

Responses to Amino Acid Imbalances and Deficiencies in Lactating Dairy Cows

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ABSTRACT

Lactating cows were exposed to large amino acid imbalances and deficiencies by i.v. infusion to characterize responses in milk production and plasma concentrations of metabolites and hormones. Six cows in early lactation were fed a basal diet of 9% CP and infused continuously for 6 d with saline (negative control), 1.1 kg/d of a complete amino acid mix (positive control), or the equivalent mix lacking Met, Lys, His, or all 3 branched-chain amino acids. All cows received all treatments in 6 successive periods in a Latin square design. Infusion of the complete amino acid mix resulted in an increase in the plasma concentrations of several essential amino acids, insulin, and glucagon. Milk protein production was stimulated by 19%, which accounted for 10% of the infused amino acid. Plasma urea, acetate, and β -hydroxybutyrate concentrations were increased. Compared with saline, the amino acid mixtures lacking Met, Lys, or His increased essential amino acids, glucose, insulin, and glucagon concentrations in plasma, and decreased growth hormone. Plasma concentration of the essential amino acid absent from the infusate fell 2-fold but milk protein yield remained within 12% of its basal value. Dry matter intakes were depressed 35% over the first 2 d of infusion of imbalanced mixtures but recovered thereafter. Milk fat yields were increased 258 and 320 g/d by mixtures devoid of Lys and His, respectively. Correction of a Met, Lys, or His deficiency did not affect hormone concentrations in plasma and milk protein yield increased 27% due entirely to increased concentration of the single amino acid in plasma. Although imbalance and deficiency generated similar amino acid profiles in plasma, it was concluded that endocrine responses to total amino acid supply during imbalance can override imperfections in the circulating amino acid profile to maintain milk protein yield at higher levels than expected from deficiency states. Both imbalance and deficiency were character-

ized by a low protein:fat ratio in milk. Infusion of a mix of amino acids lacking Val, Ile, and Leu, despite a decrease in plasma Leu to 58% of its basal value, increased milk protein and fat yields to the same extent as the complete amino acid mix.

Key words: amino acid, milk composition, plasma metabolite, hormone

INTRODUCTION

Amino acid nutrition of the lactating cow can influence the yield of protein in milk. Postruminal infusions of intact proteins, free AA, select groups of free AA, or single AA have all increased milk protein yields to various degrees (Chamberlain and Yeo, 2003). These responses are typically interpreted according to a limiting AA theory in which there is but one AA under a given set of dietary and physiological conditions whose absorptive supply can influence milk protein yield. A limiting AA phenomenon allows for efficient manipulation of milk protein yield by supplementing only one of many AA. On highly corn-based diets, Met and Lys supplies are thought to be so closely first and second limiting as to be colimiting. For diets based on temperate grasses, His is a candidate for the first-limiting AA (Chamberlain and Yeo, 2003).

Based on the large number of experiments to test responses to single AA, it is apparent that there exists a consensus that AA deficiencies are common for lactating cows. Unfortunately, quantitative evaluation of AA nutrition of the ruminant animal is beset with the difficulty of measuring transformations by ruminal microbes to which it is host. Because of the uncertainty, one rarely knows whether a particular AA supplement has corrected a deficiency or induced an imbalance. Harper et al. (1970) defined an imbalance as arising from a surplus of essential AA other than the one in limiting supply. In growing animals, an AA imbalance is accompanied by a rapid reduction in food intake mediated by appetite centers in the brain (Gietzen, 1993). Hepatic protein synthesis (Rogers, 1976) and catabolism of the limiting AA (Yuan et al., 2000) are stimulated by the additional dietary AA so that the concentration of the limiting AA falls to a lower proportion than was present in the diet, reminiscent of an essential

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AA deficiency symptom. Concentration in the anterior piriform cortex of the brain falls to an even greater extent and a detection mechanism signals intake depression (Gietzen, 1993). The low intake results in a depressed growth rate (Harper et al., 1970).

Responses of lactating ruminants to an AA imbalance have been studied little. Increasing a nominally balanced intestinal Met supply approximately 30% by abomasal infusion decreased DMI and milk production by a modest 2 kg/d (Robinson et al., 2000). A similar increase in Lys supply had no effect (Robinson et al., 2000). Varvikko et al. (1999) observed no effect on DMI or milk yield of up to 40 g/d of abomasal Met. Intravenous infusion of a mixture of Met, Lys, and Trp for 10 d into cows on a diet demonstrated to be low in His supply resulted in no change in DMI or milk yield but milk fat yield increased by 150 g/d (Kim et al., 2001). Addition of His to the infusate reversed fat yield to the basal level. In a 10-h close arterial infusion, 30 g/h of a mix of essential and nonessential AA devoid of His had no effect on DMI or milk composition, but correction of the His deficiency increased protein and reduced fat content of the milk (Cant et al., 2001). It was proposed that the protein:fat ratio of milk was a sensitive indicator of His imbalance.

Histidine has also received attention as the subject of an experiment in which removal of His from an otherwise complete mix of AA infused into the abomasum of lactating goats elicited a dramatic increase in mammary blood flow rate and clearance of His from blood by the mammary glands (Bequette et al., 2000). Consequently, despite removal of approximately 40% of incoming His, and a decrease in plasma His concentration from 73 to 8 μ M, milk protein yield decreased by only 15% (Bequette et al., 2000). This ability of the mammary glands to compensate for deficiency of a milk precursor may confound emergence of a strict limiting AA phenomenon (Cant et al., 2003).

It is widely presumed that AA deficiencies exist in lactating cows but they are poorly diagnosed and the symptoms of an AA deficiency or imbalance remain inadequately described. Objectives of the current work were to describe responses in lactation performance and blood metabolite and hormone concentrations of cows exposed to relatively large AA imbalances and deficiencies, to ascertain if such responses were general to several essential AA or more specific, and to test the hypothesis that a single essential AA limits milk protein yield.

