

## New Zealand Society of Animal Production online archive

This paper is from the New Zealand Society for Animal Production online archive. NZSAP holds a regular annual conference in June or July each year for the presentation of technical and applied topics in animal production. NZSAP plays an important role as a forum fostering research in all areas of animal production including production systems, nutrition, meat science, animal welfare, wool science, animal breeding and genetics.

An invitation is extended to all those involved in the field of animal production to apply for membership of the New Zealand Society of Animal Production at our website [www.nzsap.org.nz](http://www.nzsap.org.nz)

[View All Proceedings](#)

[Next Conference](#)

[Join NZSAP](#)

The New Zealand Society of Animal Production in publishing the conference proceedings is engaged in disseminating information, not rendering professional advice or services. The views expressed herein do not necessarily represent the views of the New Zealand Society of Animal Production and the New Zealand Society of Animal Production expressly disclaims any form of liability with respect to anything done or omitted to be done in reliance upon the contents of these proceedings.

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](https://creativecommons.org/licenses/by-nc-nd/4.0/).



You are free to:

**Share**— copy and redistribute the material in any medium or format

Under the following terms:

**Attribution** — You must give [appropriate credit](#), provide a link to the license, and [indicate if changes were made](#). You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

**NonCommercial** — You may not use the material for [commercial purposes](#).

**NoDerivatives** — If you [remix, transform, or build upon](#) the material, you may not distribute the modified material.

<http://creativecommons.org.nz/licences/licences-explained/>

## Arterio-venous differences of amino acids across the mammary gland of cows fed fresh pasture at two levels of dry matter intake during early lactation

PACHECO-RIOS, D.<sup>1,2</sup>, MCNABB, W.C.<sup>1</sup>, CRIDLAND, S.<sup>1,3</sup>, BARRY T.N.<sup>2</sup> AND LEE, J.<sup>1</sup>

<sup>1</sup>Nutrition Group, AgResearch, Grasslands Research Centre, PB 11008, Palmerston North, New Zealand.

### ABSTRACT

Four cows housed indoors in metabolism stalls were used in a cross-over experiment to test the effect of two levels of dry matter intake on amino acid metabolism during early lactation (October). Fresh ryegrass/white clover pasture was offered *ad libitum* or at 75% of the *ad libitum* intake during 2 experimental periods. Concentrations of essential amino acids (EAA) in arterial and mammary venous plasma were determined as indicators of the uptake and utilisation of individual AA for milk protein synthesis in the mammary gland.

The relative concentrations of free EAA (expressed as % of total EAA) of lysine, tyrosine, leucine, phenylalanine, methionine, tryptophan and histidine were lower in arterial plasma than those in the milk protein, suggesting these as limiting EAA. Using extraction rates of plasma as an indicator of potentially limiting amino acids, leucine, lysine and methionine appeared, in that order, as the most limiting amino acids in the *ad libitum* animals. In the intake restricted animals, the order of limitation appeared to change to methionine, lysine and leucine.

**Keywords:** amino acids; arterio-venous differences; milk protein; dairy cows.

### INTRODUCTION

Protein has become one of the most valuable milk components, both in an economic and nutritional sense. The international demand for milk protein has increased steadily in the last few years. This increase is not only the result of a greater consumption of cheese and milk powder, but also the reflection of an increased demand for specific milk protein fractions (Valeur, 1997).

Several studies have been conducted to assess whether amino acid (AA) supply to the mammary gland limits milk protein synthesis. However, most of the research on AA metabolism has been conducted with concentrate-fed animals. Manipulation of milk protein synthesis in New Zealand dairy cows requires information on the utilisation of AA by the mammary gland of pasture-fed animals. It has been postulated that the precursors for milk protein, amino acids, absorbed from the small intestine in dairy cows may be inadequate in amount and/or proportion to maximise milk protein synthesis in pasture-fed animals (Black, 1990).

