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Effect of Postruminal Glutamine Supplementation on Immune Response and Milk Production in Dairy Cows

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ABSTRACT

Seventeen multiparous Holstein cows were used to examine the effect of an increased duodenal supply of Gln on immune function and production. Cows received continuous abomasal infusions of water (control: n = 8) or 300 g/d of Gln (n = 9) for 21 d starting within 48 h of calving. There were nonsignificant increases in milk and milk protein yields in response to Gln supplementation. Glutamine treatment had no effect on plasma glucose, nonesterified fatty acids (NEFA), or β-hydroxybutyrate (BHBA) concentrations but did tend to increase plasma urea N concentration. The Gln treatment resulted in an increase of 108 μM in the plasma Gln concentration. Total essential AA concentrations decreased with the Gln treatment, whereas total nonessential AA concentrations were unaffected. T Lymphocyte proliferation did not differ between the control and Gln-treated cows. Treatment had no effect on the relative abundance of CD8 T cells but did increase the abundance of CD4 T cells. Cytokine production, as measured by IFN-γ concentration determined in vitro in concanavalin-A-stimulated peripheral blood mononuclear cells, was similar between the treatments. Over the first 3 wk following calving, Gln supplementation had limited effects on milk production, metabolic parameters, and immune function.

Key words: glutamine supplementation, immune response, transition cow, amino acid

INTRODUCTION

Although mammals can synthesize nonessential AA (**NEAA**) from other metabolic precursors, this capacity may be inadequate to meet metabolic demands under specific physiological or developmental conditions. To meet such extra demands, an additional dietary or exogenous supply is required, and under such conditions, the specific NEAA is classified as "conditionally essential." Examples include Gly, which is beneficial for newborn infants and nursing mothers (Jackson, 1991), and Pro and Arg, which enhance piglet performance (Brunton et al., 1999). Similarly, during periods of physiological stress such as trauma, burns, and postoperative recovery, supplemental Gln has proven beneficial (Lacey and Wilmore, 1990). Indeed, Gln is one of the most versatile AA in terms of metabolic uses. Notably, the immune system requires a Gln supply for lymphocyte proliferation (Newsholme, 2001) and cytokine production (Chang et al., 1999). Glutamine also acts as a precursor for purine and pyrimidine synthesis, particularly in the intestinal tract and tissues of the immune system (Gate et al., 1999) or in tissues that undergo rapid growth, such as the mammary gland at initiation of lactation. Furthermore, in porcine intestinal cells, Gln and Glu are major energy sources, contributing nearly 10-fold more to CO_2 production than does glucose (Reeds et al., 2000). In addition to these roles, milk protein synthesis also represents an important requirement because Gln and Glu constitute approximately 20% of casein (Eigel et al., 1984).

Metabolic demands for Gln may be especially high in the early postpartum cow, when the demands for milk protein synthesis are accompanied by a major increase in intestinal and mammary gland mass and activity and whole body energy expenditure. During this period, the intestine may increase its reliance on Gln as an energy source to spare glucose. Additionally, there is a deficiency of propionate to meet glucose demands, and the body must therefore rely on other gluconeogenic precursors. As a result, there may be enhanced conversion of Gln to glucose in an attempt to help satisfy demand. Such competing demands for Gln are evidenced by the lengthy depression in postcalving plasma Gln concentration. Although plasma concentrations of most AA are depressed immediately following calving,

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these are usually restored within 2 to 4 wk. This is not the case for Gln, where the lowered concentration persists for much longer (Meijer et al., 1995a; Doepel et al., 2002). This suggests that the combination of dietary supply and synthesis de novo are insufficient to meet metabolic demands, and this may result in other processes becoming compromised. This may include immune system function, and indeed immunosuppression is often experienced by the periparturient cow (Goff and Horst, 1997).

By overcoming the limitation on Gln supply, the postpartum cow could increase milk production and improve immune status. Such benefits would arise first through provision of an alternative energy supply to the gut, thus increasing glucose availability to the mammary gland, and second by increasing the arterial supply of Gln to the mammary gland and immune system. These concepts were examined in the current study, where Gln was supplemented for 3 wk immediately after parturition.

MATERIALS AND METHODS

Animals and Treatments

Twenty multiparous Holstein cows averaging 710 ± 62 kg of BW 4 wk before expected calving were housed in tie stalls in a heated barn (approximately 15°C). Twelve of the cows were surgically implanted with abomasal catheters during the dry period (mean of 31 d precalving). The remaining 8 cows were surgically implanted with chronic indwelling catheters in the mesenteric, portal, and hepatic veins (Huntington et al., 1989) in addition to the abomasal catheters. For these cows, surgeries were performed a minimum of 6 wk before calving. These cows were used in a continuation of the present study to determine the effect of Gln on nutrient splanchnic flux, and the results will be reported in a future publication. Of the cows with abomasal catheters only, one developed severe mastitis precalving and was removed from the study prior to its inception. The catheter of a second cow became displaced during her infusion; thus, she was removed from the study. One of the 8 cows with the splanchnic catheters developed an ulcerated cecum and subsequently was euthanized. Thus, 17 cows were used in the study.

The abomasal catheterization was performed as follows. Cows were fasted for 24 h prior to surgery. One hour before surgery, 3 mg/kg of ketoprofen (Anafen; Merial, Quebec, Canada) and 20 mg of acepromazine maleate (Atravet; Ayerst Laboratories, Guelph, Ontario, Canada) were injected intravenously. Cows then received paravertebral anesthesia of the right flank using lidocaine HCl 2% (Ayerst Laboratories), and the flank was prepared for laparotomy. A 15-cm-long right-flank incision was made 7 to 8 cm caudal and parallel to the 13th rib, starting 20 cm under the edge of the transverse process of the vertebra. The skin, the external and internal abdominal oblique, and the transverse muscles as well as the peritoneum were incised to expose the abdominal organs. The pyloric portion of the abomasum was exteriorized and an 8G needle was inserted through the parietal surface of the abomasal wall into the lumen ~15 cm cranial to the pylorus. Ten centimeters of 14G Tygon tubing was inserted via the needle in the abomasum. The needle was withdrawn and a purse-string suture was made around the tubing exteriorization. The tubing was sutured to the parietal surface of the abomasum and exteriorized through the dorsal surface of the cow. Thereafter, a standard omentopexy was performed.

Cows were blocked by parity, expected calving date, and previous milk production and were randomly assigned within block to 1 of 2 abomasal infusions: 10 L/d of water (control; n = 8, includes 3 cows with splanchnic catheters) or 300 g/d of L-Gln delivered in 10 L of water (Gln; n = 9, includes 4 cows with splanchnic catheters). Infusions were initiated within 48 h following parturition and were administered continuously for 21 d via a peristaltic pump. Although nutrient demands increase in the close-up dry period because of fetal growth and mammogenesis, treatments were not initiated precalving because nutrient demands are 3- to 4-fold higher postcalving, making a response to treatment more likely at that time. Additionally, the cows were placed in maternity pens from 5 d precalving to several hours postcalving, making it impossible to maintain an infusion line during the immediate peripartum period. Fresh L-Gln infusion solutions were prepared daily.

For 4 wk preceding calving, cows were fed a close-up TMR once daily at 0800 h (Table 1). Cows also received 2 kg/d of long grass hay (9.6% CP, 36.1% ADF, 60.6% NDF). After calving, a lactating cow TMR was fed twice daily at 0800 and 1600 h (Table 1). The cows also received 20 g of rumen-protected Met (Mepron; Degussa, Düsseldorf, Germany) once daily. This was mixed in a small amount of corn grain (~200 g) and given immediately before the morning feeding. Two kilograms per day of long alfalfa hay (11.9% CP, 32.9% ADF, 56.1% NDF) was also fed in the morning before the TMR was offered. During both the pre- and postpartum periods, the TMR were offered ad libitum. Moisture content of the silages was determined weekly and used to make ration adjustments. Orts were recorded daily. Cows were given free access to fresh water. Cows were milked twice a day, at 0830 and 1930 h, and milk yield was recorded at each milking.

The experimental protocol was approved by the Institutional Committee for Animal Care of the Lennoxville

Table 1. Ingredient and nutrient composition of pre- and postcalving diets

Composition	Precalving	Postcalving
	% DM	
Ingredient		
Corn silage	42.3	38.2
Grass hay[1]	16.8	19.8
High-moisture corn	13.9	19.0
Beet pulp	12.8	—
Soybean meal	6.0	11.1
Protein supplement[2]	6.0	—
Rumen bypass soybean meal[3]	—	6.7
Mineral and vitamin premix[4]	1.4	3.2
Rumen bypass fat[5]	—	1.2
Limestone	0.9	0.7
Chemical		
DM	49.8	45.9
CP	14.1	16.8
ADIP[6]	0.94	0.82
ADF	20.8	17.5
NDF	33.5	28.6
Ether extract	2.8	4.2
Lignin	2.04	1.56
Ash	6.02	7.57
Ca	0.77	1.01
P	0.50	0.57
Mg	0.35	0.30
K	1.30	1.50
Na	0.16	0.47
DCAD,[7] mEQ/kg	82	177
NE_L,[8] Mcal/kg	1.63	1.72
MP,[9] g/d	1,255	1,948

[1]Precalving hay contained 9.6% CP; postcalving hay contained 11.9% CP.

