PLASMA AND URINARY METABOLITES AS INDICES OF N UTILIZATION IN SHEEP

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SUMMARY

Concepts underlying possible application of metabolite concentration in plasma and urine as indices of N utilization are discussed. Specific aspects of N utilization by sheep are considered, with special emphasis upon an empirical relationship observed between the concentrations of glycine and all other amino acids in jugular vein plasma (G/OAA ratio) as an index of the amount of protein presented for digestion in the intestines or as an index of the retention of N by the growing sheep. Over a wide range of dietary conditions extending from hay with or without casein supplements to frozen and fresh herbage diets, log G/OAA was correlated with the amount of α-amino N entering the duodenum ($r = -0.88^{***}$; $r^2 = 0.78$) and with N retention ($r = -0.74^{***}$; $r^2 = 0.55$). High G/OAA ratios (> 0.5) were observed with diets where low amounts (< 10 g) of α-amino N were presented for digestion in the intestines. When G/OAA ratio was greater than 0.4, no group of animals in any experiment was in positive N balance. Other possible indices of the amount of protein presented for digestion in the intestine on a range of natural diets are examined, and none of these shows a closer correlation than G/OAA ratio.

MANY TECHNIQUES have been used in attempts to establish the protein requirements of animals, and to assess the ability of specific diets or supplements to meet those requirements. These have been reviewed by McLaughlan and Campbell (1969) and by Waterlow (1969) with particular reference to non-ruminants. Each approach has specific advantages and disadvantages, but collectively they provide an environment of knowledge of nutritional status within which potential diagnostic aids or ancillary tests for protein adequacy are being examined.

The chemical scoring techniques, matching amino acid composition of dietary proteins with known amino acid requirements of the animal, have been useful in pre-

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liminary screening of food proteins for potential biological value for non-ruminants. Direct application of such chemical analysis of feed proteins for ruminants is not possible because the amount and the nature of the protein digested by the animal after microbial intervention in the rumen are not simply related to dietary protein supply. Further, the ruminant's amino acid requirements are simply not known.

Measurement of the concentration of metabolites in blood and urine is finding some application in providing indices of the state of protein nutrition, and of the direction and rate of change of protein status of the non-ruminant animal (*e.g.*, Waterlow, 1969). Whether any of these metabolites provide an accurate quantitative index of a specific aspect of amino acid utilization and can complement or replace the more tedious and longer term N balance or weight gain methods of dietary protein evaluation are subjects which warrant continued attention. All such proposed methods are based upon the idea that changes in concentration of chosen metabolites uniquely reflect some specific change in metabolic patterns and will permit evaluation of the contribution of a particular metabolic process to the overall pattern of N utilization. Empirical correlations of metabolite concentrations with N retention or various aspects of N metabolism have yielded some promise of rapid prediction of efficiency of protein utilization in non-ruminants. In ruminants, the pattern of N utilization is so dependent upon "hidden" processes in the rumen, that the need for external indices which correlate with efficiency of N utilization is even greater than in non-ruminants. Recent elaborate quantitative studies have provided knowledge on the amount and nature of protein reaching the duodenum, on factors affecting rate of ammonia production in the rumen and the extent of ammonia loss from the rumen, and the extent of N recycling to the rumen and other parts of the gastro-intestinal tract. With certain diets fed under specified experimental conditions, it has been possible to construct flow diagrams, or models of major N transactions within the ruminant (*e.g.*, Nolan and Leng, 1972). The identification of the sources of greatest variability in N utilization, and of the dietary and physiological factors which influence variation should now be possible. The concept of systematic indices which will reflect particular processes of N utilization is attractive. Such indices must not only provide rapid overall evaluation of the diet's ability to sup-

port N retention at a given time, but also yield information on the processes most affecting the efficiency of N utilization.

Level of intake of total N, extent of loss of ammonia or gain of recycled N across the rumen, amounts of amino acid N reaching the intestine, the extent of digestion of dietary and microbial proteins in the intestine, and the level of urinary urea output (made up largely of losses of ammonia from ruminal fermentation plus catabolism of amino acids) are the primary parameters of N utilization. From the studies of Egan and Kellaway (1971) it is clear that separate evaluation of these variables is desirable. Attempts to predict directly N retention or the *efficiency* of N retention have generally proved much less effective and, even if successfully applied, could obscure the basis on which that efficiency was developed. For example, in studies on concentrations of urea in plasma, and on the relationship between urea and total N or urea and creatinine in the urine of young sheep (Egan and Bourke, unpubl.) it was not possible to distinguish between the contrasting conditions of (a) inefficient use of N ingested at a low level and (b) relatively efficient use of N ingested at a much higher level, without independent information on N intake, ruminal ammonia losses, or the amount of protein presented at the intestine for digestion.

