraction of portal ammonia on first pass through the liver, 200 mmol/h duodenal ammonia (Moorby & Theobald, 1999) would increase arterial concentrations by 33 μM, or approximately 2-fold. In contrast, arterial ammonia concentrations were increased 5-fold in our Experiment 3 and an estimated 10-fold in the experiment of Malik et al. (1999). It appears from the limited results that the higher the peripheral ammonaemia, the more likely milk yield and, possibly, protein percentage will be depressed.

Metabolic response

The 5-fold hyperammonaemia for 10 h caused 20% decreases in yields of milk, protein and lactose. Feed intake during the infusion interval was observed to decrease in Experiment 2. In Experiment 3, feed intake was monitored over a 24-h period that included the 10-h infusion interval and the 14-h recovery period. Intakes over 24 h were not depressed but this does not preclude the possibility that elevated intakes after the infusion compensated for an intake depression during infusion. Ammonia infusion into lactating ewes and cows did not affect intake (Malik et al. 1999, Moorby & Theobald, 1999) but urea supplementation has depressed intake (Choung et al. 1990). The intake depression we observed in Experiment 2 may be regarded as a protective response which lessens the rate of ammonia entry from the rumen. It might also have been responsible for the depression in milk yield, given that milk production decreases approximately 20% within 6–12 h of starting to fast (McGuire et al. 1995; Toerien & Cant, 2007).

Whether intake depression or some other contributing factor from ammonium acetate was responsible for the decline in milk production can be evaluated by determining whether the metabolic response of the cows corresponded to an intake depression effect or a hyperammonaemia effect. The response of lactating ruminants to a severe feed restriction or fast is not rapid owing to the continued outflow of nutrients from the reticulorumen. Typically, plasma concentrations of glucose decrease little, and acetate and gluconeogenic amino acid concentrations decrease until such time as the milk yield falls precipitously, after 18–24 h (Lomax & Baird, 1983; McGuire et al. 1995; Ndibualonji et al. 1997; Toerien & Cant, 2007). Plasma NEFA concentrations rise throughout a fast but elevated ketone body concentrations are not apparent until 24–30 h (McGuire et al. 1995; Toerien & Cant, 2007). Concentrations of essential amino acids in plasma increase as they are used less for protein synthesis and are mobilized from muscle (Ndibualonji et al. 1997). MPF and uptakes of milk precursors decrease in conjunction with the declining rate of milk synthesis (Linzell, 1967; Chaiyabutr et al. 1980). Lactose percentage in milk decreases while protein and fat percentages increase (Linzell, 1967; Drackley et al. 1991; Friggens et al. 1995). Similarities between the response to hyperammonemia observed here and the response to a restricted intake include the increase in plasma NEFA concentrations with no change in β-hydroxybutyrate, and the matching of MPF and acetate uptake with the declining milk yield. Differences include an increase in plasma

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

Table 4. Average plasma flow and balance of plasma metabolites across the udder of four cows (Experiment 3) during the last hour of a 10-h infusion into the jugular vein of 360 mmol/h ammonium acetate or sodium acetate