[2]Contains 15% corn distillers' grains, 25% corn gluten meal, 20% wheat distillers' grains, 20% Soyplus (West Central Soy, Ralston, IA), and 20% canola meal.

[3]Soyplus (West Central Soy).

[4]Precalving mineral and vitamin premix contained 3% Ca, 11% P, 12% Mg, 7% Na, 2.3% S, 120 mg/kg Co, 1,610 mg/kg Cu, 4,390 mg/kg Fe, 202 mg/kg I, 6,550 mg/kg Mn, 40 mg/kg Se, 6,630 mg/kg Zn, 730,000 IU/kg vitamin A, 246,000 IU/kg vitamin D, and 7,560 IU/kg vitamin E; postcalving mineral and vitamin premix contained 9.5% Ca, 5.5% P, 3.5% Mg, 13% Na, 2.1% S, 33 mg/kg Co, 495 mg/kg Cu, 2745 mg/kg Fe, 69 mg/kg I, 2,065 mg/kg Mn, 20 mg/kg Se, 3,000 mg/kg Zn, 500,000 IU/kg vitamin A, 65,000 IU/kg vitamin D, and 2,600 IU/kg vitamin E.

[5]Megalac (Church & Dwight Co., Inc., Princeton, NJ).

[6]Acid detergent-insoluble protein.

[7]Dietary cation–anion difference, calculated as [(%Na × 435) + (%K × 256)] – [(%Cl × 282) + (%S × 624)]; National Research Council (2001) estimated values used for Cl and S.

[8]Calculated from total diet (TMR + hay): precalving based on 14 kg DMI; postcalving based on 17 kg DMI, estimated by the National Research Council (2001).

[9]Metabolizable protein intake, calculated from total diet (TMR + hay): precalving based on 14 kg DMI; postcalving based on 17 kg DMI, estimated by the National Research Council (2001).

Research Center, and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Sampling

All diet ingredients, with the exception of the silage, were sampled weekly and composited every 4 wk; silage was composited biweekly. Milk was sampled at each milking from d 19 to 21, and aliquots were stored at –20°C.

Jugular blood samples were obtained before the morning feeding on d –25, –12, –3, 1, 4, 11, and 18 relative to calving. Treatments were initiated following the d 1 sample. For 5 of the 10 cows that had abomasal catheters only, d –25 was prior to surgery, whereas for the remaining 5 cows, d –25 was at a minimum of 10 d following surgery. For cows with splanchnic catheters, d –25 was at least 3 wk following surgery. Blood for immunological assays was collected on EDTA and kept at room temperature. At the same time, one blood sample was collected without anticoagulant for preparation of heat-inactivated autologous serum (**AS**). For determination of peripheral blood mononuclear cell (**PBMC**) AA concentrations, blood was collected on heparin and kept at room temperature. Blood for all other assays was collected on heparin or EDTA and immediately placed on ice. Plasma was obtained from this blood (15 min, 1,800 × *g* at 4°C) and frozen at –80°C until AA analysis or at –20°C until subsequent chemical analysis for urea N, NEFA, and glucose. Deproteinized blood was used for BHBA and lactate analyses. Briefly, 1 mL of whole blood was mixed with 0.9 mL of distilled water and 0.1 mL of 6 *N* perchloric acid, and the mixture was stored on ice for 1 h before being centrifuged and the supernatant collected for subsequent analysis.

Laboratory Analyses

Feed ingredient samples (with the exception of the silage samples, which were freeze-dried) were dried in a forced-air oven at 55°C for 7 d and ground to pass a 1-mm screen. Feed N was determined by combustion (Nitrogen Determinator, model FP-428; Leco, St. Joseph, MI), and CP was calculated as N × 6.25. Acid detergent fiber, NDF, lignin, and acid detergent insoluble protein were determined using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY). Ash determination was as described by AOAC (1996).

Milk N content (protein = N × 6.38) was determined by combustion (Nitrogen Determinator, model FP-428; Leco), and milk fat was measured according to the Röse–Gottlieb method (AOAC, 1996). Casein content was determined on pooled samples as described by Raggio et al. (2004).

Plasma AA were measured by isotope-dilution gas chromatography–mass spectrometry (Calder et al., 1999; Raggio et al., 2004). In brief, on the day of sampling, 1 g of plasma was added to 0.2 g of an internal

standard solution and then frozen at –80°C until analysis. On the day of analysis, plasma was deproteinized and AA concentrations were determined by gas chromatography–mass spectrometry (model HP6890, S973 mass selective detector; Hewlett-Packard, Palo Alto, CA).

Plasma NEFA were determined colorimetrically (NEFA-C kit, Wako Chemicals USA Inc., Richmond, VA) with the modifications of Johnson and Peters (1993). β-Hydroxybutyrate concentrations were determined by the procedure of Williamson and Mellanby (1974) adapted to a 96-well plate format. A colorimetric assay (Boehringer Mannheim, Dorval, Quebec, Canada) was used to determine glucose concentration (glucose oxidase/peroxidase). Plasma urea N concentrations were measured with an automatic analyzer (Technicon Autoanalyser II, Technicon Instruments Corporation, Tarrytown, NY) as previously described (Huntington, 1984). A spectrophotometric method using lactate dehydrogenase was used to measure L-lactate (Benson et al., 2002). Total leukocyte counts were determined manually using the Unopette system and a hemacytometer, and differential leukocyte counts were performed on blood smears prepared with Wright's stain.

Peripheral blood mononuclear cells were isolated from whole blood as described by Lessard et al. (2004). Briefly, blood was layered on Ficoll-Hypaque Plus (Amersham Pharmacia, Montreal, Quebec, Canada), and following centrifugation (400 × *g* for 40 min), PBMC were collected at the interface. The cells were washed twice with Hanks' balanced salt solution without Ca^{2+} and Mg^{2+} (wash solution; Gibco BRL, Toronto, Ontario, Canada) and resuspended in RPMI 1640 medium without Gln (Gibco BRL). The number of viable cells was determined by trypan blue exclusion.

To determine the proliferative response to concanavalin A (**ConA**; Sigma, St. Louis, MO), isolated PBMC were diluted to 2.5×10^6 cells/mL and 50 μL/well was plated into 96-well microtiter plates. To each well was added 100 μL of RPMI 1640 without Gln. The cells were supplemented with 5% AS and ConA at 0, 0.125, 0.5, and 1.0 μg/mL, or with 5% fetal bovine serum (**FBS**; Gibco BRL) and ConA at 0, 0.06, 0.125, and 0.5 μg/mL. Each combination of serum and ConA was plated in triplicate. Concentrations of ConA were previously determined by Lessard et al. (2004). Plates were incubated in 5% CO_2 at 37°C for 72 h; 50 μL of 5-bromo-2-deoxyuridine solution was then added (Roche Diagnostics, Laval, Quebec, Canada), and the plates were incubated for another 16 h. Cell proliferation was quantified by measuring absorbance at 370 nm. Quantification was based on the measurement of incorporation of 5-bromo-2-deoxyuridine during DNA synthesis using an anti-5-bromo-2-deoxyuridine conjugate with peroxidase. Absorbance was directly correlated with the amount of DNA synthesis, and thus the proliferative response of PBMC. Values are expressed as optical density units.