MATERIALS AND METHODS

Animals and Treatments

All animal procedures and holding facilities were approved by the Animal Care Committee at the University

Table 1. Ingredient and chemical composition (% of DM except where noted) of the basal ration fed to all cows

Ingredient composition, %	
Corn silage	60.6
High moisture corn	33.6
Straw	4.0
Vitamin/mineral premix	1.8
Chemical composition	
DM (% as-fed)	49.3
CP	9.3
CP solubility (% of CP)	44.5
NDF	32.8
ADF	19.1
Cellulose	17.9
Lignin	1.2
Fat	3.0
Ash	3.2
Calculated NE _L (Mcal/kg of DM)	1.57

of Guelph. Six rumen-fistulated, lactating Holstein cows, averaging 561 kg of BW (SE = 18 kg), 76 DIM (SE = 8 DIM), and parity 2.5 (SE = 0.3), were randomly assigned to 6 infusion treatments arranged in a Latin square design balanced for carryover effects. Two weeks before the onset of infusions, cows were fed twice daily at 0700 and 1530 h a basal TMR for ad libitum intake (Table 1) based on the low-protein diet of Wright et al. (1998). Cows remained on this diet and feeding schedule for the duration of the trial. Orts were removed, weighed, and sampled once per day and pooled weekly for DM determination. The TMR was sampled daily and pooled by the week for DM and nutrient analysis.

Treatments were continuous abomasal infusion of 3.0% saline (negative control; **NC**), 15% free AA having the profile of milk protein (positive control; **PC**), PC minus methionine (**PC-Met**), PC minus lysine (**PC-Lys**), PC minus histidine (**PC-His**), and PC minus leucine, isoleucine and valine (**PC-BCAA**). Solutions were prepared fresh daily and infused into cows at a rate of approximately 5.1 mL/min (approximately 1.1 kg/d of AA) for 6 d. Infusate bottles were weighed periodically to determine exact flow rates.

Cows were milked at 0500 and 1500 h daily and total yields were weighed and recorded. Samples of milk were collected at each milking, pooled according to yield, and stored at 4°C until analyzed.

On d 6 of infusion, cows were disconnected from their respective infusion treatments for approximately 30 min for determination of BW and insertion of a catheter into one jugular vein. Approximately 30 min after the final cow was reconnected to the infusate, blood sampling began. Blood was collected into vacutainers containing EDTA (for metabolite, insulin, and IGF-I analysis) and sodium heparin (for growth hormone and AA analysis) every 30 min between 1030 and 1400. Aprotinin (1 MIU) was added to 2 mL of whole blood with

EDTA for glucagon analysis. Samples were centrifuged immediately at $2,000 \times g$ for 15 min, and plasma was removed and stored at -20°C .

Sample Analysis

Milk samples were analyzed within 3 d of collection for protein, fat, and lactose content by infrared spectroscopy (AOAC, 1996). Results from the last 3 d of infusion were averaged for statistical analyses. Energy loss into milk was calculated from milk component yields according to Tyrrell and Reid (1965). Energy balance of cows was then estimated as NE intake in feed plus infusates minus NE expenditures for maintenance and loss in milk. Net energy content of infusates was calculated from heats of combustion of the individual AA, corrected for the energy content of all potential urea and a 64% efficiency of retention, to be 2.49, 2.49, 2.48, 2.50, and 2.19 Mcal/kg for the PC, PC-Met, PC-Lys, PC-His, and PC-BCAA treatments, respectively. Maintenance expenditures were estimated as $0.08 \text{ Mcal}/(\text{d} \cdot \text{kg}^{0.75})$ (NRC, 2001).

Plasma samples were pooled by cow and period to analyze for glucose (kit no.510-A; Sigma Chemical Co., Oakville, ON, Canada), triacylglycerol (Sigma kit no.336), acetate (Boehringer Mannheim kit; R-Biopharm GmbH, Darmstadt, Germany), BHBA (Cant et al., 1993), NEFA (NEFA C kit; Wako Chemicals GmbH, Neuss, Germany), and urea (Sigma kit no. 640-B). Plasma concentrations of AA were measured by the isotope dilution method of Calder et al. (1999) on a gas chromatograph-mass spectrometer (model HP6890, S973 mass selective detector; Hewlett Packard, Palo Alto, CA) as outlined by Raggio et al. (2004).

Growth hormone (GH), insulin, glucagon, and IGF-I concentrations in individual plasma samples were analyzed by radioimmunoassay. Growth hormone and IGF-I were iodinated and measured according to procedures described by Petittlerc et al. (1987) and Abribat et al. (1993), respectively. The bovine GH utilized for analysis was radioimmunoassay grade and was donated by A. F. Parlow (National Hormones and Pituitary Program, Bethesda, MD). Inter- and intraassay coefficients of variation for the GH analysis were 9.4 and 1.0%, respectively. Radioimmunoassay grade IGF-I was purchased from Gropep (Thebarton, SA, Australia). Inter- and intraassay coefficients of variation for IGF-I analysis were 9.6 and 4.1%, respectively. Insulin was analyzed with a commercial kit (KTSP-11002, Mediacorp, Montreal, Canada) and inter- and intraassay coefficients of variation were 3.0 and 1.3%, respectively. Glucagon was measured only in samples 4 and 8 from each cow-period with an intraassay coefficient of variation of 7.4%.