In this paper are presented data on correlation of specific metabolite concentrations or ratios of the concentrations of two metabolites, with protein reaching the duodenum and with N retention. Data have been drawn from experiments with young sheep fed a range of diets, during which were measured the intake, digestibility and retention of N, the amounts of amino acid N presented for digestion in the small intestine, the concentrations of free amino acids and urea in jugular vein plasma and concentrations and total daily outputs in the urine of urea, creatinine, allantoin, uric acid and hippuric acid (Egan, 1972).

EXPERIMENTAL

ANIMALS

Merino wethers 8 to 12 months of age at the commencement of the respective experiments were prepared with a ruminal cannula. Seven animals used in Exp. 1 and 2 also each had a duodenal re-entrant cannula placed 6 cm from the pylorus. Each animal was held in a metabolism crate and trained to all experimental procedures over a period

of 3 to 4 weeks. They were accustomed to restraint in a sling during periods of collection of duodenal digesta, and were fed hourly from an automatic feeder. All animals received weekly a supplement of vitamins A and D.

EXPERIMENT 1

Three sheep were fed each of eight diets in turn, ranging from poor quality wheaten hay (0.6% N) to barn-cured strawberry clover (3.4% N). Diets are described more completely elsewhere (Egan, 1972), and were provided at approximately 90% of *ad libitum*, in 24 equal hourly allowances each day. After 14 days prefeeding, urine and faeces were collected for a 7-day period. On the 4th day of collection, infusion of marker (EDTA-Cr) into the rumen was commenced, diluted by volume to the daily average water intake of the sheep over the preceding prefeeding period. The infusion of marker continued for 7 days, on the last 3 of which duodenal flow was measured and samples taken using a semi-automated collection and return system by which a gush of warmed stored digesta was returned to the distal duodenal cannula simultaneously with each gush of digesta from the proximal cannula. Actual flow measurements made on each day were compared and corrections made on the basis of recovery of EDTA Cr; where corrections were greater than 10%, measurements on that animal were repeated.

Blood samples were drawn from the external jugular vein on the last day of the sampling period, centrifuged immediately and 5 ml of plasma deproteinized by additions of 1.5 ml 20% trichloroacetic acid. The supernatant after centrifugation was analysed for amino acids by automated column chromatography (Technicon).

EXPERIMENT 2

Four sheep were placed together in the succession shown below, on one of three dietary regimes: wheaten straw offered to provide for a maintenance energy requirement (low protein, low level, LPL); wheaten straw supplemented with 10% casein sprayed on or in solution and dried in an oven at 85° C, offered to provide an equivalent intake of digestible energy (High protein, low level, HPL); and wheaten straw plus 10% casein at twice that level of intake (high protein, high level, HPH).

The diets were offered in a set sequence to all sheep to allow continuous monitoring of changes in N utilization in a series of nutritional changes:

$$\text{HPL} \rightarrow \text{HPH} \rightarrow \text{HPL} \rightarrow \text{LPL} \rightarrow \text{HPH} \rightarrow \text{LPL} \rightarrow \text{HPL}$$

Methods and measurements were as in Exp. 1.

EXPERIMENT 3

Seven sheep were allocated according to a latin square design to each of seven treatments:

Control: wheaten straw, 0.42% N (*ad lib.*)

and equivalent amounts of wheaten straw, supplemented as follows:

N5R	5 g casein N infused over 24 hr per rumen
N5D	5 g casein N infused over 24 hr per duodenum
N10R	10 g casein N infused over 24 hr per rumen
N10D	10 g casein N infused over 24 hr per duodenum
N10RE	10 g casein N infused over 24 hr per rumen plus 10% (dry weight) of molasses with the diet.
N10DE	10 g casein N infused over 24 hr per duodenum, also with 10% molasses added to the diet.

Measurements were made as for Exp. 1 and 2, except that duodenal digesta flow measurements were not made.