	Sodium acetate	Ammonium acetate	SEM	P†
Mammary plasma flow, l/h	652	537	75	0·326
Plasma flow/milk yield, l/kg	613	625	5	0·731
Ammonia				
Artery, μM	41	193	19	0·005
Vein, μM	50	240	16	0·002
Uptake, mmol/h	−6·6	−22·1	11	0·428
Urea				
Artery, mg N/dl	13·7	19·6	1·0	0·008
Vein, mg N/dl	14·1	20·4	1·1	0·007
Uptake, mmol/h	−111	−112	147	0·998
Glucose				
Artery, mM	3·43	3·88	0·15	0·151
Vein, mM	2·95	3·22	0·22	0·459
Extraction, %	13·8	17·7	5·0	0·788
$k{\cdot}Vol_{cap}{\cdot}N_{cap}$, l/h	100	117	57	0·963
Uptake, mmol/h	317	381	182	0·997
Acetate				
Artery, mM	2·26	2·05	0·25	0·550
Vein, mM	0·48	0·49	0·05	0·845
Extraction, %	77·6	77·7	3·6	0·791
$k{\cdot}Vol_{cap}{\cdot}N_{cap}$, l/h	1020	825	53	0·128
Uptake, mmol/h	1152	858	83	0·094
β-hydroxybutyrate				
Artery, mM	1·87	2·19	0·35	0·746
Vein, mM	1·28	1·52	0·11	0·088
Extraction, %	31·3	33·4	6·0	0·933
$k{\cdot}Vol_{cap}{\cdot}N_{cap}$, l/h	254	216	30	0·330
Uptake, mmol/h	385	357	84	0·562
Triacylglycerol				
Artery, μM	112	106	12	0·713
Vein, μM	52	49	3	0·635
Extraction, %	52·9	52·3	2·5	0·900
$k{\cdot}Vol_{cap}{\cdot}N_{cap}$, l/h	502	414	113	0·570
Uptake, mmol/h	40	31	9	0·509
Nonesterified fatty acids				
Artery, μM	75	103	8	0·054
Vein μM	104	103	11	0·807
Long-chain fatty acids				
Artery, μM	411	421	43	0·976
Vein, μM	260	251	14	0·842
Extraction, %	35·9	39·6	2·5	0·168
$k{\cdot}Vol_{cap}{\cdot}N_{cap}$, l/h	300	286	79	0·835
Uptake, mmol/h	101	95	28	0·845

† For urea and ammonia, probability of no categorical effect of infusion of ammonium compared with sodium acetate; and for all other metabolites, probability of no continuous effect of arterial plasma ammonia concentration

glucose concentration, a decrease in concentrations of several essential amino acids, and a lack of change in milk protein, fat and lactose percentages and mammary uptakes of amino acids and energy metabolites other than acetate. These differences suggest that 10 h of ammonia infusion was not long enough for an intake depression to have affected the cows' metabolism.

Similarities between the hyperammonaemia response we observed and hyperammonaemia responses in dairy cows and other species include the increase in plasma glucose concentration (Emmanuel & Edjtehadi, 1981; Symonds et al. 1981; Barej et al. 1982; Choung et al. 1990; Holeček et al. 2000) that has been attributed to decreased rates of glucose utilization in the body (Emmanuel & Edjtedahi, 1981) and/or

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

Table 5. Average concentrations of amino acids (AA, μM) in arterial plasma of four cows (Experiment 3) during the last hour of a 10-h infusion into the jugular vein of 360 mmol/h ammonium acetate or sodium acetate

	Sodium acetate	Ammonium acetate	SEM	*P*†
Met	8	12	2	0·551
Lys	108	95	1	0·066
Phe	40	35	2	0·163
Tyr	37	31	3	0·271
Thr	74	62	3	0·072
Val	225	187	6	0·039
Ile	380	274	17	0·039
Leu	163	125	2	0·004
Cln	46	57	5	0·275
Pro	70	70	6	0·820
Ser	79	78	2	0·554
Gly	237	245	15	0·735
Ala	350	360	30	0·902
Glu	156	205	25	0·396
Asp	4·7	4·2	0·3	0·215
Total AA	1·96	1·84	0·05	0·177
Essential AA	1·01	0·82	0·03	0·044
Branched-chain AA	0·77	0·59	0·02	0·012
Non-essential AA	0·94	1·02	0·05	0·459

† Probability of no continuous effect of arterial plasma ammonia concentration

Table 6. Average mammary uptakes (mmol/h) of amino acids (AA) from plasma in four cows (Experiment 3) during the last hour of a 10-h infusion into the jugular vein of 360 mmol/h ammonium acetate or sodium acetate