The effectiveness of supplementation with sources of undegradable dietary protein (UDP), or ruminally protected amino acids (mainly methionine and lysine) to increase milk yields has been assessed. However, the results have been variable (DePeters, 1992). In most cases, total mixed rations have been used, with few reports on pasture-fed animals.

In pasture-fed animals, supplementation with methionine resulted in numerically higher yields of casein during mid lactation, but not in late lactation (Pacheco-Rios *et al.*, 1997a, 1997b). As the results were inconsistent at different

stages of lactation, it was demonstrated that single-AA supplementation was not an adequate way to increase protein synthesis in a consistent manner. More research is required on the post-absorptive utilisation of AA in pasture-fed animals in order to elucidate their role as potentially limiting nutrients for milk protein synthesis.

Arterio-venous (A-V) differences across the mammary gland are indicators of the amount of precursors available for transformation into protein constituents. This measurement has been used to identify the potential amino acids which may limit milk protein synthesis in lactating cows (Derrig *et al.*, 1974; Clark *et al.*, 1977) and ewes (Davis *et al.*, 1978). In this study, the A-V differences across the mammary gland of lactating cows offered two levels of dry matter intake were analysed as part of a research project on identifying the potential limiting amino acids in pasture-fed animals.

### MATERIALS AND METHODS

#### Animals and diets

Four lactating Friesian cows in early lactation were assigned to a sequence of two levels of dry matter intake (DMI) in a 2x2 crossover design. The average days in milk of the cows was 44 (SD 14.5) with the average live weight being 498 (SD 64.2) kg at the beginning of the experiment. The 2 sixteen-day experimental periods comprised a 5-day period for diet adaptation and an 11-day period for measurements of milk production and composition, DMI and feed composition. Animals were fed individually and maintained outdoors from day 1-5, and in individual metabolism

<sup>2</sup>Institute of Food, Nutrition and Human Health, Massey University, PB 11222, Palmerston North, New Zealand.

<sup>3</sup>Institute of Molecular BioSciences, Massey University, PB 11222, Palmerston North, New Zealand.

stalls from day 6 to 16 at the Dairying Research Corporation facilities in Hamilton, New Zealand. Cows were fitted on day 11 with polyvinyl chloride (PVC, 1.0 mm ID x 1.5 mm OD) catheters in one intercostal artery and jugular and mammary (caudal superficial epigastric) veins following tranquilisation with xylazine hydrochloride (0.8 ml Rompun 2%; Bayer New Zealand Ltd., Glenfield, Auckland). Cows were allowed to recover from the surgery for one day before starting blood collections. The intercostal catheters were maintained during the two experimental periods, while both venous catheters were removed at the end of the first period and new catheters inserted on day 11 of the second period. Patency of all the catheters was ensured by daily flushing with 3 ml of heparinised saline (200 IU/ml). Animals were milked twice daily during the whole experimental period, except during the blood sampling period, when milking was conducted every two hours. During the experimental period, fresh cut ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture was offered at 6 h intervals (0300, 0900, 1500 and 2100 h).

### Treatments

The treatments tested were two levels of DMI: *ad libitum* and restricted intakes. Animals in the restricted group received 75% of the *ad libitum* intake which was established during the first five days of each experimental period.

### Measurements

On day 14 and 15, blood samples were collected from the intercostal artery and the mammary vein for each of two animals. Blood samples were collected every two hours as 1-hour integrated samples (i.e. continuous sampling for one hour, no sampling for one hour). Samples were collected at a rate of 1 ml/min using peristaltic pumps (Desaga PLG, Germany) into plastic tubes kept on ice with EDTA added as an anticoagulant. Blood was centrifuged at 3270 g (4000 rpm) at 4 °C for 15 minutes and plasma was harvested and mixed (10:1 v/v) with a solution of 200 mM phosphate buffer containing 80 mM DL-dithiothreitol (DTT). Plasma was stored at -85°C for amino acid analysis.