Statistical Analyses

Observations were subjected to ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Main effects were cow, period, and treatment. Treatment means were separated by Duncan's multiple range test. Hormone concentrations across multiple time points were analyzed using the mixed procedure of SAS. Cow and period were treated as random variables whereas treatment and time were considered fixed. Repeated measures analyses were conducted on period and time with an autoregressive order-one covariate structure. Multiple comparisons between treatments were analyzed with a Tukey-Kramer adjustment. For all statistical analyses, significance was declared at $P \leq 0.05$. Probabilities greater than 0.05 and less than or equal to 0.15 are discussed as trends.

Treatment effects were interpreted according to the definitions of Harper et al. (1970) regarding AA disproportions. Infusates lacking an essential AA were compared with NC to evaluate AA imbalances and were compared with PC to evaluate AA deficiencies.

RESULTS

The complete AA mix was infused at a rate of 1,104 g/d (Table 2). Subtractions of essential AA averaged 22 g/d for PC-Met, 75 g/d for PC-Lys, 24 g/d for PC-His, and 160 g/d for PC-BCAA. Dry matter intake was depressed on d 2 by PC-Lys compared with NC and on d 3 by PC-Met and PC-His compared with NC (Figure 1). On d 4 to 6 of infusion, DMI was not affected by any of the mixtures (Table 2) and CP intake, excluding infusates, averaged 1,243 g/d. According to the Cornell Net Carbohydrate and Protein System (CNCPS, v 5.0.38), duodenal flows of Met, Lys, His, and BCAA for NC cows were 32, 91, 32, and 251 g/d, respectively. Thus subtractions removed approximately 41, 45, 43, and 39% of the Met, Lys, His, and BCAA, respectively. Estimated supply of MP was 1,008 g/d on NC so infusions of incomplete AA mixes at 874 to 1,058 g/d (Table 2) provided approximately a 2-fold excess of AA.

Milk Production and Composition

Infusion of the complete AA mix increased yield and percentage of protein in milk compared with NC (Table 2) and, although fat yield did not significantly increase, protein:fat ratio in milk was not affected.

Infusion of AA mixtures lacking Met, Lys, or His did not affect milk protein or lactose yields relative to NC, although there was a tendency for protein yield to be lower on PC-His. Fat yield was significantly elevated by mixtures lacking Lys and His. Protein percentage in milk was not affected by AA imbalance, but lactose

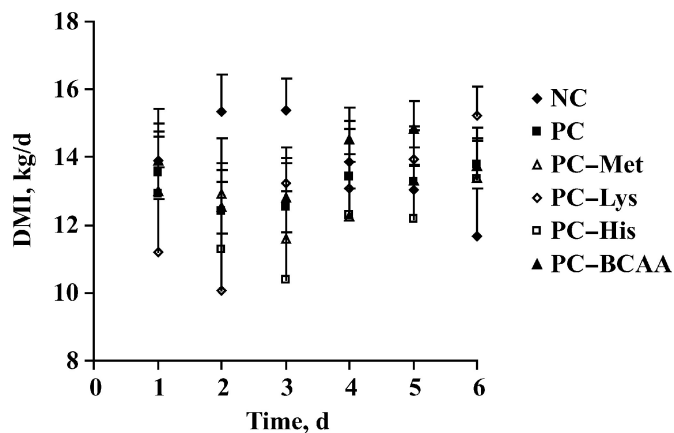
Table 2. Mean infusion rates, BW, DMI, milk production and composition, and energy balance of 6 cows during the last 3 d of a 6-d continuous abomasal infusion of 3.0% saline (NC), 15% complete AA mix (PC), or PC minus Met, Lys, His, or branched-chain AA (BCAA)

Variable	Treatment						SE	P
	NC	PC	PC-Met	PC-Lys	PC-His	PC-BCAA		
AA infusion rate, g/d	0 ^a	1,104 ^c	1,039 ^c	1,058 ^c	1,051 ^c	874 ^b	26	<0.001
BW, kg	559	568	561	568	554	571	7	0.087
DMI, kg/d	12.9	13.5	13.0	13.9	12.6	14.4	0.6	0.308
Yield								
Milk, kg/d	21.4 ^{ab}	22.7 ^{ab}	20.5 ^a	21.9 ^{ab}	20.2 ^a	23.4 ^b	0.8	0.022
Fat, g/d	728 ^a	867 ^{abc}	836 ^{ab}	986 ^{bc}	1,048 ^c	900 ^{abc}	59	0.018
Protein, g/d	585 ^a	698 ^b	557 ^a	569 ^a	512 ^a	674 ^b	27	0.001
Lactose, g/d	957	1,024	978	1,027	955	1,084	51	0.121
Percentage								
Fat	3.38 ^a	3.81 ^{ab}	4.04 ^{ab}	4.59 ^{bc}	5.26 ^c	3.80 ^{ab}	0.27	0.002
Protein	2.73 ^{ab}	3.09 ^c	2.71 ^{ab}	2.62 ^a	2.56 ^a	2.91 ^{bc}	0.07	0.002
Protein:fat	0.84 ^a	0.83 ^a	0.69 ^{ab}	0.58 ^{bc}	0.50 ^c	0.78 ^a	0.05	0.001
Lactose	4.47 ^a	4.52 ^{ab}	4.77 ^b	4.68 ^{ab}	4.71 ^{ab}	4.60 ^{ab}	0.08	0.088
Net energy balance, Mcal/d	-2.5	-1.3	-1.6	-1.2	-1.2	-1.6	0.84	0.845

^{a-c}Means with different superscripts are significantly different ($P < 0.05$).

percentage was elevated by PC-Met and fat percentage was elevated by PC-Lys and PC-His treatments. Thus, protein:fat ratio in milk was reduced by PC-Met ($P < 0.10$), PC-Lys, and PC-His. In contrast, the AA mix lacking all 3 BCAA had similar effects to the PC relative to NC. Protein yield was increased and fat yield tended to increase ($P < 0.10$) so that protein:fat ratio in milk was not affected. Lactose percentage remained similar to the NC value.