	Sodium acetate	Ammonium acetate	SEM	*P*†
Met	2·4	4·6	0·8	0·350
Lys	36·2	29·0	4·5	0·549
Phe	10·2	8·3	1·3	0·296
Tyr	12·8	9·9	1·2	0·380
Thr	15·8	14·3	1·6	0·369
Val	26·1	28·7	6·5	0·894
Ile	68·7	68·6	14·7	0·706
Leu	37·7	32·5	4·6	0·346
Cln	−2·1	2·8	4·9	0·522
Pro	3·3	6·3	3·7	0·737
Ser	13·9	12·7	1·1	0·379
Gly	−10·8	−4·5	4·1	0·558
Ala	46·8	50·9	36·5	0·992
Glu	25·9	13·0	10·2	0·420
Asp	1·2	−0·1	0·5	0·316
Total AA	271	277	47·4	0·755
Essential AA	193	196	33·4	0·744
Branched-chain AA	133	130	24·3	0·645
Non-essential AA	78	81	22·7	0·857

† Probability of no continuous effect of arterial plasma ammonia concentration

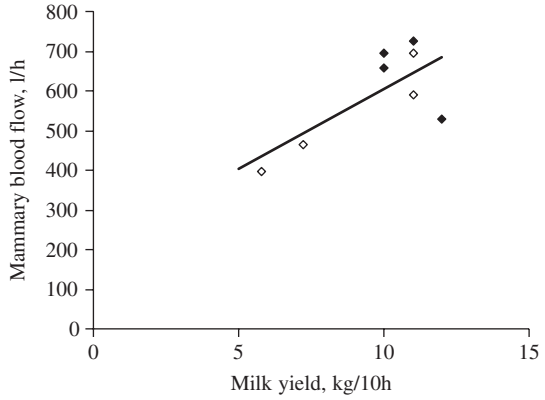


Fig. 3. Relationship between mammary blood flow and milk yield in four cows (Experiment 3) infused with 360 mmol/h ammonium acetate (open diamonds) and sodium acetate (closed diamonds) for 10-h periods on consecutive days; linear regression (solid line) is y=40·3x+202 (P=0·046, r^2=0·51).

adrenaline-induced glycogenolysis (Slawski et al. 1984; Wiechetek et al. 1989), an increase in plasma NEFA concentrations that persists in adrenalectomized animals (Slawski et al. 1984; Wiechetek et al. 1989), decreased plasma concentrations of BCAA that have been attributed to increased Gln synthesis and decreased protein degradation in muscle (Leweling et al. 1996; Holeček et al. 2000), and decreases in plasma concentrations of Lys and Thr (Holeček et al. 2000) that have not yet been fully explained.

It has been suggested that detoxification of free ammonia to urea and Gln consumes essential amino acids that might otherwise have been used for growth or lactation (Reynolds, 1992; Lobley et al. 1995). The role of the liver has been called into question by arteriovenous difference data that showed that the additional urea output during mesenteric infusion of 1100 μmol/min ammonium bicarbonate into sheep was readily accounted for by the hepatic removal of ammonia from circulation, without any need for additional amino acid catabolism (Milano & Lobley, 2001). Isotopomer experiments have shown that both N atoms on the urea molecule can derive from ammonia (Mutsvangwa et al. 1999). This small influence of the liver may explain why milk synthesis was not affected by duodenal ammonia infusion (Moorby & Theobald, 1999) that did not exceed the hepatic capacity for removal, while synthesis of milk components was depressed by jugular ammonia infusions that elevated peripheral concentrations of ammonia 5–10-fold (Experiments 2 and 3 and Malik et al. 1999).

Gln concentration in circulation typically increases during hyperammonaemia, which is thought to be due to Gln synthesis in muscle, where Ala and the BCAA, Leu, Ile and Val, provide much of the C skeleton and some N (Leweling et al. 1996; Bachmann et al. 2004). Accordingly, Ala and the BCAA concentrations decrease during hyperammonaemia (Leweling et al. 1996; Holeček et al. 2000). In addition to muscle, portal-drained viscera (Milano & Lobley, 2001) and the brain (Ott et al. 2005)

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

Table 7. Average extraction percentages of arterial amino acids (AA) by the mammary glands of four cows (Experiment 3) during the last hour of a 10-h infusion into the jugular vein of 360 mmol/h ammonium acetate or sodium acetate

