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Amino acid utilisation by the mammary gland: Whole blood versus plasma free amino acid pools.

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ABSTRACT

Utilisation of amino acids (AA) by the mammary gland of dairy cows has been studied in order to understand the responses in milk protein production to alterations in nutrient supply. One approach has been the use of balance studies, in which the amount of AA taken up from the blood by the mammary gland is compared with their respective output in milk protein(s). Although it is generally accepted that the free AA pool in blood is the major precursor for milk protein synthesis, there are differences when plasma or whole blood arterio-venous differences (A-V) are used to measure the uptake of AA by the mammary gland. Arterial and venous blood samples were obtained at 2 h intervals from cows fed fresh pasture over two 12-h sampling periods. The samples were subdivided into whole blood (WB) and plasma (P) and analysed for free AA concentrations, which were used to estimate amino acid concentrations in the erythrocyte. There were no significant differences in the A-V calculated from WB or P for the majority of AA. Plasma A-V was significantly higher than WB for threonine (13.9 vs 8.9 nmol/g blood) whilst WB A-V was higher than P for isoleucine (26.9 vs 23.3 nmol/g blood) and tyrosine (11.8 vs 10.3 nmol/g blood). Assuming the differences in A-V between WB and P can be attributed to AA exchange with the red blood cell (RBC), significant (t<0.01) exchange from RBC to the mammary gland occurred for only isoleucine (15% of WB A-V) and tyrosine (14% of WB A-V). These results indicate that plasma AA concentrations are valid measures of the immediate free amino acid pool available for uptake by the mammary gland.

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

INTRODUCTION

Global trends in the market of dairy products indicate an increase in the consumption of milk protein, both as food products and isolated compounds (Valeur, 1997; Viatte,1997). This scenario has created an increasing interest in the mechanisms controlling milk protein synthesis. Particular attention has been given to the understanding of amino acid metabolism in dairy cows, as it appears to be fundamental to devise strategies to increase both the yield and the efficiency of production of milk protein. It is well recognised that dairy cows have a requirement for a specific amount and proportion of amino acids for optimised production of milk (Tamminga & Oldham, 1980; Rulquin *et al.*, 1995). However, there is no real consensus on the quantitative and qualitative definition of the amino acid requirements for dairy cows. More quantitative studies are required on the flows and metabolic transformations of amino acids in the body of lactating dairy cows (Maas *et al.*, 1997).

The use of isotope-labeled amino acids has proven to be a useful approach to obtain insight into the utilisation of absorbed amino acids by the mammary gland and other tissues (Bequette *et al.*, 1994; Lobley *et al.*, 1996; Bequette *et al.*, 1997). A critical assumption in these studies is the definition of the pool from which the mammary gland extracts the amino acids utilised to synthesise both its constitutive protein and milk proteins. Although it has been known from early research (Cary, 1920) that free amino acids (FAA) in blood are quantitatively the major precursor pool for milk protein synthesis, some researchers have suggested a significant contribution from other precursor pools (Bequette *et al.*, 1994; Lee *et al.*, 1999). Amino acids in blood are found in two compartments, plasma and erythrocytes. The transfer of FAA from each of these compartments to the mammary gland is not clearly understood. Hanigan *et al.*, (1991) reported a significant involvement of erythrocytes in amino acid transfer to the mammary gland of dairy cows, whilst Bequette *et al.*, (1997) concluded that plasma is the main pool for amino acid uptake by mammary gland of lactating goats. Furthermore, contrasting reports on the transfer of individual amino acids by the erythrocyte have been published by the same laboratory (Hanigan *et al.,* 1991; Cant *et al.,* 1993).

Given such contrasting reports, the objective of this part of our research was to compare the AA contribution of plasma with that of whole blood to identify the main pool of free amino acids available to the mammary gland.

MATERIALS AND METHODS

Animals and diets

Four lactating Friesian cows in early lactation were used in an experiment designed to assess the utilisation of amino acids in animals fed fresh forage. The experimental animals were fitted with polyvinyl chloride catheters in the intercostal artery and mammary (caudal superficial epigastric) vein to obtain simultaneous blood samples to assess the uptake of amino acids across the udder. The experimental diets consisted of fresh cut ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture which was offered at 6 h intervals (0300, 0900, 1500 and 2100 h). Further details of the experimental design, treatments and diets are described by Pacheco-Rios *et al.*, (1998).