Comparison of the subtraction treatments with PC shows that correction of the imbalance created by omitting Met, Lys, or His resulted in an increase in protein yield and percentage in milk so that protein:fat increased. Fat yield tended to be decreased with correction of the His deficiency.

**Figure 1.** Mean daily DMI of 6 cows during 6 d of continuous abomasal infusion of 3.0% saline (NC), 15% complete AA mix (PC), or PC minus Met, Lys, His, or branched-chain AA (BCAA).

None of the treatments affected estimated energy balance despite providing an additional 2.0 to 2.8 Mcal of net energy/d.

Plasma AA Concentrations

Compared with NC, the PC treatment increased concentrations of all essential AA in plasma but His, Thr, and Lys. Despite being included in the infusate, nonessential AA concentrations were not affected except for Gly, which declined from 539 to 360 μM .

As observed for PC, mixtures lacking Met, Lys, and His increased concentrations of all essential AA but His, Thr, and Lys. In addition, Met concentration was not affected by PC-Met, whereas Lys and His concentrations on PC-Lys and PC-His, respectively, actually dropped to approximately one-half the NC value. Cysteine concentrations also dropped on PC-Met, PC-Lys, and PC-His. As with PC, plasma Gly declined on the 3 imbalance treatments. Serine concentration was elevated by PC-Met.

Infusion of PC-BCAA increased concentrations of all essential AA infused. Valine and Leu concentrations were not affected but Ile concentration tended to decrease ($P < 0.10$). None of the nonessential AA, including Gly, was affected by PC-BCAA infusion.

Correction of the Met, Lys, and His deficiencies increased concentrations of the deficient AA and Cys and, for Met and Lys correction, reduced the circulating concentrations of Tyr and Leu. Serine concentration was reduced with Met correction.

Plasma Metabolite and Hormone Concentrations

Plasma concentrations of glucose, triacylglycerol, and NEFA were not affected by infusion of the complete AA

mix. The concentration of BHBA tended to increase ($P < 0.10$). Acetate concentrations were very low on the NC treatment and almost doubled during PC infusion. Urea concentrations approximately tripled on PC vs. NC and insulin was approximately 2 times the NC concentration.

Glucose, urea, and insulin concentrations were elevated by all infusates lacking an essential AA. In addition, the PC-Met treatment caused a decrease in GH and increase in IGF-I concentrations, PC-Lys increased plasma acetate and glucagon concentrations, PC-His increased NEFA and insulin:glucagon ratio, and PC-BCAA increased the insulin:glucagon ratio.

Inclusion of the omitted AA in the infusates decreased plasma glucose and increased plasma BHBA concentrations. None of the hormones was affected by correction of AA deficiencies.

DISCUSSION

Responses to the Complete AA Mix

The stimulation of milk protein yield with PC is a well-documented effect of postruminal casein infusion (Clark et al., 1977; Rulquin, 1983; Whitelaw et al., 1986). Oftentimes, lactose and total milk yields are also elevated (Rulquin, 1983; Whitelaw et al., 1986) although such effects were not detected in this experiment. Concentrations of insulin and several essential AA were increased in plasma. An increase in circulating insulin concentrations has been detected in some (Whitelaw et al., 1986; Miettinen and Huhtanen, 1997; Mackle et al., 1999) but not all (Cohick et al., 1986; Guinard et al., 1994; Choung and Chamberlain, 1995) protein infusion experiments with dairy cows, and never to the 2-fold extent observed here. Contributing factors to the large insulin response might be that the CP content of the basal ration was lower than in other experiments and the rate of AA infusion was much higher. Amino acid supply was increased approximately 2-fold from 1.0 to 2.1 kg/d.

Milk protein yield only increased 0.11 kg/d, equivalent to 10% of the infusion, which is within the range given by Hanigan et al. (1998) for marginal efficiency of abomasal casein utilization that averaged 24% across experiments. Approximately 90% of the infused AA, then, was used elsewhere. In monogastric animals, the acute response to a protein meal or AA infusion after fasting is an elevation in both AA and insulin concentrations (Gibson et al., 1996), either of which alone stimulates protein synthesis in muscle via signal transduction pathways that share several elements in common (Davis et al., 2003; Bolster et al., 2004). In adults, insulin appears to increase net protein accretion in muscle primarily through an inhibition of protein degradation,

whereas extracellular AA continue to stimulate protein synthesis (Gibson et al., 1996). In lactating goats, protein accretion in the hindlimb was increased by 3.5 d of euglycemic insulin infusion or 7 d of euinsulinemic AA infusion (Bequette et al., 2002). Thus, the elevated concentrations of essential AA and insulin in PC cows would be expected to stimulate AA deposition in peripheral tissues. In this case, because NC cows were in negative N balance according to CNCPS calculations, the effect of PC would have been decreased mobilization of AA from body proteins. Likewise, both AA and insulin have been shown to independently stimulate milk protein secretion in dairy cows (Grinari et al., 1997; Bequette et al., 2002) although insulin appears to elicit a stronger response and the mechanisms of neither response have been verified.

In contrast to the essential AA, there is a tendency, as observed here, for the plasma concentrations of most nonessential AA to be unaffected by postruminal AA administration (Clark et al., 1977; Rulquin, 1983; Choung and Chamberlain, 1995; Miettinen and Huhtanen, 1997; Mackle et al., 1999). The concentration of Gly, despite being included in the infusate, actually decreased. A pronounced decline in plasma Gly concentration is a common feature of improved protein nutrition of ruminant and monogastric animals (Rulquin, 1983; Whitelaw et al., 1986; Miettinen and Huhtanen, 1997; Rémésy et al., 1997).

