Estimation of the duodenal amino acid supply in ruminants by amino acid analysis of the products of fermentation *in vitro*

C. Dennison and Anne M. Phillips
Department of Biochemistry, University of Natal

A method of estimating the exogenous contribution to the duodenal amino acid supply in ruminants, based upon *in vitro* fermentation, is proposed. Analyses of five grass varieties, maize and maize/grass mixtures by the proposed method, reveals that in all cases histidine is the limiting amino acid for milk production. Comparison of the milk production potential predicted from the duodenal amino acid supply with that predicted from digestibility values, suggests that amino acids might be limiting for milk production in the case of certain feedstuffs.

S. Afr. J. Anim. Sci. 1983, 13: 120 – 126

'n Metode om die eksogene bydrae tot die duodenale aminosuurverskaffing by herkouers wat op *in vitro* fermentasie gebaseer is te beraam, word voorgestel. Ontleding van vyf variëteite gras, mielie- en gras/mielie mengsels deur middel van dié metode, het aangetoon dat in alle gevalle histidien die beperkende aminosuur vir melkproduksie is. 'n Vergelyking van die melkproduksie potensiaal soos voorspel vanaf die duodenale aminosuurverskaffing met dié voorspel vanaf verteerbaarheidswaardes, het laat blyk dat aminosure dalk beperkend is vir melkproduksie in die geval van sekere voersoorte.

S.-Afr. Tydskr. Veek. 1983, 13: 120 – 126

Keywords: Amino acids, feeds, ruminants

C. Dennison* and Anne M. Phillips
Department of Biochemistry, University of Natal, P.O. Box 375, Pietermaritzburg 3200, Republic of South Africa

*To whom correspondence should be addressed

Received 25 May 1982

Introduction

In the nutrition of monogastric animals both the amino acid composition of the feed and the amounts of feed ingested contribute to the duodenal amino acid supply (DAAS), a nutritional parameter of recognized value (Scott, Nesheim & Young, 1976). In such cases, the DAAS (of exogenous origin) may be readily estimated from an analysis of the feed, together with a knowledge of the amounts of feed consumed.

In ruminants the ingested feed is modified by the action of the rumen microbes and a more or less modified feed passes through the duodenum. Nevertheless the DAAS is also recognized, in ruminants, as 'a critical point in the chain of metabolic events that link intake of food to output (of milk)' (Oldham & Tamminga, 1980) and, as those authors have noted, reliable techniques for measuring the DAAS in ruminants are needed to evaluate methods by which it may be advantageously altered.

The DAAS is most commonly measured, in ruminants, by direct sampling from live animals through duodenal cannulae (Armstrong, Savage & Harrison, 1977). This technique is expensive, as is usual with statistically valid trials using large animals, requires large amounts of feed for testing and is technically not simple. A simple means of estimating the DAAS in ruminants would therefore be advantageous.

By analogy with monogastric animals, the DAAS in ruminants could conceivably be estimated from an analysis of the feed and a knowledge of the feed intake, if the amino acid transformations effected by rumen fermentation of the feed were known. One way in which these transformations might be measured is by analysis of the products of fermentation *in vitro*. At first this might appear impracticable as the transformations *in vivo* might be considered too complex to be reproduced *in vitro*, except by a system able to mimic the *in vivo* situation closely. However, matters are greatly simplified by the essential constancy of the amino acid composition of total rumen microbial cell protein (Hungate, 1966; Purser, 1970; Alroy & Tannenbaum, 1977). Amino acid composition of the feedstuff, after digestion, would be expected to vary only between two extremes, i.e. its original composition (if it were not digested at all) and the composition of total microbial protein (if it were converted quantitatively to microbes). The extent of the conversion might reasonably be expected to be dependent upon the proteolytic activity of the rumen contents, but as this

does not vary greatly with diet (Blackburn & Hobson, 1960), this factor could be assumed to be invariant. Because of the constancy of the amino acid composition of total microbial protein, the amino acid composition of the feed after digestion would be independent of the dynamics of the microbial population effecting the digestion. It would be a function simply of (i) the composition and digestibility of the feed protein and (ii) the balance between nitrogen and energy in the feed; both of these factors being *intrinsic properties of the feed.*

The ratio of energy to nitrogen (protein + NPN) in the feed has a different effect upon the DAAS depending on which is in short supply (Swan, 1979).

(i) If the nitrogen is limiting: feed protein and NPN are converted to microbial nitrogen to an extent limited by the digestibility of the feed nitrogen sources (Figure 1a).

(ii) If the energy is limiting: digestible feed protein is sacrificed to the required extent for the liberation of energy and the surplus amino acid nitrogen is released as ammonia. This ammonia adds to the pre-existing pool of NPN which remains essentially unused (Figure 1b).

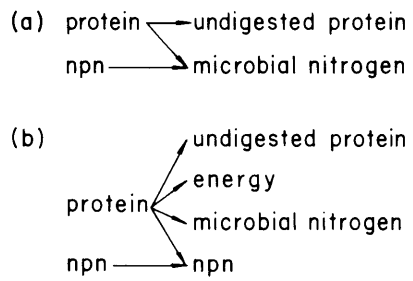

Figure 1 The fate of feed nitrogen in ruminants in the case of (a) a nitrogen limitation and (b) an energy limitation.