Characterization of blood lymphocyte populations was performed by flow cytometry analysis using the following mouse monoclonal antibodies to bovine cell surface markers: anti-CD2 (BAQ95A), anti-CD4 (IL-A11), anti-CD8 (CACT80C), anti-B cells (LCT-2A), and anti-γ-δ T-cells (GB21A). All antibodies were purchased from VMRD (Pullman, WA). Ice-cold PBS, containing BSA at 0.5%, was used to dilute antibodies and to wash the plates. All cell-labeling steps were done on ice, and all centrifugations were performed at 10°C. Isolated PBMC were resuspended in PBS–BSA and a total of 1×10^6 cells per well were plated in duplicate into 96-well U-bottomed microtiter plates (Fisher Scientific, Pittsburgh, PA). Fluorescein isothiocyanate-conjugated goat antimouse $IgG_{2a/2b}$ and the phycoerythrine-conjugated goat antimouse IgG_1 (BD Pharmingen, San Diego, CA) were used as secondary antibodies for fluorescent staining. Single labeling was performed by incubating PBMC with 50 μL of the specific antibody at 2.5 μg/mL for 20 min, followed by 2 washes. The cells were then labeled with 50 μL of the secondary antibody at 2.5 μg/mL, followed by 2 washes. Double-labeling was performed as follows to characterize CD4 and CD8 cell populations: PBMC were simultaneously incubated with 25 μL of anti-CD4 at 5 μg/mL and 25 μL of anti-CD8 at 5 μg/mL for 20 min and washed twice. Thereafter, 25 μL of each secondary antibody at 5 μg/mL was added for the labeling of cells. The PBMC were resuspended in PBS supplemented with 2% paraformaldehyde and analyzed on a Coulter Epics XL-MCL flow cytometer using Expo 32 software (Beckman Coulter, Mississauga, Ontario, Canada). Lymphocytes were gated by using forward and side light-scattering, and data were collected for 5,000 events. Background fluorescence was determined by labeling cells with secondary antibodies only. Immunofluorescence was expressed as the percentage of positive cells minus controls. The monocytes were also gated by using forward and side light-scattering, and the percentage of monocytes in PBMC samples was established as follows: (number of monocytes gated)/(number of lymphocytes gated + number of monocytes gated)·100. Interferon-γ concentrations were determined from the supernatants of PBMC that were cultured in RPMI 1640, ConA, and AS or FBS as described by Lessard et al. (2004). Concentrations are expressed in picograms per milliliter.

The lysis and subsequent analysis of free AA concentrations in the isolated PBMC were based on the method of Fukuda et al. (1982). To a known volume and density of cells, 200 μL of L-norleucine was added

as a standard. Cells were then lysed using ultrasonic disruption. The cell lysate was deproteinized, and the protein-free supernatant was analyzed for AA concentrations by gas chromatography–mass spectrometry as described by McNeil (2001).

Statistical Analysis

Before statistical analysis, daily DMI and milk yield data for the duration of the experiment were first averaged over 2 periods per week. For example, data for d 4 is the average of d 1 to 4, and data for d 7 is the average of d 5 to 7.

For lymphocyte proliferation, the baseline response at ConA = 0 was subtracted from each of the values at each level of ConA before the data were subjected to statistical analysis. The data for IFN-γ for PBMC incubated in FBS were log transformed prior to statistical analysis because the data were not normally distributed.

Repeated-measures data were analyzed using the MIXED procedure of SAS (SAS Institute, 1999). The statistical model included day, treatment (**trt**), and the day × trt interaction. For each analyzed variable, cow was subjected to 3 covariance structures: autoregressive order 1, compound symmetry, and spatial power law. The covariance structure that resulted in the smallest Bayesian information criterion was used (Littell et al., 1996). Preplanned contrasts were used to determine the effects of time, treatment, and their interaction. For variables measured pre- and postpartum, a "pre vs. post" contrast compared the precalving to the postcalving treatment data. Data from d 1 were excluded from this comparison because they were postcalving but prior to treatment. A contrast "precalving vs. d 1" determined the effect of parturition. Treatment effect was tested by the contrast describing the interaction of treatment by time [i.e., "trt × (pre vs. post)"]; the presence of an interaction indicated a treatment effect. Linear and quadratic effects of time were also tested for the treatment periods as well as the interactions "trt × time (linear and quadratic)." A significant interaction denoted that during the treatment period, the pattern over time was different between the control and Gln cows.

Daily milk yield and milk composition data from the last 7 d of treatment were reduced to weekly means prior to statistical analysis. The MIXED procedure of SAS was used (SAS Institute, 1999), with treatment considered a fixed effect and block a random effect. All data are reported as least squares means with pooled standard errors, and significance was declared at $P < 0.05$.

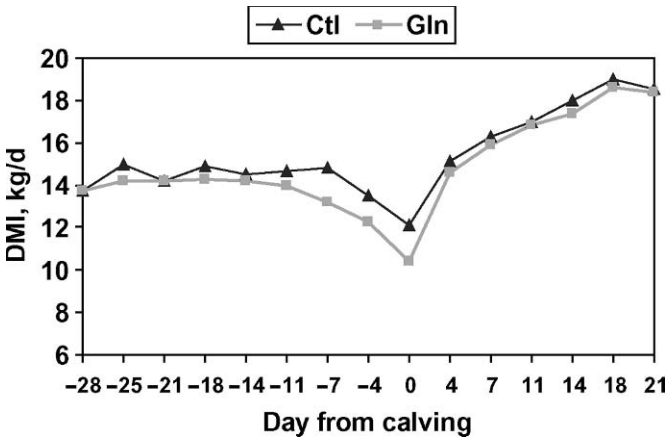

Figure 1. Dry matter intake (pooled SEM = 0.82). There were no treatment effects [trt × (pre vs. post), $P = 0.79$]. There was a precalving × calving day interaction ($P < 0.001$) and a linear effect during the treatment period ($P < 0.001$). Cows were abomasally infused with water (control, Ctl) or 300 g/d of Gln for 21 d starting within 48 h of calving. The data for day represent the average of 3- or 4-d periods, whereas the data for calving (d 0) were obtained from the day of calving only.

RESULTS

DMI and Milk Yield

Dry matter intake did not differ between the 2 treatments ($P = 0.79$), averaging 17.1 kg/d during the treatment period (Figure 1, Table 2). Intake declined steadily during the precalving period, with the lowest intake observed on the day of calving (pre vs. d 1; $P < 0.001$). Milk yield during the entire experimental period (Figure 2) was unaffected by treatment, but there was a treatment × day interaction ($P = 0.02$), primarily because of the numerical changes between the control and Gln disappearing on d 11. Milk yield and milk component yield during the final 7 d of the experimental period were also not significantly different between the treatments, although there was a 1.9 kg/d increase in milk yield and a 57 g/d increase in milk protein yield in the Gln-treated cows (Table 2). Treatment had no effect on milk protein concentration, milk casein as a percentage of CP, or milk fat concentration (Table 2).

Metabolic Parameters

The abomasal infusion of Gln was successful in generating an increase in plasma Gln concentration relative to the control treatment (Table 3). Concentrations during the treatment period averaged 227.6 ± 12.19 μM for the control cows and 352.6 ± 11.65 μM ($P < 0.001$) for the Gln-treated cows, whereas during the precalving period, concentrations averaged 307.8 ± 13.38 μM and

Table 2. DMI for the duration of the experiment, and milk production for the last 7 d of treatment[1]

Item	Treatment		SEM	P^2
	Control	Gln		
DMI, kg/d				
Precalving	14.4	13.8	0.57	
Calving	12.1	10.4	0.80	
Postcalving	17.3	17.0	0.60	0.79
Yield				
Milk, kg/d	39.3	41.2	1.91	0.49
CP, g/d	1,212	1,269	56.8	0.49
Casein, g/d	976	1,019	45.0	0.52
Fat, g/d	1,534	1,508	89.2	0.79
Milk composition				
CP, %	3.09	3.08	0.067	0.90
Casein, % of CP	80.9	80.3	0.91	0.48
Fat, %	3.92	3.74	0.167	0.46

[1]LSM are presented with the pooled SEM.

[2]For DMI, P value is for treatment × (pre vs. post); for all other data, P value is for treatment.

324.6 ± 12.67 μ*M* for the control and Gln cows, respectively.

Overall, Gln decreased ($P < 0.05$) concentrations of total essential AA (**EAA**), with an interaction [trt × (pre vs. post), $P < 0.10$] for the following individual EAA: Leu, Met, Phe, Thr, and Val. Concentrations of His, Ile, Lys, and Trp were unaffected by Gln infusion. Total NEAA (excluding Gln) concentrations were unaffected ($P = 0.21$) by treatment, but for Gly ($P = 0.05$), Ser ($P = 0.03$), and Tyr ($P = 0.04$), there was a trt × time-linear post interaction. For the control cows, Gly and Tyr concentrations increased from d 4 to 18, whereas for the Gln cows they remained constant during the same time period. In contrast, Ser concentrations in the control cows remained constant during the treatment period but decreased for the Gln cows. The overall effect of Gln relative to control was a reduction in total AA plasma concentrations ($P = 0.09$)

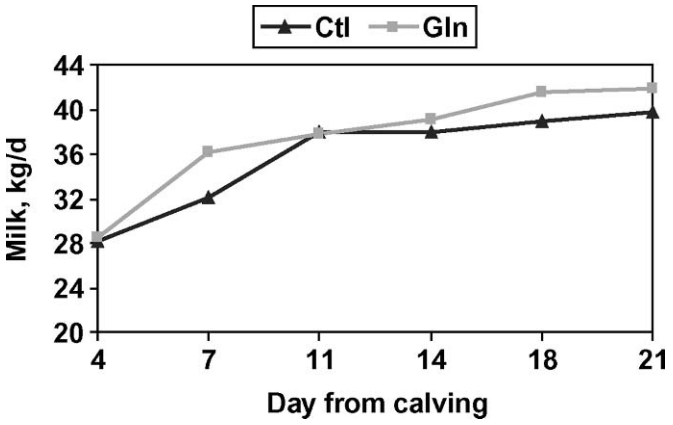

Figure 2. Milk yield during the entire treatment period (pooled SEM = 1.93). There was a treatment × day effect ($P = 0.02$). Cows were abomasally infused with water (control, Ctl) or 300 g/d of Gln for 21 d starting within 48 h of calving. The data for day represent the average of 3- or 4-d periods.

