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Amino acid supply to the small intestine of dairy cows fed pasture

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ABSTRACT

Two experiments measured the quantity and balance of amino acids available for absorption by four dairy cows fed pasture. Experiment 1 compared a diet of ryegrass/white clover pasture offered *ad libitum* or at 75% of *ad libitum* intake during October. Experiment 2, conducted in December, compared *ad libitum* diets of 100% ryegrass or 50% ryegrass and 50% white clover. Treatments were assigned according to a 2x2 crossover design.

Restricted feeding (Experiment 1) significantly reduced the total supply of amino acids to the small intestine by 23% (246 vs. 190 g amino acid nitrogen/d). Compared to an all-ryegrass diet, a ryegrass diet which contained 50% white clover (Experiment 2) significantly increased the total supply of amino acids by 10% (284 vs. 259 g amino acid nitrogen/d).

For all four pasture diets, arginine, methionine, and histidine were identified, in that order, as being most limiting, supplying 67, 71, and 77%, respectively, of the absorbable amino acid requirement. The supply of absorbable lysine from all four pasture diets was found to meet requirements of dairy cows in early to mid lactation.

Keywords: Amino acids; pasture; intestinal supply; milk protein; dairy cows.

INTRODUCTION

New Zealand dairy farmers are encouraged to produce more milk protein by a payment system that reflects international demand. To consistently and predictably increase the content of protein in milk, especially the casein component, an understanding of the post-ruminal supply of amino acids and the subsequent metabolism of amino acids by the cow is required. Estimates of the amino acid requirements of dairy cows have been made based on the proportion of essential amino acids (EAA) in milk and tissues (O'Connor *et al*., 1993). Furthermore, models such as the NRC-based Cornell Net Carbohydrate and Protein System (CNCPS; O'Connor *et al*., 1993) can make an adequate prediction of the supply of essential amino acids relative to requirements (Wu *et al*., 1997). However, the amino acids that are absorbable by lactating cows fed fresh pasture are not well documented and few data are available to validate this model for fresh pasture (van Vuuren *et al*., 1992, 1993; Younge, 1997).

Attempts have been made to identify those amino acids likely to limit milk protein by supplementing pasture-fed cows with individual amino acids. Responses, however, have been variable (Rusdi and van Houtert *et al*., 1997; Pacheco-Rios *et al*., 1997a, 1997b). Because pasture quality is variable and the order in which amino acids limit the synthesis of milk protein may change, a mechanistic approach such as that offered by the CNCPS must ultimately be used. This paper reports two experiments which were designed to characterise the supply of absorbable amino acids when lactating dairy cows in early to mid lactation were fed fresh pasture. The second objective was to identify those amino acids which were most likely to limit the synthesis of milk protein.

MATERIALS AND METHODS

Experiment 1 compared a ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) diet at two levels of intake during October and November 1996. Experiment 2 compared a predominantly ryegrass diet with a diet comprising 50% ryegrass and 50% white clover, each offered *ad libitum* during December 1996 and January 1997.

Experiment 1

Four multiparous Friesian cows were paired by milk production (21 ± 3 kg/d), live weight (492 ± 53 kg), and days of lactation (48 ± 12 d) (mean ± SD). After pairing, cows were randomly assigned to one of two levels of dry matter (DM) intake: ryegrass/white clover pasture offered *ad libitum* or restricted to 75% of *ad libitum* intake. The nitrogen (N) content of pasture was 2.6%. Cannulae were previously fitted at the dorsal rumen and proximal duodenum. Pasture was cut twice a day and fed at 0300, 0900, 1500, and 2100 h. The experiment consisted of two 14-d periods arranged in a crossover design. Cows were maintained in individual outdoor pens and adjusted to diets from d 1 to 6, during which time the level of feed restriction was established. From d 7 to 14 cows were maintained in individual metabolism stalls for measurements of milk production and composition, DM intake, and feed composition. Milk casein concentrations were determined on d 11 and 12.