The elevated plasma urea concentration on PC compared with NC indicates that AA were being catabolized faster. Although plasma glucose concentrations did not change, the 2-fold higher insulin concentration would have stimulated glucose use in the body such that entry rate of glucose must have increased to maintain euglycemia. A 4-fold increase in circulating insulin concentration increased glucose use in lactating cows by over 2,500 g/d (Grinari et al., 1997; Mackle et al., 1999), which, if every mole of AA yields 0.5 mole of glucose, is the equivalent of 3,600 g/d of AA. An increase in glucose entry rate with no effect on plasma glucose concentration has been observed in lactating cows given postruminal casein (Clark et al., 1977; König et al., 1984). In rats, increased flux through catabolic enzymes when AA concentrations are high is mediated by glucagon whose secretion is induced by the hyperaminoacidemia (Tovar et al., 2002). The absence of a large glucagon response in peripheral circulation whereas AA catabolism apparently increased in this experiment may be related to the ruminant liver remaining highly gluconeogenic irrespective of diet. In any case, where only 10% of the infused AA was incorporated into milk proteins, the majority of the infused AA appears to have been catabolized.

Table 3. Mean concentrations of metabolites and hormones in plasma of 6 cows between 1030 and 1400 of the last day of a 6-d continuous abomasal infusion of 3.0% saline (NC), 15% complete AA mix (PC), or PC minus Met, Lys, His, or branched-chain AA (BCAA)

Variable	Treatment						SE	P
	NC	PC	PC-Met	PC-Lys	PC-His	PC-BCAA		
Glucose, mM	2.11 ^a	2.16 ^{ab}	2.63 ^c	2.54 ^{bc}	2.88 ^c	2.57 ^c	0.13	0.003
Acetate, mM	0.43 ^a	0.79 ^c	0.52 ^{ab}	0.69 ^{bc}	0.60 ^{abc}	0.56 ^{abc}	0.08	0.133
BHBA, mM	1.01 ^{ab}	1.46 ^b	0.77 ^a	0.98 ^{ab}	0.80 ^a	0.86 ^a	0.17	0.099
Triacylglycerol, μ M	188	201	203	147	180	160	18	0.158
NEFA, μ M	69 ^a	96 ^{ab}	114 ^{ab}	104 ^{ab}	185 ^b	165 ^{ab}	31	0.118
Urea, mM	0.33 ^a	0.98 ^{bc}	1.23 ^c	1.19 ^c	1.24 ^c	0.83 ^b	0.11	<0.001
Insulin, pg/mL	631 ^a	1,011 ^b	1,179 ^b	1,148 ^b	1,052 ^b	965 ^b	84	<0.001
Glucagon, pg/mL	77.0 ^a	94.0 ^{ab}	95.1 ^{ab}	98.4 ^b	88.3 ^{ab}	89.6 ^{ab}	6.4	0.021
Insulin:glucagon ratio	8.2 ^a	11.4 ^{ab}	12.3 ^{ab}	12.0 ^{ab}	12.5 ^b	12.3 ^b	1.4	0.005
Growth hormone, ng/mL	6.62 ^a	4.85 ^{ab}	3.08 ^b	4.18 ^{ab}	4.26 ^{ab}	5.74 ^{ab}	0.78	0.009
IGF-I, ng/mL	110 ^a	120 ^{ab}	151 ^b	105 ^a	96.5 ^a	117 ^{ab}	11.1	<0.001

^{a-c}Means with different superscripts are significantly different ($P < 0.05$).

The 2-fold increase in plasma acetate concentration may have been a consequence of the low CP content of the basal ration. Rulquin (1983) fed a basal ration of 12% CP to lactating cows and observed an increase in plasma acetate from 1.35 to 1.47 mM with a duodenal infusion of 500 g/d casein. Others who have measured plasma acetate have reported no effect due to postruminal casein (Konig et al., 1984; Cant et al., 1993; Guinard et al., 1994; Miettinen and Huhtanen, 1997) although a 3-fold increase in acetate flux was noted (Konig et al., 1984).

It is possible that N from the PC infusate was transferred into the rumen and stimulated acetate production. Lactating cows fed adequate protein typically exhibit 3 to 5 mM plasma urea (Miettinen and Huhtanen, 1997; Wright et al., 1998), which arises mostly from rumen ammonia transfer. Given the low plasma urea concentration of 0.33 mM for NC cows (Table 3), rumen ammonia concentrations would have been deficient for microbial growth and ensuant carbohydrate fermentation. When heifers were fed a diet of 12% CP compared with 9% CP, digestibility of NDF increased from 40 to 52% due to an increase of rumen ammonia from 0.1 to 1.2 mM (Marini and Van Amburgh, 2003). Through recycling of plasma urea into the gastrointestinal tract, postruminal casein infusion increased rumen ammonia concentrations (Huhtanen et al., 1997; Bandyk et al., 2001) and NDF digestibility (Rulquin, 1982). Furthermore, as CP content of the diet fed to heifers decreased from 20 to 9% of DM, clearance of plasma urea into the gastrointestinal tract increased 9-fold (Marini and Van Amburgh, 2003). Thus, with the 9% CP basal ration fed in this experiment, a substantial proportion of the N infused on the PC treatment appears to have been transferred as urea to the rumen for stimulation of microbial growth and VFA production.

In summary, abomasal infusion of a complete AA mix at 1.1 kg/d into lactating cows consuming 1.2 kg/d CP

resulted in an increase in the plasma concentration of several essential AA, insulin, and glucagon. Milk protein secretion was stimulated by 19% and there was an apparent decrease in AA mobilization out of body proteins. Production of glucose from AA, possibly mediated by glucagon, was balanced by insulin-stimulated use of glucose. Urea produced during AA breakdown was transferred into the rumen where the N stimulated VFA production.