Plasma concentrations of most AA decreased at calving (pre vs. d 1, $P < 0.05$; Table 3). The exceptions were Met and Ser, whose concentrations did not change, and Gly, which showed an increase. Relative to precalving, concentrations of His and Phe were lower ($P < 0.01$) postcalving and remained constant. Concentrations of Lys were also lower postcalving but increased over time, whereas concentrations of Ile were higher post- vs. precalving ($P < 0.01$). With the exception of Gly, concentrations of NEAA were all lower ($P < 0.01$) postcalving vs. precalving.

Plasma urea N concentrations (Figure 3) of the control and Gln cows exhibited a different pattern during the precalving period (trt × linear effect precalving: $P = 0.02$). On d –25, the control cows (5.92 m*M*) had a lower urea N concentration than the Gln cows (7.94 m*M*), but by d –12, the concentrations were similar. Because the urea concentrations were essentially the same between the 2 treatment groups on d –12, –3, and 1, the data were reanalyzed excluding d –25. Glutamine infusion tended to increase urea N concentrations [trt × (pre vs. post), $P = 0.09$], averaging 9.3 and 10.7 m*M* for the control and Gln-treated cows, respectively. Calving had no influence on urea N concentrations (pre vs. d 1, $P = 0.59$).

Plasma glucose concentrations are shown in Figure 4. Although there was no treatment effect [trt × (pre vs. post): $P = 0.94$], there was a tendency for a trt × linear effect ($P = 0.10$). On d 4, the control cows experienced a substantial decline in concentration, whereas the Gln cows did not. Across treatments, glucose concentrations were lower (pre vs. post; $P = 0.001$) after calving (3.04 m*M* ± 0.085) than precalving (3.61 m*M* ± 0.092). Unlike glucose, lactate concentrations did not differ in the 2 periods (pre vs. post, $P = 0.83$; Figure 5), averaging 0.90 ± 0.130 m*M* in the precalving period and 0.93 ± 0.122 m*M* in the treatment period. However, concentration on d 1 (1.35 ± 0.155 m*M*) was significantly higher than precalving (pre vs. d 1, $P = 0.007$). Treatment had no effect on lactate concentrations [trt × (pre vs. post), $P = 0.98$].

Plasma NEFA (Figure 6) and BHBA (Figure 7) concentrations did not differ between treatments but did differ with physiological stage, being lower in the precalving period than the postcalving period ($P < 0.03$). Nonesterified fatty acid concentrations averaged 223.3 ± 88.38, 1,039.8 ± 103.93, and 992.6 ± 83.69 μ*M* in the precalving period, on d 1, and during the treatment period, respectively. Similarly, BHBA concentrations increased from 0.37 ± 0.156 m*M* in the precalving period

Table 3. Plasma AA concentrations (μM)[1]

Plasma AA	Trt	Day –25	–12	–3	1	4	11	18	SEM	Trt × time *P* Trt × (pre vs. post)	Trt × lin post	Trt × quad post	Time *P* Pre vs. post	Pre vs. d 1	Post lin	Post quad
EAA																
His	Control	64.8	62.1	58.6	48.9	45.9	46.4	48.0	5.72	0.99	0.35	0.18	0.001	0.001	0.75	0.13
	Gln	76.2	67.1	61.4	49.2	58.2	47.6	53.9								
Ile	Control	108.5	122.4	101.6	87.5	124.5	163.9	181.3	15.62	0.14	0.92	0.57	0.001	0.003	0.001	0.13
	Gln	123.8	115.6	113.8	71.9	100.7	154.6	160.3								
Leu	Control	170.0	171.5	152.0	126.0	140.6	187.2	207.2	16.73	0.06	0.53	0.59	0.69	0.001	0.001	0.11
	Gln	189.0	174.4	157.5	107.0	119.5	170.6	167.9								
Lys	Control	79.2	97.1	57.2	45.1	62.6	70.9	75.8	6.42	0.45	0.93	0.94	0.004	0.001	0.03	0.79
	Gln	92.8	71.7	61.0	43.1	55.3	62.3	67.5								
Met	Control	24.8	27.7	23.4	24.9	29.7	28.3	27.6	1.91	0.09	0.73	0.87	0.14	0.35	0.11	0.68
	Gln	26.9	25.2	23.3	22.9	26.8	24.4	23.6								
Phe	Control	52.9	58.4	52.2	40.6	46.5	47.5	50.7	3.25	0.08	0.50	0.49	0.001	0.001	0.49	0.77
	Gln	51.5	54.3	49.7	38.6	37.9	40.5	37.9								
Thr	Control	66.8	73.5	60.9	57.1	71.2	81.9	90.8	7.18	0.004	0.20	0.60	0.42	0.001	0.06	0.73
	Gln	74.2	67.6	53.0	37.7	56.4	53.8	60.3								
Trp	Control	48.1	46.9	33.6	21.7	28.4	39.9	47.0	3.54	0.27	0.12	0.95	0.001	0.001	0.001	0.40
	Gln	47.4	44.8	37.1	15.4	29.2	35.8	38.6								
Val	Control	228.6	248.9	207.9	160.9	200.9	269.6	309.1	21.03	0.009	0.34	0.73	0.92	0.001	0.001	0.56
	Gln	262.0	242.3	222.0	127.6	175.0	215.4	248.4								
NEAA																
Ala	Control	240.4	229.9	195.1	169.9	163.0	194.5	192.9	15.69	0.27	0.20	0.21	0.001	0.001	0.23	0.72
	Gln	245.8	211.8	192.0	141.2	165.1	155.5	164.0								
Cys	Control	111.9	118.2	99.4	67.7	88.1	91.3	88.1	5.97	0.32	0.48	0.51	0.001	0.001	0.49	0.93
	Gln	116.5	111.7	101.0	67.1	80.5	81.5	87.3								
Gln	Control	330.5	300.0	292.8	258.7	244.3	215.0	223.5	2.96	0.001	0.15	0.84	0.01	0.01	0.01	0.13
	Gln	363.7	295.1	314.9	300.7	395.5	336.1	326.2								
Glu	Control	67.5	45.1	41.4	39.1	37.8	40.8	43.5	18.77	0.30	0.28	0.75	0.001	0.001	0.33	0.69
	Gln	56.5	43.1	43.9	36.0	40.4	42.0	40.1								
Gly	Control	289.7	251.7	285.9	359.6	402.8	529.9	471.3	35.80	0.14	0.05	0.11	0.001	0.01	0.77	0.05
	Gln	282.5	252.4	316.2	330.3	443.1	426.4	392.0								
Ser	Control	93.1	86.9	87.3	90.0	73.9	79.0	77.1	7.90	0.61	0.03	0.03	0.001	0.13	0.10	0.13
	Gln	90.1	75.4	96.9	70.7	89.5	58.0	66.0								
Tyr	Control	63.7	60.8	49.1	38.0	35.8	43.1	49.1	3.45	0.04	0.04	0.54	0.001	0.001	0.04	0.71
	Gln	57.8	52.7	46.8	33.3	30.3	27.6	30.2								
Total EAA	Control	745.0	696.7	573.8	610.1	747.5	935.8	1,037.4	96.92	0.05	0.89	0.81	0.006	0.067	0.002	0.41
	Gln	838.4	853.0	433.5	512.3	588.2	801.3	854.8								
Total NEAA	Control	761.3	587.6	575.0	764.3	801.4	978.6	921.9	86.76	0.21	0.56	0.52	0.001	0.24	0.40	0.35
	Gln	738.1	747.2	432.2	678.6	758.8	790.9	779.5								
Total AA	Control	1,506.4	1,284.3	1,148.8	1,374.4	1,548.9	1,914.3	1,959.3	172.17	0.09	0.70	0.83	0.001	0.72	0.03	0.35
	Gln	1,576.5	1,600.2	865.7	1,190.9	1,347.0	1,592.2	1,634.3								

[1]LSM are presented with the pooled SEM. Trt = treatment; pre = precalving; post = postcalving; lin = linear effect; quad = quadratic effect; EAA = essential AA; NEAA = nonessential AA.