The fact that the factors which influence the DAAS are intrinsic properties of the feed, and are essentially independent of the microbial population dynamics, has the practical consequence that a simple batch *in vitro* system could be used to model the *in vivo* situation and measurement of the amino acid composition of the feed after such digestion *in vitro* could be used to predict the DAAS. The analogy between this proposed system of measuring the amino acid supply and the established method of measuring dry matter digestibility *in vitro*, should be underscored. Good correlations are obtained between digestibility values measured *in vivo* and those measured in simple batch *in vitro* systems (McLeod & Minson, 1976), despite differences in the microbial population dynamics in the two cases, because the measured value, digestibility, is again an intrinsic property of the feed.

A key consideration affecting the validity of the proposed method is that microbial protein should be synthesized with an equal energy efficiency *in vitro* and *in vivo*. This has been found to be true for bacterial protein in the case of forage diets but for concentrate diets the efficiency *in vitro* is slightly higher than that *in vivo* (Thomas & Rook, 1981).

In this communication the practicability of the proposed method of measuring the DAAS in ruminants is explored by measuring the amino acid transformations effected by the digestion *in vitro* of representative samples of forages and of maize and maize/forage mixtures. Estimates of the milk production which could be expected from a given feed are also presented.

Materials and Methods

Reagents

Artificial saliva buffer (Dennison & Marais, 1980): The artificial saliva buffer contained, per litre of solution; $NaHCO_3$ – 9,80 g; Na_2HPO_4 – 3,70 g; KCl – 0,57 g; NaCl – 0,47 g; $MgSO_4 \cdot 7H_2O$ – 0,12 g; $CaCl_2 \cdot 2H_2O$ – 0,053 g. The solution was titrated to pH 7,0 with CO_2 gas.

Procedure

Intact donor sheep were maintained on a daily diet consisting of 3 kg maize silage, 125 g maize meal and 500 g lucerne. Filtered rumen fluid was drawn from these sheep directly using a suction-strainer technique previously described (Dennison & Marias, 1980). The rumen fluid was diluted with four volumes of artificial saliva buffer at 39°C, and 50 cm^3 of the mixture was added to each of a series of bottles containing samples for digestion. The bottles used were screw-cap polythene bottles of known dry mass with a capacity of 100 cm^3. Each bottle contained a measured sample (0,5 g) of oven-dried grass, maize or maize/grass mixture of which each ingredient had been milled to pass a 1 mm screen. The solution in each bottle was swirled to suspend the sample and after flushing with CO_2 the bottle was sealed and incubated in a horizontal position at 39°C for 48 h, with intermittent gentle swirling. After 48 h, samples for amino acid analysis were transferred quantitatively into freeze-drying flasks and freeze dried. The dried samples were powdered with a mortar and pestle and analysed for amino acids as previously described (Dennison & Gous, 1980). Corresponding unfermented samples were analysed at the same time.

Samples for the assessment of digestibility were treated according to the method of Tilley & Terry (1963) for the determination of the dry matter digestibility.

The results of amino acid analysis are presented in two different ways, either as g (amino acid)/16 g N or as mol (amino acid)/100 mol (of amino acids). Each of these expressions has its drawbacks, however. The expression g (amino acid)/16 g N is too sensitive to the presence of NPN (other than ammonia) for it to be a useful indicator of the amino acid balance, although in a negative way it can indicate the presence of such NPN. Nevertheless this form of expression is most commonly used in the literature and thus comparison with published work is facilitated. A pitfall in the use of this expression should be pointed out. The expression g (amino acid)/16 g N appears to be based upon the false premise that the mass of the individual amino acids should total the mass of the protein (containing, on average, 16% N) from which they were liberated by hydrolysis. However, owing to the addition of water to each residue during hydrolysis, the liberated amino acids will total a mass about 16% greater than that of their parent protein. Summation of the amino acids in this way can give an erroneous impression of the recovery of amino acids. Consequently, in this communication, the recovery of Kjeldahl nitrogen as amino acid nitrogen is reported.

Expression of the results as mol/100 mol has the draw-

back that if one amino acid is elevated (in absolute terms), the apparent effect is that of depressing all the other amino acids, and vice versa. Nevertheless, this expression provides a useful measure of the amino acid balance which is not influenced by NPN.

Potentially the most useful expression is in terms of g (amino acid)/100 g of sample. In the experiments reported here, however, this expression is complicated by the fact that the samples, after fermentation, contain an unknown proportion of buffer salts. This is partly because certain components of the buffer are volatile and are to an indeterminate extent displaced from the solution by VFA produced by fermentation. However, the nitrogen content of each feedstuff may be used as a form of internal standard to overcome the problem of dilution of the fermented samples by salivary buffer salts, and thus to relate the amino acid composition after fermentation to the mass of feed before fermentation. This permits a calculation of the DAAS in terms of the amount of each amino acid which would be provided per 100 g or per kilogram of feed consumed. In this calculation a correction may be made for the nitrogen content of the rumen fluid, although this is relatively small and constant. In this study we have not attempted to measure the amino acids cyst(e)ine and tryptophan as we have not found a method which gives reliable values for these amino acids in feeds.