Measurements

On day 14 and 15 of each period, blood samples were collected as described previously (Pacheco-Rios *et al.*, 1998). Packed cell volume (PCV) was determined using microhaematocrit tubes by direct collection from the sampling line. At the end of the collection period, blood was subsampled for analysis of free amino acids in whole blood (WB) and plasma (P). Whole blood was haemolysed with deionised water (1:1 v/v) and mixed with 200 mM phosphate buffer containing 80 mM DL-dithiothreitol (0.1 ml of phosphate buffer per ml of blood). Plasma was obtained after blood was centrifuged at 3270 g (4000 rpm) at 4 °C for 15 minutes and prepared for amino acid analysis as described previously (Pacheco-Rios *et al.*, 1998). Both haemolysed blood and plasma samples were stored at -85°C until analysed for amino acid concentrations (Pacheco-Rios *et al.*, 1998).

Calculations

The amino acid concentrations measured in whole blood and plasma samples are expressed as nanomoles per gram of whole blood or plasma. Amino acid concentrations in erythrocytes were calculated as described by Lobley *et al.*, (1996). The proportion of whole blood amino acids present in the erythrocytes was calculated using the erythrocyte concentrations corrected by haematocrit (Equation 1). Amino acid uptake by the mammary gland was estimated as the difference between the amino acid concentrations in arterial and venous samples (A-V). For plasma, the amino acid A-V were corrected for plasma volume to allow a direct comparison with the uptake calculated from whole blood (Equation 2).

(1)

$$AA\ erythrocyte\ (\%) = \frac{(AA\ concentration\ in\ erythrocyte \times PCV)}{AA\ concentration\ whole\ blood} \times 100$$

(2)

$$AA\ uptake\ from\ plasma\ (nmol\ g^{-1} whole\ blood) = Plasma\ A-V \times (1-PCV)$$

Statistical analysis

For each amino acid, the calculated A-V from whole blood and plasma were paired according to sampling time. The uptake of each amino acid across the mammary gland was tested to be significantly different from zero using Student's *t*-test. Similarly, the difference between the uptakes calculated from whole blood and plasma was tested by means of a paired *t*-test. Both tests were conducted using the PROC MEANS of SAS (SAS Institute, 1996). Values presented are means from 35 and 26 observations (concentrations and A-V, respectively).

RESULTS

The mean ± SE of the PCV for arterial and venous samples were 27.7 ± 0.67 and 27.9 ± 0.65, respectively. Amino acid concentrations in whole blood, plasma and erythrocyte are shown in Table 1. In general, well defined chromatograms were obtained from both types of sample. However, aspartic acid and tryptophan co-eluted with unidentified peaks in some cases. Therefore, caution should be exercised in interpreting data for those amino acids. In haemolysed whole blood samples, the quantitation of arginine was hampered by hydrolysis caused by arginase present in the erythrocytes. Thus, whole blood concentration and A-V for this amino acid are not presented. Similar concentrations in plasma and whole blood (i.e. equilibrium between plasma and erythrocyte) were found for isoleucine, leucine, valine, and asparagine. The concentrations of histidine, lysine, phenylalanine, tryptophan, tyrosine, aspartic acid, glutamic acid and proline were higher in whole blood than in plasma (i.e. accumulation in the erythrocyte). For these amino acids, erythrocytes represented between 40-90% of the whole blood concentration. On the other hand, plasma concentrations were higher than whole blood for alanine, threonine, glutamine and serine.

TABLE 1: Amino acid concentrations in arterial whole blood, plasma and red blood cells, together with the proportional contribution of erythrocytes to whole blood free amino acids in lactating dairy cows fed fresh pasture (Means ± SE).