[2]Days –25, –12, and –3 are precalving; d 4, 11, and 18 are postcalving values used for contrast.

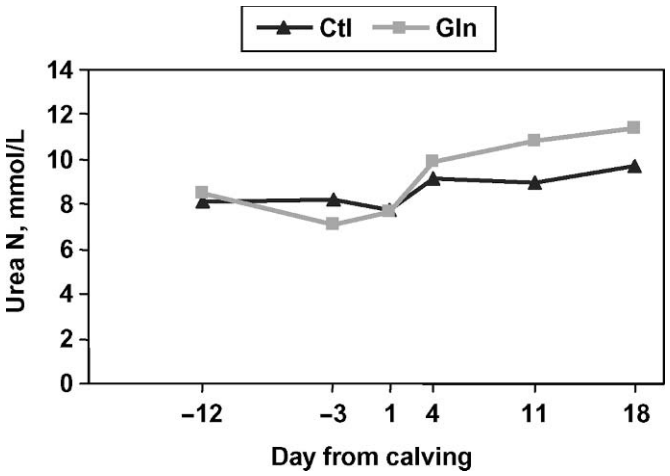

Figure 3. Plasma urea N concentrations (pooled SEM = 0.690). Data from d –25 are excluded (see text for explanation). There was a tendency for a treatment effect [trt × (pre vs. post), $P = 0.09$]. Urea N concentrations were lower before calving than during the treatment period (pre vs. post; $P < 0.001$). Cows were abomasally infused with water (control, Ctl) or 300 g/d of Gln for 21 d starting within 48 h of calving; d 1 is prior to the initiation of treatments.

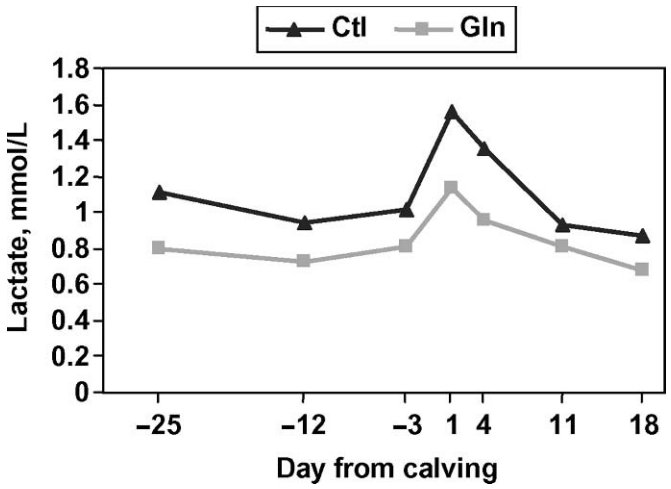

Figure 5. Blood lactate concentrations (pooled SEM = 0.227). There was no treatment effect [trt × (pre vs. post), $P = 0.98$]. Concentrations were lower precalving than on the day of calving (pre vs. d 1; $P = 0.007$). There was a linear effect during the treatment period ($P = 0.05$). Cows were abomasally infused with water (control, Ctl) or 300 g/d of Gln for 21 d starting within 48 h of calving; d 1 is prior to the initiation of treatments.

to 0.68 ± 0.169 m*M* at calving and 1.18 ± 0.151 m*M* during the treatment period.

Immunological Parameters

Total leukocyte and differential counts are shown in Table 4. Because there was no treatment effect, data were pooled and are presented by day of sampling only.

The total leukocyte count was significantly increased on d 1 compared with the precalving period (pre vs. d 1, $P = 0.001$), and then remained constant from d 4 to 18. Relative to the precalving period, the proportions of eosinophils and lymphocytes decreased on d 1, whereas that of neutrophils increased ($P < 0.005$). As a result of

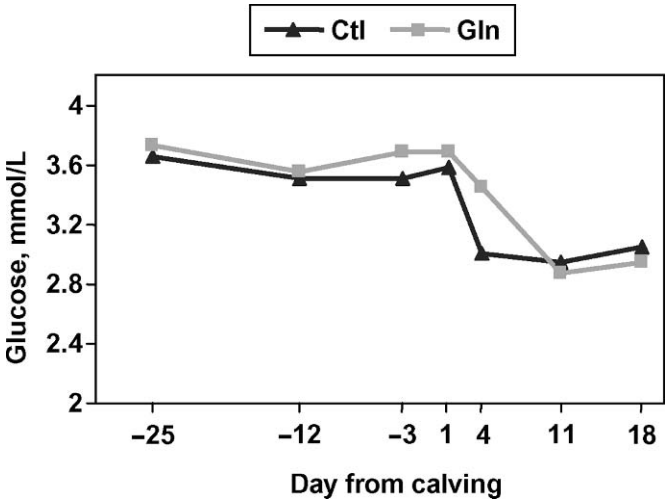

Figure 4. Plasma glucose concentrations (pooled SEM = 0.177). There was a tendency for a treatment × linear effect ($P = 0.10$). Cows were abomasally infused with water (control, Ctl) or 300 g/d of Gln for 21 d starting within 48 h of calving; d 1 is prior to the initiation of treatments.

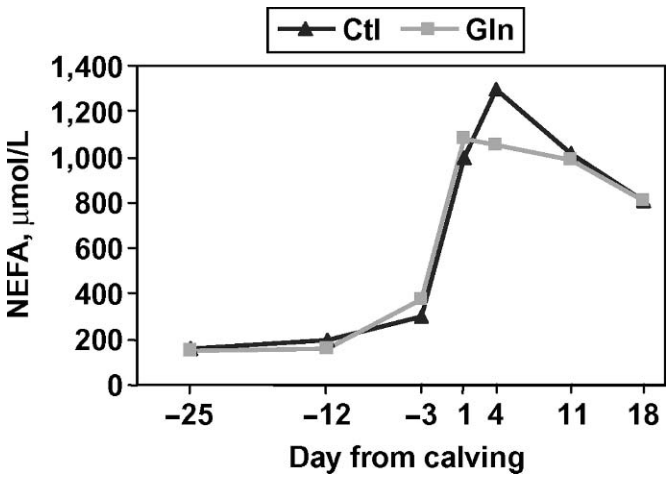

Figure 6. Plasma NEFA concentrations (pooled SEM = 152.6). There was no treatment effect [trt × (pre vs. post), $P = 0.65$]. Concentrations were lower precalving than on the day of calving (pre vs. post d 1, $P < 0.001$), and during the treatment period (pre vs. post, $P < 0.001$). There was a linear effect during the treatment period ($P = 0.05$). Cows were abomasally infused with water (control, Ctl) or 300 g/d of Gln for 21 d starting within 48 h of calving; d 1 is prior to the initiation of treatments.

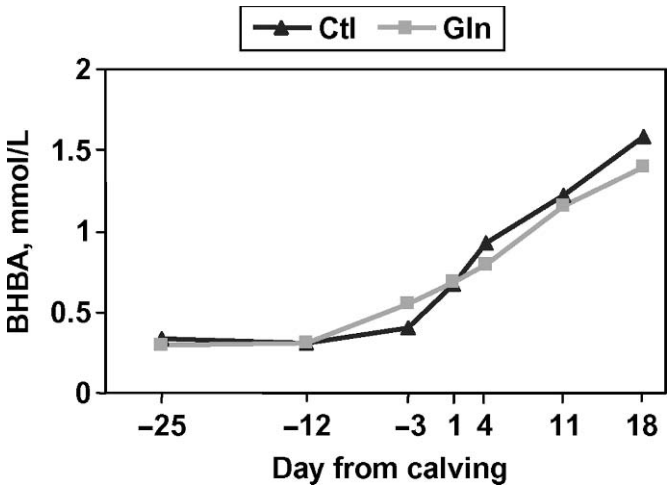

Figure 7. Blood BHBA concentrations (pooled SEM = 0.244). There was no treatment effect [trt × (pre vs. post), $P = 0.63$], but there was a linear effect during the treatment period ($P < 0.001$). Cows were abomasally infused with water (control, Ctl) or 300 g/d of Gln for 21 d starting within 48 h of calving; d 1 is prior to the initiation of treatments.

the increase in total leukocyte numbers, this resulted in no change in total numbers of lymphocytes but an increase in the number of neutrophils (linear effect, $P < 0.001$).

The proliferative response of lymphocytes, incubated either in AS or FBS, was not different between treatments (Table 5). With the exception of cells incubated at the highest ConA level, proliferation was not different between d –25 and the treatment period (pre vs. post: $P > 0.05$). For cells incubated with FBS, there was a linear increase in proliferation at the lower ConA levels ($P < 0.10$) as lactation progressed, but this effect was not observed when cells were incubated with AS.