### Laboratory methods

Samples of plasma (2 ml) were mixed with 65 µl of 3 mM methionine sulphone as an internal standard, and then deproteinised by ultrafiltration (Centrisart, molecular weight cut-off 10,000; Sartorius AG, Germany). The ultrafiltrate was collected and stored at -85°C until it was analysed for free AA concentrations.

Amino acid concentrations in plasma were measured after reverse phase HPLC separation of phenylisothiocyanate derivatives (Bidlingmeyer, 1984) using a Waters PicoTag® column and a Shimadzu LC-10/A HPLC system.

Milk samples were analysed for protein using an infra-red analyser (Milk-O-Scan 133B, Foss Electric, Hillerød, Denmark).

### Calculations

The order in which individual EAA may have been limiting milk protein synthesis was assessed using two

calculation procedures:

1) A-V differences were calculated for statistical analysis using the average values of the arterial and venous concentrations over the sampling periods. Extraction rate was then calculated by dividing the A-V difference for each amino acid by their respective concentration in arterial plasma, and the proportion expressed as a percentage. Individual EAA with the highest extraction rates were assumed to be the most limiting for milk protein synthesis.

2) Individual EAA concentrations in arterial plasma were also expressed as proportion of the total EAA in that pool. These proportions were then compared with the relative proportions of EAA in a theoretical milk protein (Mackenzie, 1997). Ratio values of plasma:milk less than unity were used to infer amino acids most likely to limit milk protein synthesis in the mammary gland.

### Statistical analysis

Means of the concentration of individual EAA for each cow were obtained and then analysed using the GLM procedure of SAS (SAS Institute, 1996). Treatments were analysed as main effects and the sequence of treatment and the interaction sequence x treatment were also analysed.

The effects of sequence were tested by using the mean square of the effect of cow within sequence as the residual error. Treatment, cow and interaction between treatment and sequence were tested using the residual error. Significant effects were declared when  $P < 0.05$  and trends at  $P < 0.10$ .

## RESULTS

Dry matter intakes and milk yields are shown in Table 1. The restriction in feed intake was achieved as planned, with the restricted group consuming, on average, 74% of the amount consumed by the *ad libitum* animals. The restriction in feed intake tended to reduce both the concentration ( $P=0.07$ ) and yield ( $P=0.09$ ) of milk protein.

Amino acid concentrations in arterial and venous plasma are shown in Table 2. No consistent treatment effects were observed in these variables. The *ad libitum* group had numerically higher arterial concentrations of isoleucine, methionine, tyrosine and valine; whilst the restricted group had numerically higher concentrations of arginine, histidine, leucine, lysine, phenylalanine, threonine and tryptophan. More consistent responses were observed in venous amino acid concentrations. The *ad libitum* cows had numerically higher venous concentrations of all the essential amino acids except arginine,

TABLE 1: Dry matter intakes and milk yields during the experimental period.

	<i>Ad libitum</i>	Restricted	SEM	Prob
Dry matter intake (kg/d)	16.7	12.3	0.26	$P < 0.01$
Milk yield (kg/d)	21.6	19.8	0.52	$P = 0.14$
Milk protein concentration (%)	3.28	3.11	0.033	$P = 0.07$
Milk protein yield (g/d)	0.70	0.61	0.021	$P = 0.09$

**TABLE 2:** Arterial and venous concentrations, A-V concentration differences and extraction % of plasma amino acids in cows fed an *ad libitum* (A) and restricted (R) intake.