Results and Discussion

The results obtained for the amino acid analysis of five grass samples, maize and mixtures of maize and *Eragrostis curvula,* both before and after fermentation *in vitro* are presented in Figures 2 & 3 where the results are expressed as mol AA/100 mol.

A feature of these results is that the various feeds in general differ more before digestion than after; in each case the trend of the changes effected by digestion being towards some common point. The results for the fermented samples,

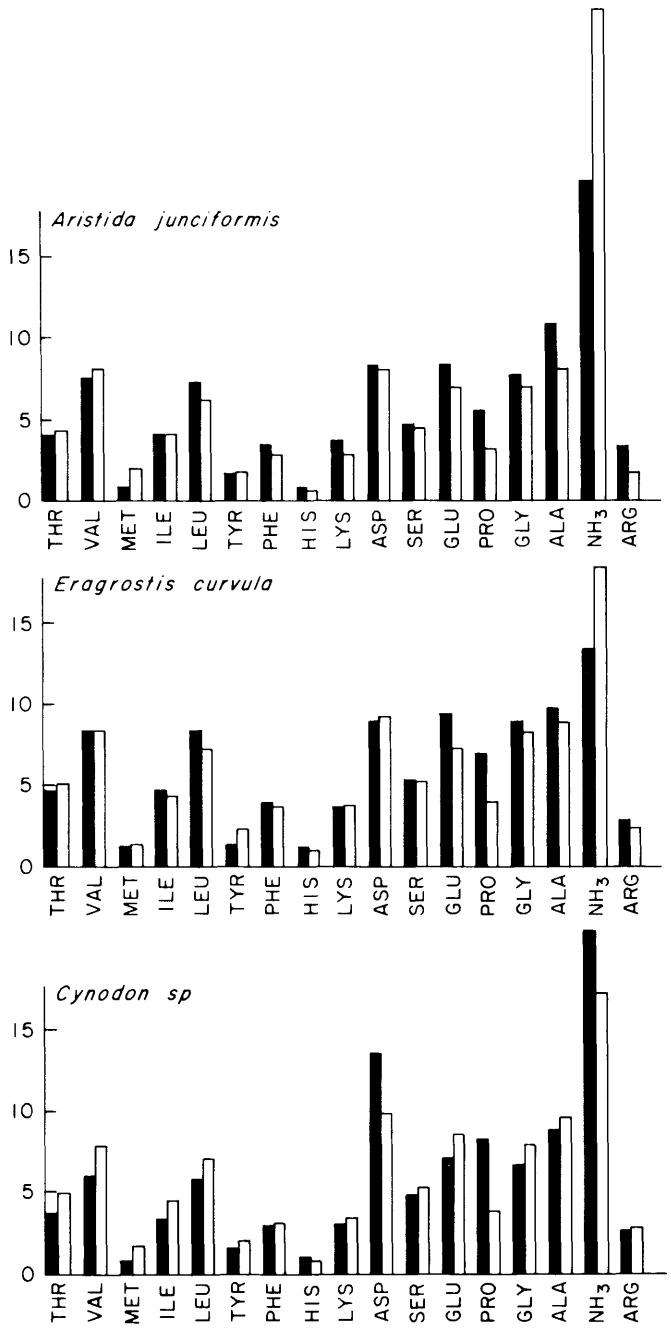

Figure 2 Amino acids in feeds (mol AA/100 mol); ■ before fermentation, □ after fermentation.