Table 6 shows the relative abundance of the T lymphocyte subpopulations. Because T cells may carry more than one receptor type, the total cell numbers for a particular day and treatment were >100%. Treatment had no effect on the relative abundance of $CD8^+$ T cells (17.1% across treatments). For $CD4^+$ cells, there was a trt × linear interaction ($P = 0.05$), primarily because of differences between the treatments on d 4. These changes were also reflected in the temporal increase in the $CD4^+$:$CD8^+$ ratio (trt × linear effect, $P = 0.04$). In comparison with d –25 values, the percentage of T cells carrying the CD2 receptor was higher for the Gln-treated cows than the control cows [trt × (pre vs. post), $P = 0.04$; Table 6]. The relative amounts of B lymphocytes tended to vary over time between the 2 treatments (trt × time quadratic, $P = 0.09$). For the control cows, the proportion of B cells was greater on d 11 vs. d 4 or 18, but this difference was not observed in the Gln-treated cows. The relative amount of monocytes (as a proportion of PBMC) was increased by the Gln infusion [trt × (pre vs. post), $P = 0.003$]. For control, the cows averaged 30.9 and 30.2% for the precalving and treatment periods, respectively, whereas for the Gln treatment, the cows averaged 25.3 and 37.2% for the 2 time periods. Lymphocyte populations did not change over the course of the treatment period (linear and quadratic, $P > 0.05$), but the percentage of monocytes decreased as lactation progressed (Table 6).

Treatment had no effect on IFN-γ concentrations (Table 7); however, there was an effect of physiological stage (pre vs. post, $P < 0.05$). Concentrations were higher precalving (1139.5 pg/mL) than postcalving (851.3 pg/mL) for PBMC incubated in FBS; however, for cells incubated in AS, this effect was not evident.

Lymphocyte Gln concentrations tended to increase with Gln infusion [trt × (pre vs. post), $P = 0.07$; Table 8]. This was due to a 20% decline in control cows, whereas Gln supplementation maintained postpartum concentrations similar to precalving. Unlike Gln, Glu concentrations were unaffected by treatment ($P = 0.93$) and averaged 15.17 nmol/10^7 cells. Lymphocyte His concentrations tended to increase ($P = 0.07$) from the pre-

Table 4. Total leukocyte and differential blood count[1]

	Day relative to calving[2]								*P* Trt × (pre vs. post)	Time *P*			
Count	–25	–12	–3	1	4	11	18	SEM		Pre vs. post	Pre vs. d 1	Post lin	Post quad
Total leukocytes, cells/μL	5,934	6,099	7,613	9,696	7,216	7,127	6,566	535.4	0.80	0.29	0.001	0.32	0.67
Differential blood count, %													
Basophils	0.4	0.5	0.7	0.3	0.6	0.6	0.8	0.24	0.55	0.55	0.38	0.58	0.66
Eosinophils	11.3	8.0	6.5	3.9	4.8	4.0	5.7	1.42	0.96	0.001	0.001	0.59	0.37
Lymphocytes	43.3	39.3	33.4	27.9	36.5	42.9	37.3	3.27	0.24	0.93	0.003	0.85	0.10
Monocytes	11.4	10.2	11.2	10.9	16.7	9.9	9.9	1.34	0.84	0.26	0.99	0.001	0.03
Neutrophils	34.0	42.0	48.6	56.9	41.3	42.6	46.4	3.77	0.27	0.54	0.001	0.31	0.76

[1]LSM are presented with the pooled SEM. Trt = treatment; pre = precalving; post = postcalving; lin = linear effect; quad = quadratic effect.

[2]Days –25, –12, and –3 values are precalving; d 4, 11, and 18 are postcalving values used for contrast.

Table 5. Lymphocyte proliferative response (optical density units) of peripheral blood mononuclear cells to concanavalin A (ConA)[1]

							Trt × time *P*			Time *P*		
		Day[2]					Trt × (pre vs. post)	Trt × lin post	Trt × quad post	Pre vs. post	Post lin	Post quad
Response	Trt	−25	4	11	18	SEM						
FBS												
ConA[3]												
0.06	Control	0.32	0.27	0.27	0.50	0.120	0.84	0.82	0.13	0.62	0.03	0.73
	Gln	0.21	0.08	0.41	0.36							
0.125	Control	0.57	0.66	0.54	0.79	0.135	0.65	0.35	0.31	0.21	0.07	0.66
	Gln	0.47	0.44	0.72	0.85							
0.50	Control	0.79	1.39	1.03	1.02	0.146	0.63	0.20	0.83	0.03	0.33	0.30
	Gln	0.72	0.97	0.88	1.02							
AS												
ConA[3]												
0.125	Control	0.12	0.08	0.08	0.10	0.059	0.38	0.66	0.29	0.92	0.36	0.42
	Gln	0.06	0.04	0.14	0.10							
0.50	Control	0.83	0.91	0.59	0.83	0.148	0.12	0.39	0.26	0.24	0.72	0.31
	Gln	0.42	0.66	0.77	0.84							
1.00	Control	0.93	1.31	1.22	1.13	0.160	0.92	0.74	0.28	0.04	0.46	0.28
	Gln	0.72	1.12	0.77	1.05							

[1]LSM are presented with the pooled SEM. Trt = treatment; pre = precalving; post = postcalving; lin = linear effect; quad = quadratic effect; FBS = fetal bovine serum; AS = autologous serum.

[2]Day −25 is precalving; d 4, 11, and 18 are postcalving values used for the contrasts.

[3]ConA concentrations are micrograms per milliliter.

calving (0.34 nmol/10^7 cells) to the postcalving (0.44 nmol/10^7 cells) period for the control cows, but remained constant for the Gln-treated cows (0.49 and 0.45 nmol/10^7 cells for the precalving and treatment periods, respectively; Table 8). Glutamine supplementation resulted in a decrease in both Thr and Val concentrations ($P = 0.03$). Valine concentrations for the control cows were 3.40 and 3.79 nmol/10^7 cells during the precalving and treatment periods, respectively, whereas for the Gln cows, concentrations were 6.60 and 5.02 nmol/10^7 cells for the same time periods. Essential AA concentrations did not differ between the precalving period and d 1, with the exception of Thr and Val, which tended to decrease at calving. For the NEAA, Ala tended to decrease at calving, Asn and Asp decreased at calving, and Gln increased at calving.

Table 6. Characterization of peripheral blood mononuclear cell subpopulations (%)[1]

							Trt × time *P*			Time *P*		
		Day					Trt × (pre vs. post)	Trt × lin post	Trt × quad post	Pre vs. post	Post lin	Post quad
Subpopulation	Trt	−25	4	11	18	SEM						
T lymphocytes												
CD2	Control	64.1	57.5	69.2	68.7	4.53	0.04	0.20	0.15	0.02	0.13	0.36
	Gln	48.4	63.5	62.7	64.5							
CD4	Control	30.6	29.9	36.4	37.5	3.45	0.11	0.05	0.41	0.001	0.42	0.40
	Gln	24.0	37.3	35.7	34.1							
CD8	Control	16.9	16.2	18.7	19.5	2.55	0.71	0.75	0.28	0.24	0.12	0.83
	Gln	13.8	15.5	15.3	17.7							
γ-δ	Control	11.8	12.8	14.7	12.0	1.81	0.27	0.92	0.02	0.91	0.47	0.55
	Gln	9.8	9.6	7.7	8.6							
CD4:CD8[2]	Control	2.2	2.2	2.3	2.5	0.38	0.33	0.04	0.93	0.13	0.24	0.57
	Gln	1.9	3.0	2.4	2.1							
B lymphocytes	Control	13.4	15.7	18.9	12.3	3.07	0.37	0.42	0.09	0.95	0.69	0.34
	Gln	16.4	14.4	13.6	15.5							
Monocytes	Control	30.9	39.2	26.0	25.5	2.48	0.01	0.73	0.84	0.01	0.01	0.01
	Gln	25.3	45.1	33.4	33.0							

[1]LSM are presented with the pooled SEM. Trt = treatment; pre = precalving; post = postcalving; lin = linear effect; quad = quadratic effect.

[2]Data are presented as ratios, not as percentages.

Table 7. Interferon-γ concentrations in supernatants of concanavalin A-stimulated peripheral blood mononuclear cells (pg/mL)[1]

	Day relative to calving									Trt × time *P*			Time *P*		
	Control				Gln										
Supernatant	–25	4	11	18	–25	4	11	18	SEM[2]	Trt × (pre vs. post)	Trt × lin post	Trt × quad post	Pre vs. post	Post lin	Post quad
FBS	1,124	801	841	706	1,156	934	837	1,025	—	0.61	0.51	0.19	0.05	0.92	0.83
AS	1,046	909	785	581	1,317	1,262	1,146	919	275.3	0.85	0.98	0.97	0.25	0.20	0.81

[1]LSM are presented with the pooled SEM. Trt = treatment; pre = precalving; post = postcalving; lin = linear effect; quad = quadratic effect; FBS = fetal bovine serum, AS = autologous serum.