Amino acid	Arterial plasma concentration (mM)			Venous plasma concentration (mM)			A-V difference (mM)			Extraction (%)		
	A	R	SEM	A	R	SEM	A	R	SEM	A	R	SEM
Arginine	73.2	83.3	10.62	48.5	50.7	8.84	24.7	32.5	1.79□	34.6	40.1	3.16
Histidine	33.3	43.2	5.25	26.6	34.6	5.39	6.8	8.6	0.47	20.6	20.9	3.91
Isoleucine	104.0	102.0	11.51	70.9	63.6	9.44	33.1	38.3	2.14	31.4	37.9	2.11
Leucine	115.4	125.2	16.50	70.2	69.8	12.82	45.2	55.4	3.73	38.9	44.5	2.44
Lysine	74.2	81.6	14.44	47.0	43.2	11.73	27.2	38.4	2.71□	36.8	48.7	4.61
Methionine	27.9	26.3	4.15	17.6	13.8	2.97	10.3	12.5	1.25	36.7	49.1	3.28
Phenylalanine	47.6	49.9	4.65	34.1	31.8	3.50	13.5	18.1	1.28	28.3	36.2	0.89*
Threonine	132.5	137.1	21.36	114.1	114.2	20.15	18.4	22.9	1.66	15.0	20.4	3.31
Tryptophan	5.1	5.4	0.80	4.9	4.5	0.86	0.2	0.9	0.21	4.3	15.4	4.94
Tyrosine	45.7	39.7	5.78	32.6	22.7	4.67	13.2	16.9	1.57	28.7	43.5	3.53□
Valine	249.1	232.3	21.83	206.0	183.5	21.72	43.1	48.7	0.45*	17.1	21.2	2.03

□ P &lt; 0.10

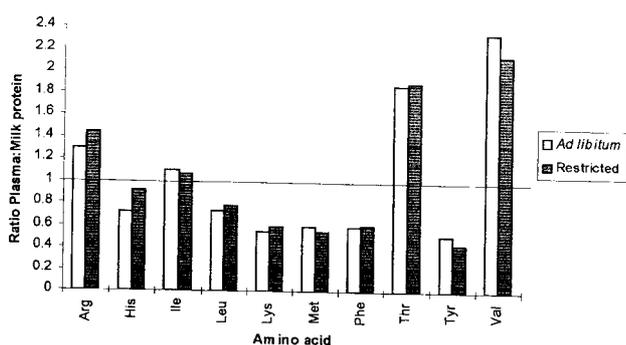
\* P &lt; 0.05

histidine and threonine. However, none of these responses were significant.

A-V differences were consistently higher in those animals with restricted DM intakes (Table 2). This effect was significant for valine (P=0.01); whilst arginine (P=0.09) and lysine (P=0.10) approached statistical significance.

Using the plasma extraction % of EAA as an indicator of limiting AA for milk protein synthesis, it was found that leucine, lysine and methionine were the three most limiting AA for animals offered pasture *ad libitum*. The order of limitation changed in cows with restricted intakes to methionine, lysine and leucine.

Individual EAA were expressed as a proportion of total EAA, and these values compared with the relative proportion of EAA in milk protein. The ratio of the relative proportions of amino acids in arterial plasma versus milk protein was used as an indicator of potentially limiting amino acids (Figure 1). For both levels of intakes, lysine, tyrosine, leucine, phenylalanine, methionine, tryptophan and histidine were present in plasma in lower proportions than in milk protein.

**FIGURE 1:** Ratio of the relative proportions of amino acids in arterial plasma and milk protein in cows offered *ad libitum* or restricted intakes, compared with values for a theoretical milk protein (dotted line).

## DISCUSSION

The extraction of plasma amino acids calculated in this experiment are similar to those published elsewhere for cows fed concentrate-based diets (Derrig *et al.*, 1974; Yang *et al.*, 1986; Illg *et al.*, 1987; Cant *et al.*, 1993), except for methionine, lysine and isoleucine. In these cases, the extraction percentages observed are lower than the published data (35-50 vs 50-70%). Assuming that a relatively low concentration of a particular EAA together with a high extraction of that AA by the mammary gland identifies a potentially limiting AA (Clark *et al.*, 1977) our results indicated that leucine, lysine and methionine were the three main limiting AA for the *ad libitum*-fed animals. However, the order of limitation appeared to change for restricted animals to methionine, lysine and leucine. Davis *et al.* (1978) found a similar sequence of potentially limiting AA in their experiment with lactating sheep, while Clark *et al.* (1977) reported that methionine and lysine were the most critical amino acids.