RESULTS AND DISCUSSION

Results of correlation analysis of data from Exp. 1 are shown in Table 1 as r^2 values. Variability in plasma urea concentration under the "continuous" feeding conditions with herbage diets could be associated with: 72% of variability in urinary N loss, but less than 60% of variability in N intake; in N loss across the reticulo-rumen; in α-amino N reaching the duodenum; or in N retention. Variability in ruminal ammonia concentration was associated with 63% of the variability in N loss across the rumen, with 69% of variability in urinary N loss, but with only 38% of variability in α-amino N at the duodenum and 29% of variability in N retention. For some of these parameters, the same types of correlation analyses have been presented for separate experiments (Egan and Kellaway, 1971) and r^2 values are similar though experimental conditions are dissimilar. Of all the individual free plasma amino acids, the concentration of glycine was most closely correlated ($r = -0.84$, $r^2 = 0.72$) with the amount of α-amino N reaching the duodenum. It is not intended to discuss further the relationships between other individual amino acids or groups of amino acids here. When glycine concentration was expressed as a ratio relative to all other

TABLE 1: CORRELATIONS (r^2 values) OF VARIABLES FOR HERBAGE DIETS FED IN EQUAL HOURLY ALLOWANCES (Data from Exp. 1)

Variables	*N Intake*	*N Loss across Rumen*	*α-amino N at Duodenum*	*Urinary N Loss*	*N Retention*
Concentrations:					
Plasma urea	0.59	0.53	0.58	0.72	0.56
Ruminal ammonia	0.63	0.72	0.38	0.69	0.29
Plasma glycine	—	—	0.72	—	0.59
Outputs:					
Urinary urea	0.82	—	0.58	0.84	0.63
Urinary uric acid + allantoin	0.59	—	0.68	0.40	0.54
Ratios:					
Glycine/other amino acids (log G/OAA)	0.64	—	0.78	—	0.55
Urinary uric acid + allantoin / urea	—	—	0.46*	—	—
	—	—	0.35†	—	—
Urinary uric acid + allantoin / creatinine	—	—	0.56*	—	—
	—	—	0.41†	—	—
Urinary urea / creatinine	0.65*	0.49*	0.52*	0.64*	—
	0.52†	0.37†	0.40†	0.36†	—
Urinary urea / total N	0.70*	0.62*	0.56*	0.73*	—
	0.63†	0.60†	0.35†	0.50†	—

*Bulked urine.
†Single micturition sample.

amino acids (G/OAA ratio) the logarithm of that ratio was highly correlated with the amount of α-amino N reaching the duodenum ($r = -0.88$, $r^2 = 0.78$).

Similar relationships between glycine and other groups of amino acids, and the amounts of protein reaching the intestines have been demonstated (Hogan *et al.*, 1968; Reis, 1970). Data from Exp. 2 (Table 2) derived under quite dissimilar experimental conditions and also data derived from that presented by Hogan *et al.* (1968) were examined similarly. The values derived from Hogan *et al.* were calculated assuming total plasma amino acid concentration to be the sum of all given amino acids plus a

TABLE 2: THE INTAKE OF APPARENTLY DIGESTIBLE ENERGY, AND THE INTAKE, DIGESTION, EXCRETION AND RETENTION OF N BY SHEEP ON EACH OF SEVEN SUCCESSIVE DIETARY TREATMENTS, AND THE RATIO OF GLYCINE TO ALL OTHER AMINO ACIDS IN THE JUGULAR PLASMA

Treatment	*HPL*	*HPH*	*HPL*	*LPL*	*HPH*	*LPL*	*HPL*
Intake:							
App. dig. E intake (kcal/day)	1411	2893	1402	1365	2829	1355	1296
N Intake (g/day)	10.0	20.0	9.9	4.3	20.2	4.3	9.8
App. dig. N intake (g/day)	8.5	17.3	8.3	1.3	17.5	1.7	8.3
Amino acid N digested (g/day)	6.0b	13.8c	6.3b	3.4a	14.3c	3.2a	6.4b
Percentage of app. dig. E derived from amino acids	22.9b	24.3b	22.8b	11.7a	23.7b	12.0a	21.9b
Urinary N (g/day)	8.2b	11.1c	8.8b	3.0a	9.8bc	4.2a	7.9b
N Retention (g/day)	+ 0.3b	+ 6.2c	− 0.5ab	− 1.7a	+ 7.7c	− 2.5a	+ 0.4b
Glycine/other amino acids	0.51	0.27	0.47	0.73	0.20	1.22	0.57

Note: Data sharing the same letters are not significantly different. $P < 0.05$.

constant of 8% to account for amino acids for which results were not reported in that paper. Collectively, all data (shown in Fig. 1 as the untransformed values) were correlated so that variability in log G/OAA ratio was associated with 76% of the variability in the amount of α-amino N reaching the duodenum. The conditions of the experiments and the nature of the protein reaching the duodenum in the various experiments undoubtedly varied considerably. The energy intakes and efficiency of utilization of N varied greatly in Exp. 1 and 2 (*e.g.*, Table 2 for Exp. 2). High G/OAA ratio (> 0.5) were observed with diets yielding less than 10 g of α-amino N at the duodenum, and reached a maximum of 1.2 (*i.e.*, glycine constituted more than half of all free plasma amino acids) when α-amino N yield at the duodenum was only 3.2 g/day. However, as the amount of α-amino N reaching the duodenum rose above 12 g/day sensitivity of the ratio was greatly decreased (see Fig. 1). Though the approach has thus far been empirical, the data suggest that further exploration of the G/OAA ratio is warranted. The patterns of meta-