AA Imbalances

Infusion of AA mixtures lacking Met, Lys, or His resulted in no change in milk protein yields compared with NC, whereas PC-BCAA stimulated protein yield to the same extent as did PC. Thus, PC-BCAA responses will be considered separately from the following discussions of AA imbalances and deficiencies in lactating dairy cows.

Plasma concentration of the AA absent from the infusate on PC-Met, PC-Lys, and PC-His treatments decreased to approximately one-half its respective NC value (Table 4), although the Met effect was not significant because of difficulties with measuring low Met concentrations. These concentrations decreased, not because of loss into milk, but because of more efficient catabolism or deposition into body proteins. Essential AA (particularly Leu) and insulin, which are stimulatory to muscle and liver protein synthesis (Davis et al., 2003; Bolster et al., 2004), were elevated during imbalance (Table 3). On the other hand, glucagon, which stimulates activity of AA-degrading enzymes in the liver (Pestaña, 1969; Tovar et al., 2002), was also increased, and plasma glucose and urea concentrations were higher, indicating faster breakdown of AA. Kinetically, for the deficient AA to be broken down or incorporated into protein at a faster rate during imbalance (which it must for the concentration in plasma to fall),

Table 4. Mean concentrations of AA in plasma of 6 cows between 1030 and 1400 of the last day of a 6-d continuous abomasal infusion of 3.0% saline (NC), 15% complete AA mix (PC), or PC minus Met, Lys, His, or branched-chain AA (BCAA)

AA, μM	Treatment						SE	P
	NC	PC	PC-Met	PC-Lys	PC-His	PC-BCAA		
Met	15 ^a	38 ^b	7 ^a	44 ^b	35 ^b	47 ^b	5	<0.001
Phe	43 ^a	71 ^b	82 ^b	85 ^b	81 ^b	85 ^b	4	<0.001
Tyr	41 ^{abc}	32 ^a	42 ^{bcd}	49 ^{cd}	36 ^{ab}	51 ^d	3	0.001
His	24 ^b	32 ^{bc}	34 ^{bc}	33 ^{bc}	10 ^a	42 ^c	3	<0.001
Trp	31 ^a	50 ^b	60 ^b	56 ^b	57 ^b	59 ^b	3	<0.001
Thr	127 ^{ab}	138 ^{ab}	151 ^{ab}	166 ^b	116 ^a	220 ^c	12	<0.001
Val	155 ^a	374 ^b	394 ^b	397 ^b	364 ^b	169 ^a	46	0.002
Ile	74 ^a	140 ^b	151 ^b	154 ^b	129 ^b	65 ^a	13	<0.001
Leu	108 ^a	187 ^b	249 ^c	245 ^c	215 ^{bc}	63 ^a	18	<0.001
Lys	51 ^b	70 ^b	64 ^b	33 ^a	54 ^b	95 ^c	6	<0.001
Gly	559 ^a	360 ^{bc}	362 ^{bc}	321 ^c	365 ^{bc}	509 ^{ab}	49	0.081
Ala	219 ^{ab}	237 ^{ab}	219 ^{ab}	207 ^b	179 ^b	277 ^a	21	0.091
Ser	156 ^{ab}	147 ^a	303 ^c	166 ^{ab}	190 ^{ab}	202 ^b	17	<0.001
Cys	78 ^a	86 ^a	59 ^b	57 ^b	56 ^b	85 ^a	3	<0.001
Glu	197	176	203	204	166	187	12	0.115
Gln	34	33	55	46	32	51	7	0.092

^{a-c}Means with different superscripts are significantly different ($P < 0.05$).

the change in V_{\max} or K_m of the process must be large enough to overcome the 50% decrease in substrate concentration. Because insulin concentration increased to a greater extent than glucagon concentration, because the stimulatory BCAA concentrations were higher on PC-Met, PC-Lys, and PC-His than on any of the other treatments (Table 4), and because protein synthesis has a lower K_m than AA catabolism for AA (Rogers, 1976), we conclude, as did Rogers (1976), that stimulation of sequestration of all AA in body proteins siphoned the most deficient AA out of plasma.

Harper et al. (1970) concluded that the accumulation of one or more essential amino acids in blood instigated the food intake depression seen with AA-imbalanced, -deficient, and high-protein diets, and that the low concentration of one essential AA during imbalance and deficiency would further exacerbate the depression. Typically, consumption of an AA-imbalanced diet by rats leads to an immediate depression in DMI by approximately 50%, but after a few days, intake begins to increase and returns to basal levels by 5 to 7 d (Gietzen, 1993). We noted a 35% depression in DMI on d 2 and 3 of infusion of PC-Met, PC-Lys, and PC-His, which is a smaller depression and for a shorter time than observed in rats. It has been noted that the severity of the imbalance influences the duration of the adaptive period in rats (Harper et al., 1970). Recycling into the rumen of urea formed in the liver from infused AA would have allowed microbial synthesis of the deficient AA and thereby lessened the magnitude of the imbalance. The reduction in DMI, mediated by appetite centers in the brain (Gietzen, 1993), is considered responsible for low growth rates manifest during imbalance

(Harper et al., 1970). In our experiment, there were no differences in DMI between treatments during the last 3 d of infusion when performance measures were taken for statistical analyses but the depression in DMI immediately before the observation period likely contributed to the mammary responses to imbalance noted here. Acute responses of the milk-synthesizing apparatus to AA infusions can be detected within a few hours of the change in plasma AA concentrations (Cant et al., 2001) and are often recorded after 4 d of infusion (Cant et al., 1993; Mackle et al., 1999), so the use of d 4 to 6 averages was considered appropriate to describe acute imbalance effects. Responses to a long-term AA imbalance of the magnitude imposed here might be quite different.