Amino acid	Concentration (nmol/g)			Contribution of erythrocytes
	Whole blood	Plasma	Erythrocyte[1]	to whole blood (%)
Essential				
Arginine	na	81 ± 3.1	na	na
Histidine	60 ± 1.9	36 ± 2.0	121 ± 1.8	58 ± 1.4
Isoleucine	99 ± 2.8	102 ± 3.6	92 ± 4.4	26 ± 1.0
Leucine	136 ± 4.9	121 ± 5.1	176 ± 6.0	37 ± 1.0
Lysine	97 ± 3.0	77 ± 2.9	149 ± 6.3	43 ± 1.3
Methionine	34 ± 1.4	28 ± 0.9	52 ± 4.6	40 ± 1.5
Phenylalanine	58 ± 1.7	50 ± 1.5	78 ± 3.1	38 ± 1.1
Threonine	90 ± 7.1	126 ± 6.6	1 ± 15.8	-11 ± 6.6
Tryptophan	13 ± 0.8	5 ± 0.2	33 ± 1.7	76 ± 1.1
Tyrosine	55 ± 1.5	44 ± 1.6	84 ± 2.4	43 ± 1.0
Valine	219 ± 8.5	231 ± 9.5	192 ± 11.5	24 ± 1.2
Non-essential				
Alanine	146 ± 5.9	154 ± 5.3	123 ± 9.5	23 ± 1.4
Asparagine	47 ± 1.4	48 ± 1.7	44 ± 2.7	27 ± 1.6
Aspartic acid	85 ± 10.3	5 ± 0.2	297 ± 37.0	93 ± 0.6
Glutamine	125 ± 5.5	227 ± 9.8	-140 ± 17.7	-32 ± 4.7
Glutamic acid	177 ± 8.1	60 ± 2.1	472 ± 22.0	75 ± 0.9
Glycine	388 ± 11.1	271 ± 8.3	698 ± 30.2	50 ± 1.0
Proline	99 ± 2.9	76 ± 3.0	158 ± 5.7	45 ± 0.9
Serine	91 ± 5.1	106 ± 5.1	56 ± 8.3	15 ± 1.8

[1] Calculated from Equation 1.

Irrespectively of the type of sample, arterio-venous differences indicated a significant ($P < 0.01$) mammary uptake of all the amino acids reported, with exception of aspartic acid, glycine and tryptophan, for which the A-V measured from both whole blood and plasma were not different from zero. For most amino acids, there was no difference in uptake calculated using WB or P (Table 2). Calculated plasma uptake was higher than that from WB for threonine (+56%; $P < 0.05$). On the other hand, whole blood uptakes were significantly higher than those from plasma for isoleucine (+15%; $P < 0.01$) and tyrosine (+14%; $P < 0.01$).

TABLE 2: Arterio-venous differences calculated from whole blood or plasma amino acid concentrations in lactating dairy cows fed fresh pasture (Means ± SE).

Amino acid	A-V differences				
	Whole blood	P^1	Plasma[1]	P^1	P^2
Essential					
Arginine	na		19.4 ± 1.07	**	na
Histidine	3.6 ± 0.59	**	4.9 ± 0.52	**	NS
Isoleucine	26.9 ± 1.40	**	23.3 ± 1.36	**	**
Leucine	36.8 ± 1.75	**	34.9 ± 1.87	**	NS
Lysine	23.6 ± 1.27	**	22.9 ± 1.38	**	NS
Methionine	8.0 ± 0.43	**	7.8 ± 0.44	**	NS
Phenylalanine	12.2 ± 0.62	**	11.1 ± 0.60	**	NS
Threonine	8.9 ± 1.44	**	13.9 ± 1.50	**	*
Tryptophan	0.8 ± 0.44	NS	0.2 ± 0.11	NS	NS
Tyrosine	11.8 ± 0.65	**	10.3 ± 0.62	**	**
Valine	30.5 ± 1.28	**	29.9 ± 2.44	**	NS
Non-essential					
Alanine	12.8 ± 1.02	**	12.6 ± 1.85	**	NS
Asparagine	9.3 ± 0.39	**	9.7 ± 0.54	**	NS
Aspartic acid	-0.5 ± 1.39	NS	-0.1 ± 0.24	NS	NS
Glutamine	14.6.± 2.23	**	16.9 ± 2.76	**	NS
Glutamic acid	30.3 ± 2.52	**	28.7 ± 1.28	**	NS
Glycine	2.5 ± 2.40	NS	2.6 ± 2.81	NS	NS
Proline	6.0 ± 0.81	**	6.9 ± 0.98	**	NS
Serine	7.1 ± 1.02	**	8.4 ± 1.59	**	NS