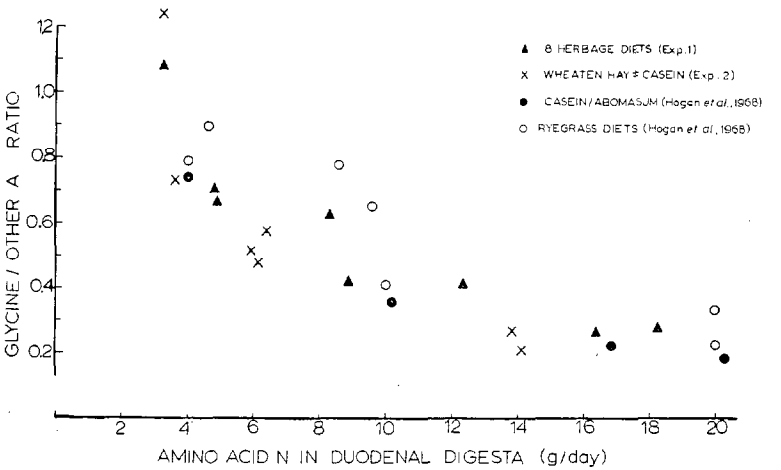

FIG. 1: *Relationship between amino acid N in duodenal digesta (g/day) and the ratio between free plasma glycine and all other amino acids (untransformed). (For log of ratio,* $r^2 = 0.76$.)

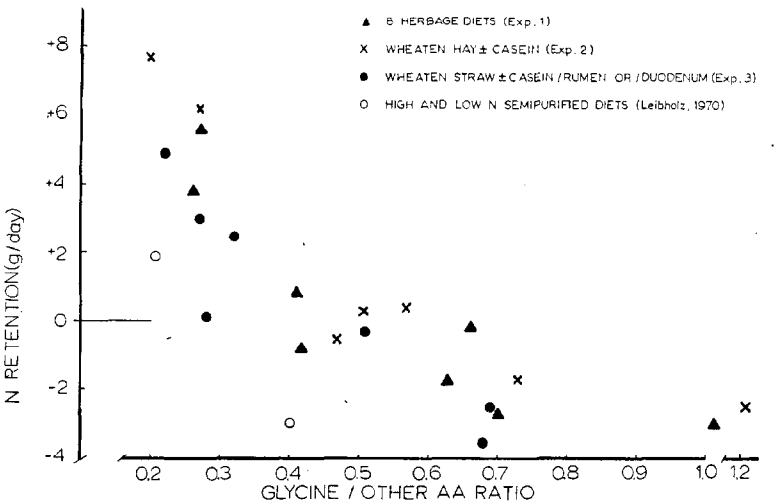

FIG. 2: *Relationship between the ratio of free plasma glycine and all other amino acids, and N retention (untransformed). (For log of ratio,* $r^2 = 0.56$.)

bolism of glycine with differing availabilities of protein and energy are a necessary basis for such studies. The high glycine concentration in plasma is paralleled by high concentrations in tissues; glycine constitutes 30% of the free amino acid in the tissues, and of all cellular free glycine 80% is present in muscle tissue (Christensen *et al.*, 1948). Whether with low amino acid uptake from the intestines, glycine concentration in plasma is elevated relative to other amino acids, through increased relative rate of synthesis, decreased relative rate of utilization, or mobilization from the cellular free glycine pool remains to be determined. Other possible indices, such as allantoin plus uric acid excretion in the urine (which may reflect the amount of microbial nucleic acid absorbed and which conceivably could correlate with protein yield at the duodenum), showed poorer correlations in Exp. 1 with the various parameters of N utilization, as shown in Table 1.

In correlating log G/OAA ratio with N retention (taken as an index of amino acid utilization in net protein synthesis) it was found that only 55% of variability in one parameter could be associated with variability in the other. Data from Exp. 1, 2 and 3, with two points derived from data presented by Leibholz (1970) for sheep fed high- and low-protein semipurified diets, are shown in Fig. 2. From this it seems unlikely that the relationship between glycine and other amino acids is dependent upon the extent of retention of N, or the efficiency of utilization of absorbed amino acids, but more upon the amounts of amino acids absorbed from the small intestine. Further interpretation of these observations, and an appraisal of the reliability of this particular ratio as a metabolic index of amino acid absorption awaits more detailed examination of the characteristics of glycine metabolism.

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