	Sodium acetate	Ammonium acetate	SEM	*P*†
Met	35·2	69·4	21·6	0·515
Lys	53·6	56·4	1·6	0·825
Phe	38·8	44·8	6·9	0·676
Tyr	50·4	58·8	1·5	0·253
Thr	34·4	44·8	3·8	0·201
Val	16·8	31·7	5·1	0·201
Ile	27·2	49·2	8·9	0·247
Leu	35·5	48·8	3·5	0·129
Cln	−15·1	7·6	23·1	0·544
Pro	7·0	16·9	8·7	0·537
Ser	26·8	32·2	4·8	0·464
Gly	9·1	2·5	3·6	0·434
Ala	14·0	25·0	13·6	0·671
Glu	26·7	12·6	10·1	0·441
Asp	38·7	0·0	27·5	0·594
Total AA	20·8	28·7	3·2	0·260
Essential AA	29·2	46·3	6·7	0·235
Branched-chain AA	26·1	43·8	6·5	0·217
Non-essential AA	11·3	14·9	2·2	0·472

† Probability of no continuous effect of arterial plasma ammonia concentration

also consume BCAA during hyperammonaemia, presumably to provide C for Gln synthesis. In Experiment 3, we found that BCAA, along with Lys and Thr, decreased in arterial concentration in hyperammonaemic dairy cows. However, uptake of these amino acids from circulation by the mammary glands was not affected. These five amino acids are all part of the group II essential amino acids that are taken up by the mammary glands in excess of their output into milk (Mepham, 1982) so that deficiencies are relatively rare. Removal of approximately half of the Leu, Ile and Val from the absorptive amino acid supply had no effect on yields of milk and protein from early lactation cows (Weekes et al. 2006). Thus, though we find evidence that ammonia detoxification in non-hepatic tissues alters the arterial supply of essential amino acids for milk synthesis, there is no evidence that the decreases in amino acid concentrations were responsible for the decline in milk component production during hyperammonaemia.

Milk synthetic response

The effect of ammonium acetate infusion on the profile of circulating metabolites in Experiment 3 shares more in common with responses to hyperammonaemia in non-lactating, non-ruminant species than with responses to fasting in lactating ruminants. We conclude that the decrease in milk yield was not caused by a depression in feed intake but, alternatively, the depressions in intake and milk yield are independent and may constitute two prongs of a coordinated response to hyperammonaemia.

MPF was estimated from Phe+Tyr arteriovenous differences by a method that assumes that these two amino acids are not taken up from red blood cells or peptides, and they are transferred without loss into milk proteins. These issues have been discussed by others using Phe+Tyr to estimate MPF (Cant et al. 1993; Mackle et al. 2000; Thivierge et al. 2002) and the possibility of deviation from the assumptions, which could shift the accuracy by 10–20%, is considered acceptable to detect treatment differences. For example, the contribution of peptides to mammary Phe+Tyr supply would have to change by several fold to be responsible for a 20% difference in MPF. However, because of the error inherent in calculating a difference between two measures of Phe+Tyr concentration in plasma, the MPF estimate is not very precise. According to the SE of 75 l/h in Experiment 3 (Table 4), the difference in MPF between treatments would have to be 178 l/h to be detected as significant at $P<0{\cdot}05$ with a power of 0·80. This means that if MPF decreased to the same degree as milk yield, i.e,. 20% or 130 l/h, it would not be detectable. Regression analysis shows that MPF was significantly related to milk yield (Fig. 3) which, given the decline in milk yield, may suggest that MPF was actually decreased by hyperammonaemia.

A similar argument involving SE could be applied to the metabolite uptakes from plasma except that none of the uptake means, barring acetate, were arithmetically decreased by ammonium acetate infusion. Thus, errors do not appear to be masking a true effect of treatment. Amino acid uptakes were not decreased during hyperammonaemia, yet arterial concentrations of many essential amino acids were depressed, which manifested itself, for Leu, as an increased extraction percentage (Table 7). The only uptake affected was that of acetate. Aside from acetate, then, the decrease in milk yield and MPF was not accompanied by or consequent to a decrease in mammary utilization of milk precursors.