The maintenance of milk protein yield on PC-Met, PC-Lys, and PC-His within 12% of the NC value, despite a 50% decrease in concentration of one essential AA, attests to the great flexibility of the lactating cow to maintain milk production under widely nonideal nutritional conditions. The mammary glands display a broad repertoire of maneuvers to obtain milk precursors from blood independent of their circulating concentrations. These include control over transmembrane transport and local blood flow rate (Bequette et al., 2000). We have suggested that the mammary glands operate according to a centrally dictated setpoint for milk production (Cant et al., 1999), of which this experiment provides an example. The setpoint is thought to be dictated by hormones such as those of the somatotrophic axis. Insulin-like growth factor-1 is arguably the main controller of mammary epithelial cell number once lactation has been established in cows, and IGF-I concen-

trations were not decreased by AA imbalance. In fact, plasma IGF-I actually increased on PC–Met. Likewise, insulin, which is stimulatory to milk protein yield, was elevated during AA imbalance (Table 3). These endocrine responses may have allowed the mammary glands to overcome a 50% decrease in circulating concentrations of one essential AA.

Plasma GH was lower or tended to be lower for PC–Met, PC–Lys, and PC–His than for NC. Improvement in protein nutrition of animals generally increases the GH stimulation of hepatic IGF-I release. Through a feedback mechanism, the elevated IGF-I concentration inhibits pituitary GH release so that plasma GH declines (Clarke et al., 1993; Polkowska et al., 1996). Several postruminal infusion experiments have shown no effect of casein on plasma GH of lactating cows (Konig et al., 1984; Cohick et al., 1986; Guinard et al., 1994) but Whitelaw et al. (1986) noted a decline. Concentrations of IGF-I were not reported. In this experiment, IGF-I concentration in plasma was increased by PC–Met but not by the other imbalance treatments nor the full AA profile. Although it is surprising, then, that PC had no effect on IGF-I, the expression of GH receptor and IGF-I mRNA by porcine hepatocytes in culture was decreased by removal of only certain essential AA from the medium (Brameld et al., 1999); Met, His, Ile, and Leu removals had no effect. Here, it appears that Met, Lys, and His removals had no effect on the GH-depressing effects of the AA infusions. That an imbalanced AA supply can stimulate IGF-I or suppress GH release may point to the insulin sensitivity needed to dispose of an AA or glucose load during hyperaminoacidemia.

Milk fat yields were increased 258 and 320 g/d over NC by PC–Lys and PC–His, respectively (Table 2). Similarly, Kim et al. (2001) observed a 150 g/d increase in milk fat yield when 8 g/d of Met, 28 g/d of Lys, and 2.5 g/d of Trp were infused i.v. for 10 d into cows deficient in His supply. On the other hand, a short, 10-h intraarterial infusion of 300 g of an AA mixture lacking His but otherwise complete had no effect on milk fat yield or percentage (Cant et al., 2001). The PC–Lys response indicates that the milk fat stimulation is not specific to a His imbalance but may be more general. For example, an excess of Met often results in higher milk fat production (Varvikko et al., 1999). Elevated Ser concentrations in plasma when Met was absent from the infusate indicates a decreased synthesis of choline, which may have prevented a milk fat response from being manifest on PC–Met, in which choline is purported to influence milk fat secretion (Sharma and Erdman, 1988). However, it is difficult to speculate on such a matter while the mechanism of the milk fat stimulation remains obscure. There was little change in plasma concentrations of the milk fat precursors acetate,

BHBA, or long-chain fatty acids ($3 \times$ triacylglycerol + NEFA) and there was no correlation between milk fat yields and any or all of the milk fat precursors (data not shown). Plasma acetate was increased on PC–Lys, probably due to urea recycling into the rumen as already discussed, but lack of a significant effect on acetate for PC–His may actually reflect a pull, not a push, into mammary lipogenesis. The significantly higher NEFA concentration on PC–His was primarily due to one cow exhibiting a concentration of 414 μ M. Thus, an explanation of the milk fat stimulation is not readily forthcoming from plasma metabolite data and must reside in the mammary response. We have previously suggested (Cant et al., 1999) that the stimulation of mammary blood flow observed during His deficiency (Bequette et al., 2000), were it to occur during AA imbalance, could inadvertently supply more milk fat precursors to the mammary glands without any elevation of their concentrations in plasma. In this way, the mechanisms invoked by the mammary glands to maintain milk protein yields are stimulating milk fat yields. There is also the possibility that AA themselves were converted to fat in the mammary glands, although 150 g of milk fat from 38.5 g of excess AA in the experiment of Kim et al. (2001) does not seem likely. Alternatively, shifts in intracellular metabolism to accommodate the imbalanced AA load may have provided NADPH to stimulate lipogenesis or an easy shunt of tricarboxylic acid cycle intermediates into fat.

In conclusion, 6 d of AA imbalance in lactating cows increased essential AA, glucose, insulin, and glucagon concentrations in plasma, and decreased GH. Sequestration of AA in body proteins was stimulated, or mobilization inhibited, to such an extent that the AA missing from the infusate fell in plasma to approximately half its basal concentration. The mammary glands, due perhaps in part to insulin and IGF-I effects, upregulated control points in the AA supply system to maintain milk protein yields despite the low concentration of a single essential AA in plasma. Milk fat yields were elevated on PC–Lys and PC–His by more than 300 g/d with no apparent relationship to acetate, BHBA, or long-chain fatty acid concentrations in plasma.