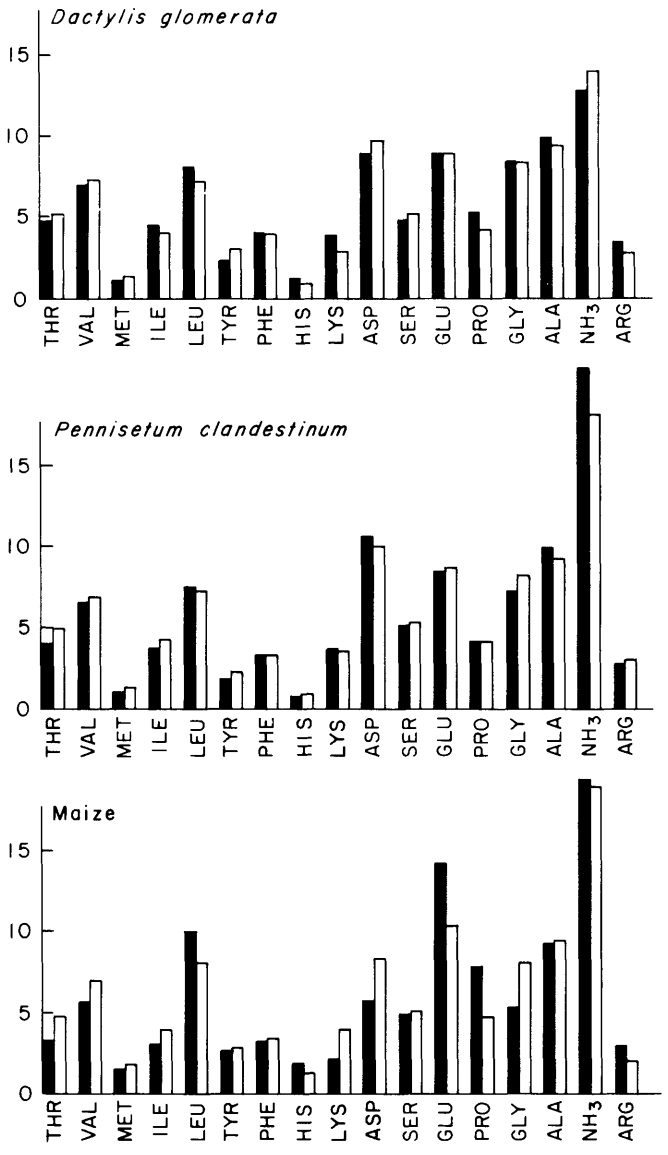

Figure 3 Amino acids in feeds (mol AA/100 mol); ■ before fermentation, □ after fermentation.

expressed as a percentage of the corresponding amino acid in bacterial protein (Table 1), indicate that for most amino acids fermentation effects a change towards the composition of microbial protein. This is consistent with the established theory that feed protein is degraded and resynthesized into microbial protein (Figure 1). However, exceptions to the general trend are provided by the amino acids valine and serine, where an already sufficient level is further increased by fermentation, and histidine where a generally deficient level is further reduced by fermentation. No consistent trend could be observed in the case of lysine and arginine.

Analysis of the feed after fermentation provides a means of measuring the maximum extent of microbial protein synthesis. Comparison of the amino acid composition of the fermented feed with that of bacterial protein (Table 1) reveals that lysine is the limiting amino acid in most cases. The level of this limiting amino acid therefore places an upper limit on the extent to which bacterial protein could have been produced from each of the feeds.

A comparison of the balance of amino acids in all ingredients, after fermentation, with that of milk protein amino acids (Table 2) shows that for milk production histidine is the most limiting essential amino acid followed by lysine.

Table 1 Amino acids in samples before and after fermentation as a percentage of amino acids in bacterial protein[a]

	A. junciformis		*Cynodon sp*		*D. glomerata*		*P. clandestinum*		*E. curvula*		Maize	
	before ferm.	after ferm.	before ferm.	after ferm.	before ferm.	after ferm.	before ferm.	after ferm.	before ferm.	after ferm.	before ferm.	after ferm.
THR	82,2	101,7	81,0	102,7	91,5	104,9	83,1	102,5	89,2	102,5	64,9	97,6
VAL	125,0	155,1	104,9	131,1	111,4	119,4	114,7	114,0	131,5	141,3	91,9	115,7
MET	46,7	113,3	39,2	82,9	51,7	66,3	55,0	67,9	56,7	62,5	73,8	85,0
ILE	79,0	93,7	70,8	91,7	88,1	84,4	77,6	84,9	89,0	90,7	59,8	82,7
LEU	115,3	114,4	96,9	111,9	124,4	114,8	124,0	118,1	128,3	117,3	163,7	127,5
TYR	57,1	72,1	57,1	78,5	77,1	105,6	64,7	80,0	41,5	85,0	93,2	100,9
PHE	107,4	100,3	97,7	100,0	122,9	122,1	108,9	106,1	115,3	118,2	100,5	103,7
HIS	81,3	62,6	78,8	65,6	85,0	73,5	61,3	73,8	76,9	73,1	119,4	80,6
LYS	67,0	61,2	57,4	63,7	67,8	53,1	70,2	66,6	62,5	68,5	36,5	73,5
ASP	89,5	102,6	153,9	107,8	92,7	104,6	119,6	104,4	93,0	103,1	62,6	91,7
SER	121,1	138,7	135,6	140,0	124,9	136,2	142,4	143,6	134,0	139,8	132,7	134,4
GLU	85,7	84,3	77,7	88,7	90,4	91,7	91,3	91,4	83,8	77,0	150,3	108,2
PRO	159,0	107,8	248,3	112,2	147,1	119,0	124,1	119,8	191,0	119,5	230,7	139,3
GLY	91,9	98,7	83,3	95,7	99,3	100,7	90,4	100,1	101,9	101,9	66,3	97,8
ALA	122,1	108,3	106,1	108,7	110,5	106,5	116,4	105,5	105,2	103,1	106,1	107,7
ARG	93,3	53,6	75,2	77,6	96,9	76,4	81,4	87,6	74,8	67,1	82,6	56,7

[a] $\frac{\text{mol \% AA in sample}}{\text{mol \% AA in bact. pr.}} \times 100$

Table 2 Amino acids in fermented samples as a percentage of amino acids in milk protein[a]