It is possible that hormones mediated the 20% decline in milk yield. Adrenaline release from the adrenal glands is stimulated by ammonia (Slawski et al. 1984) and exogenous adrenaline reduces milk yield (Gorewit & Aromando, 1985; Leenanuruksa & McDowell, 1985). Exogenous insulin can stimulate milk synthesis (Mackle et al. 2000) but plasma insulin concentration is generally increased during hyperammonaemia (Strombeck et al. 1981; Slawski et al. 1984) so insulin does not make a likely candidate to explain the decreased milk yield. To our knowledge, effects of hyperammonaemia on the galactopoietic hormones growth hormone and IGF-1 have not been studied.

Conclusion

Malik et al. (1999) infused 46 mmol/h ammonium bicarbonate into the jugular vein of lactating ewes, to an arterial concentration of ammonia we estimate to have been 10-times normal. The observed 24% decrease in milk protein yield supports the hypothesis that ammonia detoxification alters availability of amino acids for productive purposes. Moorby & Theobald (1999) created a much smaller

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

hyperammonaemia in lactating dairy cows by duodenal infusion of 200 mmol/h ammonium acetate to an estimated arterial concentration twice normal. There was no effect on milk production, a 4% increase in milk fat percentage and only a 2% decline in casein percentage. They concluded that ammonia detoxification had a negligible impact on amino acid supplies for productive purposes. We infused 360 mmol/h ammonium acetate into the jugular vein of dairy cows to an arterial concentration 5-times normal at 193 μM, and observed a 20% decrease in milk, protein and lactose yields. Circulating concentrations of several essential amino acids decreased but mammary uptakes of amino acids and energy metabolites, excluding acetate, were not affected by the hyperammonaemia. Mammary acetate uptake decreased in proportion to the drop in milk yield and mammary plasma flow. A depression in feed intake may have caused the decrease in milk production. Further research should focus on the possible role of adrenaline in mediating the depression in milk synthesis during hyperammonaemia.

The authors thank Sarah Armstrong, Donna Benschop, Kathleen (Brown) Shore, Jodi Calberry, Peter deVries, Susy Kilby, Sue (Edmonds) Sinclair, Bev Livingston, Paul Luimes, Angela Martin, Lori Sheehan, Melanie Sippel, Chanelle Toerien, Tammi Weekes and Erin Winter for assistance with animal care, sampling and laboratory analysis. Financial support for this work was provided by NSERC Canada and the Ontario Ministry of Agriculture and Food. Norm Purdie was in receipt of a Dairy Research Development Corporation postgraduate award.

References

Bachmann C, Braissant O, Villard A-M, Boulat O & Henry H 2004 Ammonia toxicity to the brain and creatine. *Molecular Genetics and Metabolism* **81** 852–857

Barej W, Harmeyer J, Drost H & Libau H 1982 The effect of hyperammonaemia on plasma glucose, insulin, glucagon, and adrenaline levels in sheep. *Zentralblatt fuer Veterinaermedizin Reihe A* **29** 197–206

Brown DL, Taylor SJ, DePeters EJ & Baldwin RL 1989 Influence of sometribove, USAN (recombinant methionyl bovine somatotropin) on the body composition of lactating cattle. *Journal of Nutrition* **119** 633–638

Bruckmaier RM, Wellnitz O & Blum JW 1997 Inhibition of milk ejection in cows by oxytocin receptor blockade, alpha-adrenergic receptor stimulation and in unfamiliar surroundings. *Journal of Dairy Research* **64** 315–325

Cant JP, DePeters EJ & Baldwin RL 1993 Mammary uptake of energy metabolites in dairy cows fed fat and its relationship to milk protein depression. *Journal of Dairy Science* **76** 2254–2265

Cant JP & McBride BW 1995 Mathematical analysis of the relationship between blood flow and uptake of nutrients in the mammary glands of a lactating cow. *Journal of Dairy Research* **62** 405–422