Correction of an AA Deficiency

Correction of a Met, Lys, or His deficiency increased the concentration of the deficient AA in plasma and no other in a consistent manner, with the exception of Cys. Tyrosine and Leu concentrations decreased when Met and Lys deficiencies were corrected, which may have been a consequence of the 28% greater milk protein yields. Because insulin and IGF-I concentrations in plasma were not affected by the corrections, the most

likely cause of the increase in milk protein yield was the elevated concentration of the one essential AA restored to the infusate. The same cannot be said of the protein response to an imbalance, which, although there are similarities between imbalance and deficiency, allows a distinction to be drawn. As an example, between PC-Lys and NC, Lys concentration in plasma increased from 33 to 51 μM and milk protein yield did not change. In contrast, between PC-Lys and PC, Lys concentration increased from 33 to 70 μM and milk protein yield was elevated by 129 g/d. These yield responses do not follow an expected dose-response curve in which the marginal stimulation of yield is greater at the lower concentrations of AA. Rather, endocrine responses to total AA supply during imbalance can override imperfections in the AA profile to maintain milk protein yields at higher levels than expected from deficiency experiments.

Correction of a deficiency decreased plasma glucose and increased BHBA concentrations (Table 3). Whitelaw et al. (1986) suggested that postruminal casein could decrease the energy balance of a lactating cow by increasing both milk yield and fat mobilization from adipose tissue. Such an effect of a single AA would produce the changes in circulating glucose and BHBA concentrations observed here but none of the hormones or milk and lactose yields support it. Catabolism of AA may simply have provided more tricarboxylic acid cycle intermediates to burn BHBA and thereby spare glucose during a single AA deficiency than otherwise.

Milk fat yield was depressed by 181 g/d with His correction and there was a tendency for fat percentage to be reduced with corrections of both the Lys and His deficiencies (Table 2). Thus, imbalance and deficiency states are both characterized by a low ratio of protein:fat in milk. The fat elevation has attracted attention in recent years as an unexplained consequence of AA supplementation (Cant et al., 2003; Chamberlain and Yeo, 2003).

Responses to an AA Mix Lacking BCAA

Infusion of a mix of AA lacking BCAA did not affect the protein:fat ratio in milk compared with NC, and neither did restoration of the BCAA supply (Table 2). Both protein and fat yields were stimulated by PC-BCAA in the same manner as by PC, and so neither an imbalance nor a deficiency state can be declared. Although Val and Ile concentrations in plasma did not change with PC-BCAA compared with NC, Leu fell to 58% of its basal value, which is similar to the changes in Met, Lys, and His concentrations that elicited noticeable effects on milk component secretion. Concentrations of several essential AA (Phe, Tyr, Trp, Thr, and

Lys) were higher or tended to be higher on PC-BCAA than on PC, and Gly was also elevated ($P < 0.10$), which suggests that the PC-BCAA infusate did not inhibit mobilization of AA from body pools to the same extent as the other deficient infusates or PC. In fact, mobilization may have increased to supply BCAA used in milk protein production. The stimulation of milk protein yield on PC-BCAA compared with NC would have been, in this case, responsible for the decline in plasma Leu. That scenario lies in distinct contrast to the stimulation of use in nonmilk protein pathways by PC-Met, PC-Lys, and PC-His. The difference appears to be due to Leu, which is an AA metabolized less in the liver and more in the periphery, where it can signal activation of protein synthesis and inhibition of protein degradation (Bolster et al., 2004). Thus, infusion of an AA mix devoid of one essential AA but containing Leu stimulates AA sequestration in peripheral tissues, dropping the missing AA in plasma, leaving the mammary glands to their own devices to maintain milk protein yield. Infusion of a mix lacking Leu does not stimulate peripheral AA use, which permits the endocrine and AA signals to stimulate milk protein yield. These differences in AA partitioning due to Leu could be exploited to advantage in managing milk synthesis and BW change throughout lactation. Such an effect of Leu could have accounted for the stimulation of N retention by BCAA in growing pigs fed a Met-deficient diet (Langer and Fuller, 2000).

Limiting AA

The stimulation of protein synthesis by hormones and AA to different degrees in mammary and nonmammary tissues leads to an abrogation of the classic "limiting AA" response in milk protein yield. Here, there was no difference in milk protein yield whether Met, Lys, or His was absent from the infusate. According to the law of the minimum conventionally used to describe essential AA responses, only one AA should have completely prevented a change in milk protein yield; the other omissions should have stimulated milk protein yield to the point of a second limitation. If anything, the tendency for protein yield to be lower on PC-His than on NC indicates that milk protein was not accorded a priority for His use. In other words, it was not incorporation into milk protein that caused plasma His to fall but use elsewhere. That the expected pattern of a sequential order of limitations did not emerge can be attributed to use of AA in nonmilk protein pathways, and the ability of the mammary glands to overcome a deficit in circulating milk precursor supply.

CONCLUSIONS

We have induced large AA imbalances and deficiencies in lactating cows. The lack of a prolonged DMI de-

pression, the recycling of plasma N into the rumen for microbial AA synthesis, and endocrine regulation of milk synthesis all serve to dampen the impact of disproportionate supplies of AA on milk protein yield. Infusion into the bloodstream of a mix of AA lacking one essential AA caused concentration of that essential AA to fall in plasma. High insulin and other essential AA concentrations in plasma implicated protein synthesis as being responsible for the decrease. However, not all essential AA imbalances yielded equivalent responses. When BCAA were absent, milk protein secretion was stimulated; when other essential AA were absent, indirect evidence suggests that synthesis of proteins in the body was stimulated. In comparison to a complete AA mix, removal of one essential AA also caused the concentration of that AA to fall in plasma but milk protein yield decreased. Stimulation of insulin and IGF-I secretion in response to total AA supply during imbalance appears to have allowed the mammary glands to overcome imperfections in the circulating AA profile to maintain milk protein yield at higher levels than expected from deficiency responses.

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