Following a primer dose, Cr EDTA and Yb acetate were separately infused (1.4 ml/minute) into the rumen from d 7 to 13. Infusion from d 7 to 11 was designed to attain steady state concentrations in the rumen. Duodenal contents were sampled every 4 h (approximately 250 ml) on d 12 and 13, with sample times advanced by 2 h in the second 24 h. Steady state duodenal samples were prepared in whole digesta and fluid phases. Digesta was stored at 4 ^{0}C during collection, frozen at -18 ^{0}C, and subsequently dried at 95 ^{0}C and ground through a 1-mm sieve. Duodenal samples were analysed for DM, organic matter, total N, NH_3-N, purine and amino acid content. Duodenal sampling continued every 4 h for 24 h during the run down period following cessation of infusions on d 13. Digesta was frozen for subsequent analysis of Yb content or centrifuged at 1500 g for 10 min and supernatent frozen for Cr analysis.

From d 12 to 14 ruminal digesta was collected twice a day with sampling times advanced by 4 h each day. Bacteria were isolated from the digesta sample (see Kolver 1997), freeze dried, and bulked after being ground through a 1-mm sieve. Bacterial samples were analysed for ash, N, purine and amino acid content. Concentrations of amino acids were measured following reverse phase HPLC separation of phenylisothiocyanate derivatives (Bidlingmeyer *et al*., 1984) using a Waters PicoTag column and a Shimadzu LC-10/A HPLC system.

Experiment 2

Four multiparous Friesian cows, three of which had been used in Experiment 1, were paired by milk production (19 ± 2 kg/d), live weight (503 ± 32 kg), and days of lactation (128 ± 11 d) (mean ± SD). After pairing, cows were randomly assigned to one of two pasture treatments: a ryegrass diet or a diet comprising 50% ryegrass and 50% white clover (DM basis), each offered *ad libitum.* The 100% ryegrass diet contained 2.44% N and the 50% ryegrass/white clover diet contained 3.1% N. Pastures of ryegrass and of white clover were cut twice a day. The quantities of fresh ryegrass and white clover required for the mixed diet were determined using a microwave DM. Experimental design, sampling schedule, and animal management were as for Experiment 1, with the exception that cows were fed at 1000, 1600, 2200, and 0400 h.

Data were analysed using the general linear model procedure of SAS 6.12 according to a crossover design. The analysis tested for cow, period, and treatment, and their interactions. All means presented are least squares means tested with the PDIFF option in SAS 6.12. Because only four cows were used in this experiment, significant effects were declared at P<0.05 and trends at P<0.15.

RESULTS

Cows fed restricted amounts of pasture consumed 77% of the amount consumed by cows fed *ad libitum* (Table 1). Restriction of feed intake significantly reduced the concentration of milk protein and tended (P=0.14) to reduce the concentration of casein. In Experiment 2, including white clover in the ryegrass diet resulted in a significantly higher DM intake, and tended to increase milk protein concentration (P=0.15).

TABLE 1. Dry matter intake and milk yield during the experimental period.

	Experiment 1				Experiment 2			
	Ad libitum	Restricted	SED	P <	Ryegrass	50% WC	SED	P <
Dry matter intake (kg DM/d)	16.6	12.8	0.47	0.02	18.5	21.2	0.26	0.01
Milk yield (kg/d)	21.5	20.9	1.08	0.62	20.7	21.9	0.94	0.31
Milk protein (%)	3.35	3.06	0.006	0.001	3.15	3.18	0.017	0.15
Milk casein protein (%)	2.34	2.12	0.096	0.14	2.25	2.24	0.045	0.76
Milk protein yield (g/d)	0.71	0.64	0.039	0.21	0.65	0.70	0.025	0.16

Restricted feeding resulted in a significantly lower intake of N, and subsequent flow of total N and non-NH_3 N to the duodenum (Table 2). In Experiment 2, including white clover in a ryegrass diet significantly increased the N intake by cows, but had no significant effect on flow of total N or non-NH_3 N to the duodenum. Flow of bacterial N was significantly reduced by 40% when intake was restricted (Experiment 1), but was not significantly changed when white clover was included in a ryegrass diet (Experiment 2) (Table 2).

TABLE 2. Nitrogen supply to the small intestine.