[2]SEM not available for FBS data because the data were log transformed.

DISCUSSION

Milk Yield and DMI

The numerical increase in milk yield (1.9 kg) with Gln supplementation was higher than the significant response (1.3 kg) observed in 6 cows also infused with 300 g/d of Gln at 6 wk postpartum (Meijer et al., 1995b, Experiment 1), but the interanimal variation in the current study precluded statistical significance. Unlike the experiment by Meijer et al. (1995b, Experiment 1), Plaizier et al. (2001) also did not observe any response in milk yield in cows averaging 63 DIM at the initiation of a study in which they received a postruminal infusion of Gln of up to 300 g/d for 4 d. No milk response was also reported by Meijer et al. (1995b, Experiment 2), but in the latter study, Gln decreased DMI. Milk protein content and yield were not significantly affected by an increased duodenal supply of Gln, as previously observed by Plaizier et al. (2001) and Meijer et al. (1995b).

Dry matter intake was not influenced by Gln supplementation. This observation is similar to that previously reported, even though the stage of lactation and the length of infusion varied between studies. In the study by Plaizier et al. (2001), DMI was not different among cows (63 DIM) receiving incremental amounts (from 0 to 300 g/d) of Gln postruminally for 4 d. Meijer et al. (1995b) reported no difference in DMI between cows receiving 0 or 300 g/d of Gln abomasally in one study, but did observe a decrease in intake with Gln supplementation in a subsequent study using the same cows and with the same level of supplementation. The decline in intake during the last 7 d precalving is consistent with previously published results (Doepel et al., 2002) and may be associated with animal factors (physical fill, endocrine changes) and dietary factors, as discussed by Grummer et al. (2004).

Metabolic Parameters

Glutamine infusion resulted in plasma Gln concentrations 108 μM higher than those in control cows, showing quite clearly that the abomasal infusion was successful in delivering Gln to the duodenum and that at least part of it escaped catabolism across the intestine and was absorbed into the bloodstream.

The lower concentration of total EAA in the Gln-treated cows may be the result of different metabolic effects of Gln. There is evidence that Gln stimulates protein synthesis (Reecy et al., 1996) or decreases protein degradation (Vom Dahl and Häusinger, 1996). Improvements in net anabolism (greater milk protein output) would lower concentrations of EAA. However, Plaizier et al. (2001) also observed a trend of decreased plasma concentrations of EAA with Gln treatment in cows at a later stage of lactation without any effect on milk protein yield. In that study, as in the current study, increased urea concentrations indicated increased ureagenesis, which has been suggested to elevate hepatic removal of EAA (Lobley et al., 1995).

As DIM progressed, the Gln concentration decreased linearly, indicating an increasing demand with advancing lactation. Previous studies have also demonstrated that Gln concentrations postcalving remain below precalving concentrations for up to 6 wk postpartum (Meijer et al., 1995a; Doepel et al., 2002).

The decrease in plasma AA concentrations on d 1 relative to precalving likely reflects the substantial reduction in DMI at calving and an increase in protein synthesis for growth of the mammary gland. However, by d 18, plasma concentrations of EAA in the control cows (with the exception of His) increased and returned to, or were even higher than, precalving values. This result suggests that relative to the other EAA, His may have been limiting. Unlike the EAA, the concentrations of most of the NEAA were lower on d 18 than during the precalving period. This contrasts with the results of Doepel et al. (2002) in which NEAA concentrations, with the exception of Gln at 21 d postcalving, were equivalent to precalving levels. In that study, dietary protein was higher and milk yield lower than in the current study.

The tendency for the increase in plasma urea N concentrations with the Gln treatment is probably the result of Gln deamination in the gut and liver. The excess

Table 8. Lymphocyte AA concentrations (nmol/10^7 cells)[1]

AA	Trt	Day –25	–12	–3	1	4	11	18	SEM	Trt × time *P* Trt × (pre vs. post)	Trt × lin post	Trt × quad post	Time *P* Pre vs. post	Pre vs. d 1	Post lin	Post quad
EAA																
Arg	Control	0.45	0.52	0.59	0.57	0.54	0.48	0.48	0.064	0.63	0.79	0.39	0.24	0.29	0.12	0.97
	Gln	0.45	0.46	0.58	0.52	0.48	0.47	0.40								
His	Control	0.27	0.36	0.38	0.37	0.43	0.45	0.44	0.084	0.07	0.66	0.61	0.43	0.79	0.49	0.46
	Gln	0.45	0.58	0.45	0.48	0.40	0.50	0.47								
Ile	Control	0.47	0.49	0.45	0.48	0.43	0.44	0.40	0.132	0.75	0.25	0.39	0.13	0.73	0.55	0.20
	Gln	0.50	0.56	0.65	0.59	0.42	0.57	0.52								
Leu	Control	0.65	0.72	0.80	0.80	0.65	0.61	0.60	0.110	0.98	0.67	0.22	0.03	0.67	0.32	0.34
	Gln	0.66	0.70	0.89	0.73	0.65	0.74	0.54								
Lys	Control	0.37	0.39	0.39	0.45	0.39	0.36	0.37	0.044	0.22	0.61	0.48	0.10	0.18	0.20	0.77
	Gln	0.37	0.33	0.40	0.36	0.34	0.32	0.28								
Met	Control	0.20	0.25	0.25	0.28	0.27	0.22	0.21	0.044	0.11	0.76	0.18	0.06	0.16	0.04	0.71
	Gln	0.24	0.29	0.35	0.31	0.25	0.26	0.20								
Phe	Control	0.25	0.27	0.38	0.33	0.26	0.26	0.25	0.045	0.48	0.24	0.19	0.26	0.12	0.14	0.17
	Gln	0.30	0.27	0.30	0.36	0.30	0.34	0.20								
Thr	Control	0.79	1.00	0.97	0.93	0.93	0.92	0.91	0.108	0.03	0.83	0.26	0.03	0.10	0.64	0.29
	Gln	0.97	0.99	1.07	0.79	0.78	0.90	0.72								
Val	Control	3.56	3.89	2.77	2.91	3.61	4.29	3.48	1.102	0.03	0.81	0.54	0.18	0.09	0.68	0.07
	Gln	5.53	7.50	6.78	5.09	4.76	6.02	4.29								
NEAA																
Ala	Control	2.90	3.33	3.30	2.89	2.47	2.49	2.63	0.301	0.57	0.20	0.08	0.001	0.10	0.57	0.13
	Gln	2.91	3.12	3.33	2.78	2.31	2.75	1.88								
Asn	Control	0.62	0.80	0.70	0.58	0.59	0.69	0.67	0.095	0.10	0.63	0.18	0.003	0.02	0.51	0.03
	Gln	0.92	0.81	0.83	0.68	0.56	0.80	0.58								
Asp	Control	7.60	7.71	8.32	5.18	6.41	6.57	7.43	0.759	0.40	0.34	0.07	0.001	0.001	0.29	0.30
	Gln	7.87	7.08	6.13	5.23	4.97	6.27	5.02								
Gln	Control	3.10	3.62	4.44	4.15	3.32	2.76	2.83	0.364	0.07	0.14	0.16	0.03	0.05	0.003	0.80
	Gln	3.24	3.63	4.72	4.47	4.34	4.10	2.95								
Glu	Control	14.69	14.17	16.08	15.31	14.56	16.06	13.91	1.317	0.93	0.70	0.55	0.79	0.47	0.36	0.02
	Gln	15.17	15.47	16.60	16.87	15.25	17.54	13.67								
Gly	Control	23.04	27.37	26.13	27.77	23.71	27.84	22.53	4.192	0.46	0.89	0.26	0.25	0.65	0.81	0.006
	Gln	25.32	28.23	27.04	27.06	19.58	30.42	19.25								
Pro	Control	1.58	1.97	1.76	1.73	1.66	1.79	1.74	0.251	0.34	0.77	0.10	0.20	0.34	0.88	0.03
	Gln	2.12	2.25	1.98	1.84	1.63	2.29	1.60								
Ser	Control	2.12	2.53	2.39	2.44	1.95	2.13	2.11	0.297	0.04	0.47	0.18	0.001	0.22	0.98	0.06
	Gln	2.65	2.67	2.95	2.22	1.79	2.32	1.64								
Tyr	Control	0.73	0.92	0.73	0.92	1.52	1.52	1.34	0.467	0.23	0.12	0.48	0.007	0.90	0.37	0.27
	Gln	1.56	2.02	2.55	1.86	1.84	2.58	2.48								

[1]LSM are presented with the pooled SEM. Trt = treatment; pre = precalving; post = postcalving; lin = linear effect; quad = quadratic effect; EAA = essential AA; NEAA = nonessential AA.