										Bacterial protein	
	Aristida[b]	Coast cross[c]	Cock's foot[d]	Kikuyu[e]	*Eragrostis*[f]	75% E[f] 25% M[g]	50% E 50% M	25% E 75% M	Maize	Kaufmann (1979)	Weller (1957)
THR	122,4	123,7	126,4	123,4	123,4	122,3	119,4	119,3	117,6	106,1	120,3
VAL	161,0	136,0	123,9	118,3	146,6	137,0	125,9	123,1	120,0	93,7	103,7
MET	119,8	87,7	70,0	71,7	65,9	66,5	70,5	77,4	89,8	110,1	105,7
ILE	112,4	110,0	101,2	101,7	108,8	103,9	103,3	99,0	99,2	119,9	119,9
LEU	89,4	87,4	89,7	92,2	91,7	94,0	96,5	99,5	99,5	80,2	78,1
TYR	66,9	73,0	98,1	74,4	79,0	78,1	79,5	84,8	93,6	98,4	92,9
PHE	97,4	97,2	118,7	103,1	114,7	106,4	104,4	99,9	100,9	110,0	97,2
HIS	47,6	48,8	46,5	54,8	54,6	49,3	49,3	60,9	60,0	74,4	74,4
LYS	57,3	59,7	49,7	62,3	64,2	63,8	62,4	66,6	68,9	112,4	93,7
ASP	151,5	159,2	154,5	154,1	152,1	149,5	142,6	141,7	135,4	166,4	147,7
SER	89,9	90,8	88,3	93,2	90,6	91,2	89,3	91,0	87,1	73,5	64,8
GLU	51,3	54,0	55,8	55,6	46,9	52,5	60,1	62,6	65,7	66,7	60,8
PRO	41,2	42,9	45,5	45,8	45,6	50,4	54,5	55,6	53,2	37,3	38,2
GLY	309,2	299,7	315,5	313,7	319,2	30,6	282,9	267,2	306,0	284,8	313,3
ALA	231,1	231,9	227,2	235,2	220,1	223,2	229,9	229,2	229,8	172,8	213,4
ARG	88,9	128,9	126,9	145,5	111,4	82,9	109,5	98,2	94,2	134,4	166,0

[a] $\frac{\text{mol \% AA in ferm. samples}}{\text{mol \% AA in milk protein}} \times 100$

[b] *A. junciformis* [c] *Cynodon sp* [d] *D. glomerata* [e] *P. clandestinum* [f] *E. curvula* [g] Maize

However, the protein in the feed after digestion consists of microbial protein and undegraded feed protein (plus in an *in vivo*, endogenous component which will be ignored here). If the undegraded feed protein is intrinsically undigestible and therefore unavailable to the animal as well as to the microbes, the only protein available to the animal will be microbial protein. In this case the limiting amino acid for milk production is also histidine (Table 2).

The results of amino acid analysis of mixtures of maize and *E. curvula,* expressed as mol amino acid/100 mol are presented in Figure 4. Again, the difference in amino acid composition of all of the mixtures was greater before fermentation than after. As has been argued above, this is probably due to the synthesis of bacterial protein, of essentially constant composition, from the different feed proteins. However, the results also suggest that separate feed ingredients might be digested essentially independently, as the results obtained for the mixtures after fermentation are no different from the results predicted by simple proportion from the results of the separate ingredients.

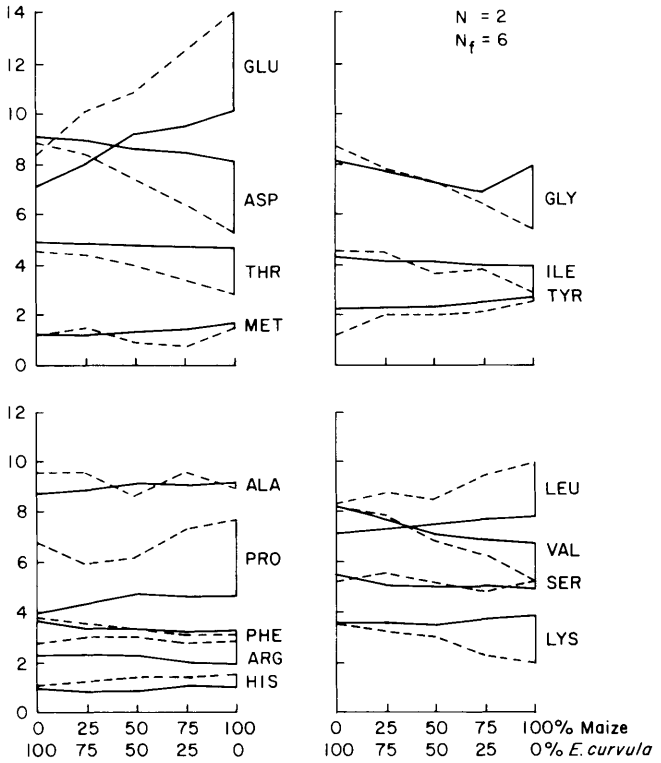

Figure 4 Amino acids (mol AA/100 mol) in 5 combinations of *E. curvula* and Maize ..... before fermentation _____ after fermentation. N = number of replicates analysed before fermentation. N_f = number of replicates analysed after fermentation.