The relative proportions of amino acids present in a 'theoretical' milk protein (Mackenzie, 1997) resembled the required proportions of absorbable EAA for lactating cows according to the Cornell Net Carbohydrate and Protein System (Wu *et al.*, 1997; Table 3). The similarity between the two values validates the use of the milk amino acid composition as means to identifying potentially limiting AA. The comparison of the relative proportions of EAA in plasma and milk protein was used by Spires *et al.* (1975) and Clark *et al.* (1977) to determine the order of potential limiting amino acids for milk protein synthesis in dairy cows. Using different post-ruminal infusates, they consistently found that lysine, methionine and phenylalanine were the three most limiting amino acids in cows fed total mixed rations of alfalfa hay and maize-soybean concentrate. Similar results were found in our experiment, in which the calculations of the relative proportions of EAA in plasma and milk protein (Table 3) indicated that

**TABLE 3:** Relative proportions (g AA/100g total EAA) of essential amino acids in milk protein, arterial plasma and the required absorbable EAA.

	Plasma <i>Ad libitum</i> DMI	Plasma Restricted DMI	Milk Protein <sup>1</sup>	Absorbable required <sup>2</sup>
Arginine	8.1	9.0	6.3	12.4
Histidine	3.7	4.7	5.1	5.8
Isoleucine	11.5	11.0	10.4	11.4
Leucine	12.7	13.5	17.4	17.2
Lysine	8.2	8.8	14.8	16.0
Methionine	3.1	2.8	5.2	5.0
Phenylalanine	5.2	5.4	8.9	8.8
Threonine	14.6	14.8	7.8	8.7
Tryptophan	0.6	0.6	2.6	2.7
Tyrosine	5.0	4.3	9.8	NA
Valine	27.4	25.1	11.7	12.0

<sup>1</sup> From Mackenzie, 1997.

<sup>2</sup> From Wu *et al.*, 1997 using the Cornell Net Carbohydrate and Protein System.

tyrosine, lysine, phenylalanine and methionine are the most limiting amino acids in animals fed *ad libitum* pasture, whilst the order of potential limitation changed for restricted animals to tyrosine, methionine, lysine and phenylalanine. Data for tyrosine is not available in the studies mentioned above, making it difficult to draw a conclusion about the role of this AA as a limiting nutrient for milk protein synthesis.

Using two different approaches to determine the role of specific amino acids as limiting nutrients for milk protein synthesis, it appears that methionine and lysine are involved as co-limiting amino acids for milk protein synthesis in pasture-fed dairy cows. Other amino acids that may be involved as limiting AA are leucine and phenylalanine. An important finding is the change of order of limitation due to restriction in feed intake. In this study, the two approaches indicated that methionine may have a more important role during feed restrictions. This observation is supported by previous work (Pacheco-Rios *et al.*, 1997a, 1997b) in which methionine supplementation had positive effects during mid but not during late lactation, where feed restriction is less likely to occur.

More research is required to take into account the effects of dietary restriction on mammary blood flow (MBF) and consequently, AA uptake by the mammary gland. Cant *et al.* (1993) reported extraction percentages that compensated for reductions in MBF. In this way, the final AA uptake was not affected in spite of the lower MBF measured in cows fed a high level of dietary fat. A potential reduction in MBF due to feed restriction may explain the increase in extraction percentage observed in this study.

With the present results, and further research on the relationship between uptake of AA and their output in milk, it is expected to identify the potential limiting AA for milk protein synthesis in the pasture-fed dairy cow. This information is necessary to design supplementation strategies which give positive and consistent responses in milk protein yield.