[2]Days –25, –12, and –3 are precalving; d 4, 11, and 18 are postcalving.

N will eventually contribute to hepatic ureagenesis, and therefore to the increase in circulating urea N concentrations. Plaizier et al. (2001) also reported that postruminal Gln infusion resulted in a modest numerical increase in urea concentration.

Plasma glucose concentrations averaged over the treatment period were unaffected by the Gln infusion. This does not preclude the conversion of Gln to glucose, or the sparing of glucose at the intestinal level. Homeostatic controls maintain circulating glucose concentrations within a narrow range; thus, increases in gluconeogenic activity are not reflected in plasma concentrations. For example, in human subjects (Perriello et al., 1997) following a Gln infusion of 11.4 μmol/kg per min, postabsorptive plasma glucose concentrations remained unchanged relative to pretreatment levels, but the conversion of Gln to glucose increased from 0.66 to 4.48 μmol/kg per min. In the present study, the maximum supply of glucose precursor offered by the supplemental Gln (41 mmol/h) represents up to 10% of glucose production in early lactation. The decrease in glucose concentrations after calving is typical of this period (Doepel et al., 2002; Reynolds et al., 2003) and is indicative of the high glucose demands imposed by milk synthesis.

Lactate concentrations were not different in the pre- and postcalving periods. This is in contrast to the values reported by Reynolds et al. (2003), in which lactate concentrations were lower postcalving than precalving. This may be related to the manner in which the data

were analyzed. Reynolds et al. (2003) compared each sampling day postcalving with d 19 precalving, whereas in the present study, the average across all sampling days within a period was compared. When the data from d 19 are compared with d –25, then one can see a relative change in concentration similar to that reported by Reynolds et al. (2003).

The gradual rise in plasma NEFA concentrations precalving followed by the rapid increase at calving has been observed in many studies (see the review by Drackley, 1999). The spike at calving suggests that NEFA concentrations are regulated by feed intake and hormonal status. Similar to NEFA concentrations, BHBA concentrations were lower in the precalving than the postcalving period. This is indicative of body fat mobilization and the limited capacity of the liver for oxidation. The steady rise in BHBA concentrations after calving is similar to that reported by Veenhuizen et al. (1991) for cows that received a ketosis induction protocol, and indicates that the cows were in negative energy balance. Averaged across treatments, cows were in greater negative net energy balance on d 21 (–7.9 Mcal/kg) than on d 4 (–4.8 Mcal/kg).

Immunological Parameters

Total leukocyte count increased on the day of calving primarily because of an increase in neutrophils, in line with previous reports (Preisler et al., 2000). Unlike the total leukocyte count, lymphocyte proliferation was consistent over the periparturient period. This contrasts with the results of Kehrli et al. (1989), who reported that the proliferative response induced by ConA in Holstein heifers was depressed during the first week postpartum relative to the response 2 wk prepartum, and that the reduction in proliferative response induced by phytohemagglutinin P began at 2 wk prepartum. The differences observed between the 2 studies may be related to the frequency and time of sampling and to culture conditions. In the study by Kehrli et al., blood samples were obtained weekly from 5 to 3 wk precalving and 3 times weekly for the 2 wk surrounding calving, whereas in the present study, samples were obtained only once precalving (d –25) and once weekly postcalving. It is possible that a depression in proliferative response would have been observed had the sampling frequency precalving been increased. Although there was no overall time effect, at low ConA concentrations, cells incubated with FBS showed a linear increase in their proliferative response after calving. These results suggest that lymphocyte proliferation in these particular culture conditions is impaired shortly after calving relative to 3 wk postcalving. This parturition effect is supported by previously reported studies showing that as lactation progresses, lymphocyte response to mitogens increases (Van Kampen and Mallard, 1997). However, this same response was not observed when the cells were incubated with AS, suggesting that factors in cow serum may influence the lymphocyte response to ConA. As reported in Mallard et al. (1998), blood factors such as cortisol, growth hormone, and IGF-I contribute to the variation in the blastogenesis response in periparturient cows. The lack of lymphocyte proliferation response to Gln suggests that the Gln supply from intestinal absorption and endogenous synthesis was adequate to meet the needs of the immune cells, and that a more catabolic state is required to induce a Gln deficiency.

On average, over the periparturient period the population of T lymphocytes carrying the CD4 receptor was similar to that previously reported (Van Kampen and Mallard, 1997; Kimura et al., 1999). Kimura and colleagues reported a reduction precalving followed by an increase postcalving, but this did not occur in the current study. The various results are difficult to compare because the sampling schedules were different between studies and short-term responses may have occurred within the critical time leading to calving. The difference between the control and Gln-treated cows in the abundance of $CD4^+$ cells during the treatment period was primarily due to the difference on d 4. For the control cows, the percentage of $CD4^+$ cells was the same on d –25 and 4, whereas for the Gln-treated cows, there was an increase of more than 50% from d –25 to 4. The $CD4^+$ cells play a role in both the humoral and cell-mediated immune systems, so the increase at d 4 with the Gln treatment would indicate, at least in the short term, an improvement in immunocompetence. The relevance of this observation to the overall health of the postpartum cow may be limited because the populations of $CD4^+$ cells in the 2 treatments were similar on d 11 and 18.

The ratio of $CD4^+$:$CD8^+$ was higher on d 4 for the Gln-treated cows than for the control cows. In humans, a ratio of 2 or higher is indicative of immunocompetence (Harp et al., 1991), so the elevated ratio on d 4 in the Gln cows may be an indication of a short-term improvement in immune status. Nonetheless, ratios exceeded 2 for all cows postcalving and, based on the human data, this might indicate these animals were in a sound immunocompetent state. Thus, responses to additional Gln might be marginal. For other animals, under less favorable conditions, such improvements in ratio may be more beneficial.

To the authors' knowledge, this is the first study to report the effects of Gln on monocyte abundance. The monocyte population was significantly increased as a result of the Gln treatment. Monocytes, which play an

important role in controlling infection in the postparturient period, are known to require Gln for cytokine production. It appears that Gln may also be required for monocyte proliferation, and that it is the dual action of Gln on cell numbers and cytokine production that contributes to the protective effects of monocytes.

Glutamine supplementation had no effect on the production of IFN-γ, in contrast with findings in humans (Rohde et al., 1996). This may be due to differences in incubation conditions, in which 300 μM of Gln was added to the medium for the human studies but none was supplemented in the current work. Even though plasma concentrations in the present study were roughly equal to that in the media, this does not ensure that the same response would be generated, as a variety of hormonal and metabolic factors would likely be influencing the response in the animal. Of course, there may also be an innate difference between human and ruminant lymphocytes in terms of IFN-γ. Certainly, glucose and Gln metabolism by lymphocytes differs between ruminants and rats (Wu and Greene, 1992). Rat lymphocytes preferentially used Gln as their major energy substrate, whereas the bovine lymphocytes preferred glucose.

Blood lymphocyte Gln concentrations were similar to those reported by McNeil (2001) in sheep and tended to be higher in the Gln-treated cows than in the control cows. This is probably due to the elevation in plasma Gln concentrations in the Gln-infused cows. Based on a K_m of 142 μM (as in human lymphocytes; Schröder et al., 1990), the 35% elevation in plasma Gln concentrations would increase the rate of transport of Gln into lymphocytes by 16%. Nonetheless, the Gln concentrations are relatively low, only 25% those of Glu, again in line with findings in sheep lymphocytes (Lobley et al., 2001). This is despite the fact that the rate of Glu transport into cells is 10-fold lower than that of Gln (McDermott and Butler, 1993) and suggests that Glu is synthesized in the cells using Gln as the precursor. This was confirmed by studies in vitro showing that rapid Gln entry is accompanied by release of ammonia from the amide N and export of Glu. In addition, studies in sheep in vivo demonstrated that 65% of lymphocyte Glu was derived from plasma Gln. The rates of influx and efflux of metabolites (including Gln, Glu, and Ser) through Na^+-dependent transporters determine cell volume, and this regulates cellular activities (Häusinger et al., 1994). Thus, increased movements of Gln may alter lymphocyte metabolism even if intracellular concentrations are unaltered or are changed only minimally.

CONCLUSIONS

Glutamine supplemented postruminally had limited effects on metabolism, immune status, and cow productivity over the first 3 wk postcalving. Numerical increases in milk and milk protein yield may suggest the relief of a certain limitation on cow productivity with infusion of Gln. Immunocompetence, as measured by lymphocyte proliferation, IFN-γ concentrations, and changes in T-cell subpopulations, was not enhanced by the additional duodenal Gln supply over the 3-wk treatment period.

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