Table 3 Kjeldahl N of feedstuffs recovered as amino acid nitrogen (g amino acid N/100 g of Kjeldahl N)

Feedstuff	Before fermentation	After fermentation
A. junciformis	65,3	83,6
Cynodon sp	57,5	96,1
D. glomerata	64,8	79,4
P. clandestinum	51,3	79,5
E. curvula (E)	65,4	88,8
75% E + 25% M	70,5	84,9
50% E + 50% M	77,8	85,7
25% E + 75% M	84,9	97,4
Maize (M)	79,5	102,4

The results of amino acid analysis of the different feed ingredients expressed as g amino acid/16 g N are presented in Figure 5. These results indicate a general increase in the levels of most amino acids upon fermentation. This means that after fermentation a greater proportion of the nitrogen is recovered as amino acids (Table 3). These results could indicate that amino acids are synthesized from NPN, which is not unexpected (Figure 1), but they could also simply reflect an analytical difficulty in recovering amino acids from forage proteins. Similar low recoveries of nitrogen as amino acid nitrogen have been reported for forages by Fishman & Evans (1978) and Hodgson (1964).

The amino acids which would be supplied per kilogram of feed after fermentation are presented in Table 4. Milk production from this supply of amino acids depends upon the manner and extent to which the protein is digested and absorbed by the animal. If the fermented feed is digested as a whole, with no change in the proportions of the amino acids, the protein production might reasonably be expected to be limited by the supply of histidine; the limiting amino

Table 4 Amino acid (g) supplied after fermentation by 1 kg of feed

	A. junciformis	*Cynodon sp*	*D. glomerata*	*P. clandestinum*	*E. curvula*	Maize
THR	2,25	8,58	7,43	9,64	6,20	5,53
VAL	4,06	13,11	10,26	12,91	10,10	7,69
MET	1,20	3,49	2,35	3,25	1,90	2,52
ILE	2,25	8,41	6,64	8,80	6,05	5,18
LEU	3,52	13,05	11,46	15,56	9,98	10,24
TYR	1,42	5,79	6,81	6,62	4,46	5,10
PHE	1,98	7,47	7,87	8,92	6,36	5,33
HIS	0,58	1,93	1,52	2,45	1,49	1,74
LYS	1,89	7,21	5,00	8,47	5,46	5,35
ASP	4,74	18,77	15,54	20,46	13,07	10,61
SER	2,06	7,86	6,52	9,11	5,67	5,17
GLU	4,46	17,83	15,74	20,72	11,37	14,81
PRO	1,58	6,29	5,70	7,59	4,72	5,37
GLY	2,32	8,45	7,61	9,97	6,50	4,83
ALA	3,17	12,06	10,13	13,22	8,29	8,05
ARG	1,27	6,76	5,58	8,61	4,13	3,17