	Experiment 1				Experiment 2			
	Ad libitum	Restricted	SED	P <	Ryegrass	50% WC	SED	P <
Total N intake (g/d)	425.5	333	11.03	0.01	446.1	660.9	10.77	0.003
N Flows (g/d)								
Total N	536.1	404.2	17.82	0.02	494.0	539.0	21.9	0.18
Non-NH_3 N	505.0	373.0	20.3	0.02	459.0	494.0	22.9	0.27
Bacterial N	310.1	185.2	16.41	0.02	269.2	284.6	14.05	0.39

Restricted feeding (Table 3) significantly reduced the total flow of amino acids to the duodenum (g AAN/d). Feed restriction significantly reduced the flow of 14 of the 17 individual amino acids by approximately 23%, and tended to reduce the flow of methionine (P=0.06), serine (P=0.06), and cystine (P=0.09). In Experiment 2, including white clover in a ryegrass diet significantly increased total amino acid flow by approximately 10% and significantly increased the flow of 11 of the 17 individual amino acids. Flow of alanine (P=0.06), cystine (P=0.07), isoleucine (P=0.12), and proline (P=0.06) tended to increase when white clover was fed. Glycine and methionine appeared to be unchanged by the addition of white clover.

The required proportions of absorbable essential amino acids (EAA) for lactating cows used in the CNCPS (Wu *et al*., 1997) was used to determine amino acid adequacy of the diet (Table 4). For all diets fed in Experiments 1 and 2, the most limiting amino acids were arginine, methionine, and then histidine, which supplied approximately 67, 71, and 77% of amino acid requirement, respectively.

TABLE 3. Amino acid supply to the small intestine (g AAN/d).

	Experiment 1				Experiment 2			
	Ad libitum	Restricted	SED	P <	Ryegrass	50% WC	SED	P <
Total amino acid flow	246.4	189.5	6.68	0.01	259.1	284.3	3.94	0.02
Total EAA flow	91.7	70.2	1.83	0.01	95.4	107.6	1.62	0.02
Alanine	23.0	17.8	0.71	0.02	23.7	26.2	0.65	0.06
Arginine	7.6	5.8	0.17	0.01	8.0	9.2	0.17 17	0.02
Aspartamine	24.9	20.2	0.77	0.03	25.5	28.5	0.57	0.03
Cystine	3.1	2.6	0.16	0.09	3.8	4.1	0.09	0.07
Glutamine	25.5	20.1	0.67	0.02	25.3	28.3	0.68	0.05
Glycine	44.4	31.3	2.03	0.02	52.4	52.2	1.79	0.92
Histidine	4.3	3.4	0.19	0.05	4.0	4.5	0.11	0.05
Isoleucine	10.0	6.9	0.36	0.01	10.8	12.2	0.51	0.12
Leucine	17.7	14.0	0.54	0.02	18.4	20.5	0.36	0.03
Lysine	14.1	11.1	0.18	0.01	14.5	16.4	0.16	0.01
Methionine	3.3	2.5	0.22	0.06	3.4	3.8	0.19	0.17
Phenylalanine	8.7	6.6	0.42	0.04	8.9	10.2	0.20	0.02
Proline	12.2	9.7	0.41	0.03	10.6	11.8	0.29	0.06
Serine	14.6	11.9	0.66	0.06	14.7	16.6	0.29	0.02
Threonine	13.9	11.1	0.58	0.04	14.4	16.3	0.31	0.03
Tyrosine	7.2	5.7	0.32	0.04	7.8	8.9	0.10	0.01
Valine	12.1	8.8	0.23	0.01	12.9	14.6	0.35	0.04

TABLE 4. Relative proportions (g AA/100g total EAA) of essential amino acids available for absorption and the required absorbable essential amino acids.

	Experiment 1		Experiment 2		Required
	Ad libitum	Restricted	Ryegrass	50% WC	Absorbable AA[1] EAA
Arginine	8.3	8.2	8.4	8.5	12.4
Histidine	4.6	4.9	4.2	4.2	5.8
Isoleucine	10.8	9.8	11.3	11.3	11.4
Leucine	19.3	19.9	19.3	19.0	17.2
Lysine	15.4	15.9	15.2	15.3	16.0
Methionine	3.6	3.5	3.6	3.5	5.0
Phenylalanine	9.5	9.4	9.3	9.4	8.8
Threonine	15.2	15.8	15.1	15.1	8.7
Valine	13.2	12.5	13.5	13.6	12.0

[1]From Wu *et al*., (1997)