Chaiyabutr N, Faulkner A & Peaker M 1980 Effects of starvation on the cardiovascular system, water balance and milk secretion in lactating goats. *Research in Veterinary Science* **28** 291–295

Choung J-J, Chamberlain DG, Thomas PC & Bradbury I 1990 The effects of intraruminal infusions of urea on the voluntary intake and milk production of cows receiving grass silage diets. *Journal of Dairy Research* **57** 465–478

Culea M & Hachey D 1995 Determination of multiply labeled serine and glycine isotopomers in human plasma by isotope dilution negative-ion chemical ionization mass spectrometry. *Rapid Communications in Mass Spectrometry* **9** 655–659

Dhondt G, Houvenaghel A, Peeters G & Verschooten F 1973 Influence of vasoactive hormones on blood flow through the mammary artery in lactating cows. *Archives of International Pharmacodynamics* **204** 89–104

Drackley JK, Veenhuizen JJ, Richard MJ & Young JW 1991 Metabolic changes in blood and liver of dairy cows during either feed restriction or administration of 1,3-butanediol. *Journal of Dairy Science* **74** 4254–4264

Emmanuel B & Edjtehadi M 1981 Glucose biokinetics in normal and urea-treated sheep (*Ovis aries*). *Comparative Biochemistry and Physiology* **68B** 555–560

Friggens N, Emmans GC, Robertson S, Chamberlain DG, Whittemore CT & Oldham JD 1995 The lactational responses of dairy cows to amount of feed and to the source of carbohydrate energy. *Journal of Dairy Science* **78** 1734–1744

Gorewit RC & Aromando MC 1985 Mechanisms involved in the adrenalin-induced blockade of milk ejection in dairy cattle. *Proceedings of the Society for Experimental Biology and Medicine* **180** 340–347

Holeček M, Šprongl L & Tichý M 2000 Effect of hyperammonemia on leucine and protein metabolism in rats. *Metabolism* **49** 1330–1334

Keiding S, Munk OL, Roelsgaard K, Bender D & Bass L 2001 Positron emission tomography of hepatic first-pass metabolism of ammonia in pig. *European Journal of Nuclear Medicine* **28** 1770–1775

Lacasse P & Prosser CG 2003 Mammary blood flow does not limit milk yield in lactating goats. *Journal of Dairy Science* **86** 2094–2097

Leenanuruksa D & McDowell GH 1985 Effects of prolonged intravenous infusions of adrenaline on glucose utilization, plasma metabolites, hormones and milk production in lactating sheep. *Australian Journal of Biological Sciences* **38** 197–208

Leweling H, Breitkreutz R, Behne F, Staedt U, Striebel J-P & Holm E 1996 Hyperammonemia-induced depletion of glutamate and branched-chain amino acids in muscle and plasma. *Journal of Hepatology* **25** 756–762

Linzell JL 1967 The effect of very frequent milking and of oxytocin on the yield and composition of milk in fed and fasted goats. *Journal of Physiology* **190** 333–346

Linzell JL 1974 Mammary blood flow and methods of identifying and measuring precursors of milk. In *Lactation, A Comprehensive Treatise* (Eds BL Larson & VR Smith) pp. 143–225. New York, USA: Academic Press

Lobley GE, Connell A, Lomax MA, Brown DS, Milne E, Calder AG & Farningham DAH 1995 Hepatic detoxification of ammonia in the ovine liver: possible consequences for amino acid catabolism. *British Journal of Nutrition* **73** 667–685

Lomax MA & Baird GD 1983 Blood flow and nutrient exchange across the liver and gut of the dairy cow. Effects of lactation and fasting. *British Journal of Nutrition* **49** 481–496

MacKenzie SL 1987 Gas chromatographic analysis of amino acids as the n-heptafluorobutyryl isobutyl esters. *Journal of the Association of Official Analytical Chemists* **70** 151–160