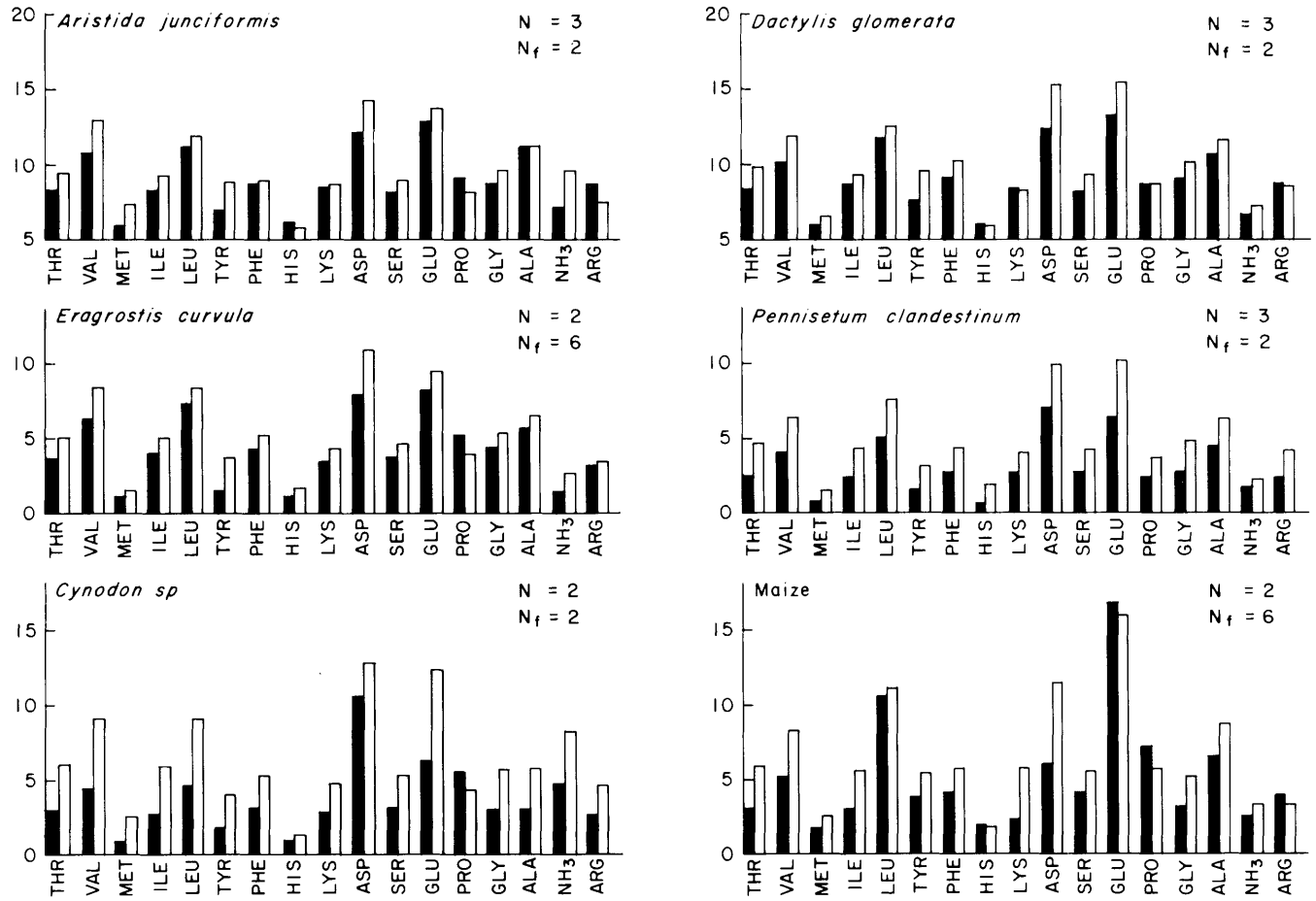

Figure 5 Amino acids in feeds (g AA/16 g N); ■ before fermentation, □ after fermentation. N = number of replicates analysed before fermentation. N_f = number of replicates analysed after fermentation.

acid in the fermented feedstuff (Table 2). On the other hand if the undegraded portion of the fermented feedstuff is intrinsically undigestible and therefore unavailable to the animal, only the microbial amino acids might be available to it. An upper limit to the microbial protein level is provided by the level of the limiting amino acid in the fermented feed, lysine being the most common limiting amino acid in this regard (Table 1). The milk protein production from this supply of microbial protein would again be limited by histidine (Table 2) but in this case by the histidine level in the microbial protein.

In either of the above cases, however, the exact amount of milk which would be produced is difficult to estimate accurately as the absorbed amino acids enter an amino acid pool to which reserve protein might also contribute and from which amino acids are drawn for both maintenance and production in a proportion determined by the plane of nutrition. Nevertheless by using the not implausible assumptions that the available amino acids are absorbed to the extent of 75% (Kaufmann, 1979) and that the amino acids must be supplied in the blood in a 50% excess over their secretion in milk (Tamminga & Oldham, 1980), it is possible to estimate the milk protein production possible from the DAAS, given the distribution of amino acids between maintenance and production. The theoretical upper limit of milk protein production may be estimated by considering the case when all the amino acids are channelled to production and none to maintenance.

The results of such an estimation of the milk protein and milk production from the amino acid supply is compared with an independent estimate, based upon digestibility (Bredon & Stewart, 1979), in Table 5. This comparison suggests that *P. clandestinum* and *E. curvula* provide a deficiency of energy relative to amino acids, while coast cross grass *(Cynodon sp.), D. glomerata* and especially maize provide a deficiency of amino acids relative to energy, for the middle-range dairy cow considered. These imbalances can nevertheless be overcome by an appropriate mixture of ingredients, for example *E. curvula* and maize (Table 5). Bearing in mind that the estimates of the amino acids provided are very likely over-estimates, as the upper limit condition has been invoked at different stages in the estimate, it would appear that amino acid deficiencies might not be unusual in dairy cattle. This is at odds with the conclusion reached by Hogan (1974) based on different considerations, but in general terms our results do not appear to conflict with the empirical observations upon which the established practice of dairy-cow feeding is based.

The method of estimating the DAAS which is put forward in this paper cannot account for differences in the rate of passage of digesta, especially in the case of high intakes of feeds containing milled concentrates where the rate of passage is not directly linked to the rate of digestion. In such cases the proposed method would tend to over-estimate the conversion of feed protein to microbial protein. This limitation does not apply significantly to forages, however, and

Table 5 Milk production from 1 kg of feed: (I) if total AAs available for absorption (II) if only bacterial protein AAs available for absorption (III) predicted from energy content

I	*A. juncifformis*	*Cynodon sp*	*D. glomerata*	*P. clandestinum*	*E. curvula*	75% E 25% M	50% E 50% M	25% E 75% M	Maize
HIS (g) (Table 4)	0,58	1,93	1,52	2,45	1,49	1,36	1,21	1,54	1,74
Milk protein (g)[a]	9,3	39,7	31,3	50,4	30,6	28,0	24,9	31,7	35,8
Milk (l)[b]	0,3 – 0,4	1,1 – 1,6	0,9 – 1,3	1,4 – 2,0	0,9 – 1,2	0,8 – 1,1	0,7 – 1,0	0,9 – 1,3	1,0 – 1,4
II									
LYS (g) (Table 4)	1,89	7,21	5,00	8,47	5,46	5,04	4,69	5,21	5,35
Bacterial protein (g)	21,7	82,9	57,5	97,4	62,8	57,9	53,9	60,0	61,5
Bacterial HIS	0,41	1,57	1,09	1,85	1,19	1,1	1,0	1,1	1,17
Milk protein (g)[a]	7,6	29,2	20,3	34,4	22,1	20,5	18,6	20,5	21,8
Milk (l)[b]	0,2 – 0,3	0,8 – 1,2	0,6 – 0,8	1,0 – 1,4	0,6 – 0,9	0,6 – 0,8	0,5 – 0,7	0,6 – 0,8	0,6 – 0,9
III									
Digestible dry matter (g)	286	675	637	588	453	587	656	715	795
Milk (l)[c]	–	1,2	1,1	0,8	0,4	0,8	1,1	1,4	1,6