[1]Calculated from Equation 2.
P^1 Probability that the uptake is not different from zero.
P^2 Probability that the difference between whole blood and plasma is not different from zero.
** P <0.01 Student's t
* P <0.05 Student's t
NS Non-significant

DISCUSSION

The concentrations of amino acids in either blood or plasma of lactating dairy cows have been well documented as part of numerous studies on amino acid metabolism. However, most of the data available has been obtained from animals fed different types and amounts of concentrates. Furthermore, only a few reports have included comparisons between the amino acid concentrations measured in blood and plasma. This study represents a first step towards a better characterisation of the amino acid utilisation by lactating cows fed fresh pasture as the sole diet. The concentrations measured in plasma in pasture-fed animals measured in the present study are comparable to those of concentrate-fed cows (Pacheco-Rios, review of 37 reports, unpublished data) with exception of methionine (higher in pasture-fed animals) and alanine, glutamate and glycine (higher in concentrate-fed animals). The differences in concentrations between blood and plasma are similar to those published overseas (Cant *et al.*, 1993).

Plasma is generally regarded as the blood compartment from which amino acids are extracted by the mammary gland for protein synthesis. However, erythrocytes have been reported as a major reservoir for some amino acids in blood. The results in this study support the concept that the erythrocyte accumulates, to a lesser or greater extent, amino acids present in the blood. The percent contribution of erythrocytes to whole blood amino acids found in this study is similar to previous published reports for ruminants (Danilson *et al.*, 1987) and monogastrics (Proenza *et al.*, 1994). For some amino acids, the accumulation can be related to particular requirements in the red blood cell. An example of this situation is the accumulation of glutamic acid and glycine required for the intracellular synthesis of glutathione, an antioxidant tripeptide (Tunnicliff, 1994). However, the purpose of the accumulation of other amino acids in the erythrocyte remains obscure and it has given origin to the suggestion that erythrocytes contribute actively to the AA exchange between blood and tissues. Therefore, some authors have proposed that using plasma AA concentrations would underestimate the uptake by tissues, as the contribution from red blood cells is neglected (Heitmann & Bergman, 1980; Danilson *et al.*, 1987; Hanigan *et al.*, 1991). By means of arterio-venous differences, these studies have shown that red blood cells were involved in AA transfer across the portal drained viscera (PDV) of sheep (Heitmann & Bergman, 1980), the hindlimb of calves (Danilson *et al.*, 1987) and the mammary gland of dairy cows (Hanigan *et al.*, 1991).

Hanigan *et al.*, (1991) reported an erythrocyte involvement in the transport of most amino acids to the mammary gland, and stated that plasma A-V would underestimate the uptake compared with the A-V from whole blood. However, later research from the same group (Cant *et al.*, 1993) found that plasma A-V would overestimate the uptake of some amino acids by the mammary gland. In contrast to those studies, in which "spot" samples were pooled and then assayed for amino acid concentrations, the present data reflects the differences between whole blood and plasma within each hourly-integrated sample. In the present study, whole blood and plasma uptakes were significantly different only for histidine, isoleucine, threonine, tyrosine and asparagine. From these amino acids, the erythrocyte appeared to contribute 14% of the total uptake of isoleucine and tyrosine by the mammary gland. Although the present results can not rule out the transfer of other amino acids from erythrocytes to the mammary gland, it appears that such transfer, if existent, contributes only a small proportion of the total amino acid flux to the mammary gland. This finding supports the reports of Lobley *et al.*, (1996) and Bequette *et al.*, (1997) who found a negligible involvement of the erythrocyte in amino acid transfer between tissues, using ^{13}C labeled amino acids. However, the uptake of free amino acids has been found to be insufficient to account for the output of some amino acids in goat's milk protein (Bequette *et al.*, 1994). The involvement of pools other than blood free amino acid (i.e. peptides, protein turnover) is still to be determined for lactating dairy cows fed fresh forages.

From the results in the present study, it can be concluded that, for most of the amino acids studied, plasma uptakes are adequate measures of whole blood free amino acid uptakes by the mammary gland. These results add to the understanding of the post-absorptive utilisation of amino acids by the lactating dairy cow, which is required to devise strategies for optimisation of milk protein synthesis.

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