Mackle TR, Dwyer DA, Ingvartsen KL, Chouinard PY, Ross DA & Bauman DE 2000 Effects of insulin and postruminal supply of protein on use of amino acids by the mammary gland for milk protein synthesis. *Journal of Dairy Science* **83** 93–105

Malik B, Nicol AM & Van Houtert M 1999 Source of excess nitrogen affects nutrient partitioning in lactating ewes. *Proceedings of the New Zealand Society of Animal Production* **59** 158–161

McGuire MA, Bauman DE, Dwyer DA & Cohick WS 1995 Nutritional modulation of the somatotropin/insulin-like growth factor system: response to feed deprivation in lactating cows. *Journal of Nutrition* **125** 493–502

Mepham TB 1982 Amino acid utilization by lactating mammary gland. *Journal of Dairy Science* **65** 287–298

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press

Milano GD & Lobley GE 2001 Liver nitrogen movements during short-term infusion of high levels of ammonia into the mesenteric vein of sheep. *British Journal of Nutrition* **86** 507–513

Moorby JM & Theobald VJ 1999 Short communication: The effect of duodenal ammonia infusions on milk production and nitrogen balance of the dairy cow. *Journal of Dairy Science* **82** 2440–2442

Mutsvangwa T, Buchanan-Smith JG & McBride BW 1999 Effects of in-vitro addition of ammonia on the metabolism of ^{15}N-labelled amino acids in isolated sheep hepatocytes. *Canadian Journal of Animal Science* **79** 321–326

Ndibualonji BB, Dehareng D & Godeau JM 1997 Effects of starvation on plasma amino acids, urea and glucose in dairy cows. *Annales de Zootechnie* **46** 163–174

Neter J, Wasserman W & Kutner MH 1985 *Applied Linear Statistical Models 2nd Edn.* Homewood IL, USA: Richard D Irwin

Ott P, Clemmesen O & Larsen FS 2005 Cerebral metabolic disturbances in the brain during acute liver failure: From hyperammonemia to energy failure and proteolysis. *Neurochemistry International* **47** 13–18

Prosser CG, Davis SR, Farr VC & Lacasse P 1996 Regulation of blood flow in the mammary microvasculature. *Journal of Dairy Science* **79** 1184–1197

Reynolds CK 1992 Metabolism of nitrogenous compounds by ruminant liver. *Journal of Nutrition* **122** 850–854

SAS Institute, Inc. 2000 *SAS/STAT User's Guide: Version 8.* Cary NC, USA: SAS Institute, Inc

Slawski Z, Barej W & Wiechetek M 1984 The participation of adrenal medullary hormones in the metabolic effects of hyperammonemia. *Zentralblatt fur Veterinamedezin Reihe A* **31** 481–488

Strombeck DR, Rogers, QR & Stern JS 1981 Effects of ammonia infusion on plasma glucagon, insulin and amino acids in intact, pancreatectomized and adrenalectomized dogs. *American Journal of Veterinary Research* **42** 810–818

Symonds HW, Mather DL & Collis KA 1981 The maximum capacity of the liver of the adult dairy cow to metabolize ammonia. *British Journal of Nutrition* **46** 481–486

Thivierge CM, Bernier JF, Dubreuil P & Lapierre H 2002 The effect of jugular or abomasal infusion of amino acids on milk yield in lactating cows fed a protein deficient diet. *Reproduction Nutrition Développement* **42** 1–13

Toerien CA & Cant JP 2007 Duration of a severe feed restriction required to reversibly decrease milk production in the high-producing dairy cow. *Canadian Journal of Animal Science* **87** 455–458

Weekes TL, Luimes PH & Cant JP 2006 Responses to amino acid imbalances and deficiencies in lactating dairy cows. *Journal of Dairy Science* **89** 2177–2187

Wiechetek M, Podgurniak P, Zabielski R & Podgurniak M 1989 The effect of adrenal denervation on the metabolic effects of hyperammonemia in sheep. *Canadian Journal of Physiology and Pharmacology* **67** 1062–1066

https://doi.org/10.1017/S0022029908003634 Published online by Cambridge University Press