## ACKNOWLEDGMENTS

This research was supported by the New Zealand Foundation for Research, Science and Technology. The authors thank the Dairying Research Corporation for providing the facilities; and the skillful assistance of Vicki Carruthers, David Phipps, Erna Jansen and Peter Neil (Dairying Research Corporation); Tricia Harris, Brian Treloar, Penny Back and Jason Peters (Nutrition Group, AgResearch Grasslands) and Nicolas Lopez-Villalobos (Institute of Veterinary, Animal and BioMedical Sciences, Massey University). The senior author also thanks the National Autonomous University of Mexico for providing the scholarship for his studies.

## REFERENCES

- Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. 1984. Rapid analysis of amino acids using pre-column derivatisation. *Journal of Chromatography* **336**:93-104
- Black, J. L. 1990. Nutrition of the grazing ruminant. *Proceedings of the New Zealand Society of Animal Production* **50**:7-27
- Cant, J. P.; DePeters, E. J.; Baldwin, R. L. 1993. Mammary amino acid utilization in dairy cows fed fat and its relationship to milk protein depression. *Journal of Dairy Science* **76**:762-774
- Clark, J. H.; Spires, H. R.; Derrig, R. G.; Bennink, M. R. 1977. Milk production, nitrogen utilization and glucose synthesis in lactating cows infused posturally with sodium caseinate and glucose. *Journal of Nutrition* **107**:631-644
- Davis, S. R.; Bickerstaffe, R.; Hart, D. S. 1978. Amino acid uptake by the mammary gland of the lactating ewe. *Australian Journal of Biological Science* **31**:123-132
- DePeters, E. J.; Cant, J. P. 1992. Nutritional factors influencing the nitrogen composition of bovine milk: a review. *Journal of Dairy Science* **75**:2043-2070
- Derrig, R. G.; Clark, J. H.; Davis, C. L. 1974. Effect of abomasal infusion of sodium caseinate on milk yield, nitrogen utilization and amino acid nutrition of the dairy cow. *Journal of Nutrition* **104**:151-159
- Illg, D. J.; Sommerfeldt, J. L.; Schingoethe, D. J. 1987. Lactational and systemic responses to the supplementation of protected methionine in soybean meal diets. *Journal of Dairy Science* **70**:620-629
- Mackenzie, D.D.S. 1997. Milk composition as an indicator of mammary gland metabolism. *Proceedings of the Nutrition Society of New Zealand*. **22**: 126-136.
- Pacheco-Rios, D.; McNabb, W.C.; Hill, J.P.; Barry, T.N.; Mackenzie, D.D.S. 1997a. The effects of methionine supply upon milk composition and production of dairy cows in mid-lactation. *Proceedings of the New Zealand Society of Animal Production* **57**: 147-150.
- Pacheco-Rios, D.; McNabb, W.C.; Hill, J.P.; Barry, T.N.; Mackenzie, D.D.S. 1997b. The effects of methionine supplementation upon milk composition and production of dairy cows in late lactation. *Proceedings of the Nutrition Society of New Zealand*. **22**: 184-191.
- SAS Institute Inc. 1996. SAS User's Guide. 6.11. Cary, NC
- Spires, H. R.; Clark, J. H.; Derrig, R. G.; Davis, C. L. 1975. Milk production and nitrogen utilisation in response to postprandial infusion of sodium caseinate in lactating cows. *Journal of Nutrition* **105**:1111-1121
- Valeur, J. 1997 Milk protein production and market prospects. In Milk composition, Production and Biotechnology (Ed. Welch, R. A. S., Burns, D. J. W., Davis, S. R., Popay, A. I., and Prosser, C. G.) 93-103. Cambridge, U.K.: CAB International.
- Wu, Z.; Polan, C. E.; Fisher, R. J. 1997. Adequacy of amino acids in diets fed to lactating dairy cows. *Journal of Dairy Science* **80**: 1713-1721
- Yang, C. M. J.; Schingoethe, D. J.; Casper, D. P. 1986. Protected methionine and heat-treated soybean meal for high producing dairy cows. *Journal of Dairy Science* **69**:2348-2357