DISCUSSION

The flow of individual amino acids calculated in this experiment were similar to those published elsewhere for cows fed fresh pasture (van Vuuren *et al*., 1992, 1993; Younge, 1997), once flow was adjusted for differences in DM intake. The 23% decrease in the flow of amino acids in cows fed a restricted amount of pasture (Experiment 1) appeared to largely be accounted for by the 23% reduction in DM intake, and consequently N intake. The 13% difference in DM intake between ryegrass and ryegrass/white clover pasture (Experiment 2) only resulted in a 10% difference in amino acid flow. Although N intake was higher on the ryegrass/white clover diet compared with the ryegrass diet, loss of N in the form of NH_3-N was greater on the ryegrass/white clover diet (data not shown), which resulted in a similar flow of non-NH_3 N to the duodenum (Table 2). All individual amino acids appeared to be reduced to a similar extent when pasture intake was restricted (Experiment 1), and increased by a similar margin when white clover was included in a ryegrass diet (Experiment 2), the exception being glycine and methionine in Experiment 2.

The flow of non-NH_3 N to the duodenum was greater than the N intake in three of the four diets tested in Experiments 1 and 2. This is in contrast to other studies (van Vuuren et al., 1992, 1993; Younge, 1997) which report lower flows of non-NH_3 N relative to N intake when pasture is fed to dairy cows. This inconsistency is surprising, but may relate to an increased recycling of N to the rumen as a result of the three ryegrass diets containing only moderate levels of protein (15-16%). This may also explain why total AA N flow to the duodenum represented 49 to 58% of non-NH_3 N flow (Table 2), when other comparable studies (van Vuuren et al., 1992, 1993; Younge, 1997) report a higher proportion of AA N (61 to 73% of non-NH_3 N).

Relative to published amino acid requirements (Wu *et al*., 1997), arginine, methionine, and then histidine appeared to be potentially limiting amino acids. Interestingly, the individual amino acids and the order of limitation were not different between cows fed *ad libitum* or restricted pasture, or when ryegrass pastures included a significant level of white clover. This result is in contrast to a companion experiment (Pacheco-Rios *et al*., 1998) that measured the extraction of amino acids by the mammary gland. In that study leucine, lysine, and methionine appeared, in that order, as the most limiting amino acids in dairy cows fed fresh pasture *ad libitum*. The order of limitation appeared to change to methionine, lysine, and leucine, when intake was restricted. The apparent inconsistency in order of amino acid limitation between the experiment of Pacheco-Rios *et al*., (1998) and our experiments may reflect differing post-absorptive metabolism between individual amino acids. However, Wu *et al*., (1997) states that the absorbable amino acid requirements (when expressed as a percentage of total essential amino acids) (Table 4) are comparable with amino acid requirement percentages determined from duodenal digesta. For example, the methionine and lysine requirements of Wu *et al*., (Table 4) are comparable with requirements for intestinal methionine (5% of essential amino acids) and lysine (15-16% of essential amino acids) identified by Schwab (1996). Both the current study and that of Pacheco-Rios *et al.,*(1988) identified methionine as being limiting. Methionine (and lysine) have been identified as the two most limiting amino acids when lactating cows are fed a variety of diets (Schwab, 1996). This may not be surprising given that methionine is first limiting in ruminally synthesised microbial protein, and is often present in lower amounts relative to total EAA in feed proteins. Lysine, however, appeared to be adequate in the pasture diets fed in Experiment 1 and 2, when compared with the 15-16% of EAA recommended by Schwab (1996). Calculations based on data presented by van Vuuren *et al*., (1992, 1993), and Younge (1997) revealed that absorbable lysine from pasture diets was also not limiting in these studies. Our finding that methionine may be potentially limiting is in agreement with calculations based on the data of van Vuuren *et al*., (1992, 1993) that indicate methionine represented 2.6% of EAA flow. Younge (1997), however, reported that methionine supply from fresh pasture was able to meet the methionine requirements of lactating dairy cows.

Further experiments are being conducted to characterise the absorbable amino acids available in dairy cows during late lactation. This matrix of amino acid supply across stage of lactation, pasture type, and level of intake will be used to evaluate the predictive capability of models such as the CNCPS. Ultimately this will allow a mechanistic prediction of protein nutrition and milk protein production to be made for different grazing situations.

CONCLUSION

Methionine, but not lysine, appeared to limit milk protein production across a range of pasture type and level of intake for cows in early to mid lactation. Arginine and histidine were also identified as being potentially limiting.

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