[a] Calculated from HIS value (Table 4) and milk protein composition (Bigwood, 1963) assuming 75% absorption from intestine and 65% incorporation into milk protein by mammary gland (Oldham & Tamminga, 1980)

[b] Assuming milk protein content ranges from 2,5% (at high yield) to 3,5% (at low yield) (Kaufmann, 1979)

[c] Predicted from digestibility values, assuming 500 kg cow, 3,5% butter fat (Bredon & Stewart, 1979)

with the advantages of simplicity and the small amounts of sample required, the *in vitro* method might prove useful in the evaluation and improvement of forages.

Acknowledgements

We wish to thank Mrs E.M. Stephenson for her invaluable technical assistance in this project. The work was supported by financial grants from the Department of Agriculture and the University of Natal Research Fund.

References

ALROY, Y. & TANNENBAUM, S.R., 1977. Considerations in predicting phenotypic modifications in amino acid profiles of total cell micro-organisms. *Biotechnol. Bioeng.* 19, 1145 – 1153.

ARMSTRONG, D.G., SAVAGE, G.P. & HARRISON, D.G., 1977. Digestion of nitrogenous substances entering the small intestine with particular reference to amino acids in ruminant stock. *Proc. 2nd Int. Symp. on Protein Metabolism & Nutrition,* p55 – 60.

BIGWOOD, E.J., 1963. Nitrogenous constituents and the nutritive value of human and cow's milk colostrum. *World Rev. Nutr. and Dietetics* 4, 97 – 116.

BLACKBURN, T.H. & HOBSON, P.N., 1960. Proteolysis in the sheep rumen by whole and fractionated rumen contents. *J. Gen. Microbiol.* 22, 272 – 281.

BREDON, R.M. & STEWART, P.G., 1979. In: Feeding and Management of Dairy Cattle in Natal. Part 1, Rev. Natal Region, Dept of A.T.S., Pietermaritzburg, p.155.

DENNISON, C. & GOUS, R.M., 1980. The amino acid composition of selected South African feed ingredients. *S. Afr. J. Anim. Sci.* 10, 9 – 17.

DENNISON, C. & MARAIS, J.P., 1980. The influence of ruminant salivary buffer salts upon the *in vitro* microbial digestion of forages. *Agroanimalia,* 12, 1 – 6.

FISHMAN, M.L. & EVANS, J.J., 1978. Amino acid composition of Coastal Bermuda grass. *J. Agric. Food Chem.* 26, 1447 – 1451.

HODGSON, H.C., 1964. The protein amino acid composition and nitrogen distribution in two tropical grasses. *J. Sci. Fd Agric.* 15, 721 – 724.

HOGAN, J.P., 1974. Quantitative aspect of nitrogen utilization in ruminants. *J. Dairy Sci.* 58, 1164 – 1177.

HUNGATE, R.E., 1966. The Rumen and its Microbes. Academic Press, New York, p.312.

KAUFMANN, W., 1979. In: Feeding Strategy for the High Yielding Dairy Cow. Eds. Broster, W.H. & Swan, H. Granada Publishing. London, p.90 – 113.

McLEOD, M.N. & MINSON, D.J., 1976. The analytical and biological accuracy of estimating the dry matter digestibility of different legume species. *Anim. Fd Sci. Technol.* 1, 61 – 72.

OLDHAM, J.D. & TAMMINGA, S., 1980. Amino acid utilization by dairy cows. I. Methods of varying amino acid supply. *Livest. Prod. Sci.* 7, 437 – 452.

PURSER, D.B., 1970. Nitrogen metabolism in the rumen: micro-organisms as a source of protein for the ruminant animal. *J. Anim. Sci.* 30, 988 – 1001.

SCOTT, M.L., NESHEIM, M.C. & YOUNG, R.J., 1976. Nutrition of the chicken. 2nd edn, M.L. Scott & Associates, Ithaca, New York, p.92.

SWAN, H., 1979. In: Feeding Strategy for the High Yielding Dairy Cow. Eds. Broster, W.H. & Swan, H., Granada Publishing, London, p.49 – 67.

TAMMINGA, S. & OLDHAM, J.D., 1980. Amino acid utilization by dairy cows. II. Concept of amino acid requirements. *Livest. Prod. Sci.* 7, 453 – 463.

THOMAS, P.C. & ROOK, J.A.F., 1981. In: Recent Developments in Ruminant Nutrition. Eds. Haresign, W. & Cole, D.J.A., Butterworths, London, p.157 – 183.

TILLEY, J.M.A. & TERRY, R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Brit. Grassld Soc,* 18, 104 – 111.

WELLER, R.A., 1957. The amino acid composition of hydrolysates of microbial preparations from the rumen of sheep. *Austr. J. Biol. Sci.* 10, 384 – 389.