THE USE OF AMINO ACID PROFILES TO MEASURE MICROBIAL PROTEIN OUTFLOW FROM THE RUMEN

D.J. COTTLE*+ and J.V. NOLAN*

SUMMARY

An evaluation is made of the potential usefulness of the amino acid profile method for quantitating the unfermented feed, bacterial, protozoa1 and other components in abomasal or duodenal digesta. A simpler method of calculation based on multiple regression analysis is proposed and applied to results obtained in a number of laboratories.

INTRODUCTION

The measurement of the flow of microbial protein from the rumen is essential in studies of protein metabolism in the ruminant. Various markers (RNA, DAPA, EAP, ^{15}N, ^{35}S and specific amino acid (a.a.) profiles have been used to identify microbial materials in abomasal or duodenal digesta. Digesta flow markers (^{15}Cr-EDTA, ^{103}Ru-P must also be used to determine the flow of digesta and hence of microbial N to the lower tract. Various workers have compared techniques (see Ling and Buttery 1978; Siddons et al. 1979). Estimates of the proportion of microbial in digesta are usually highest when using RNA, followed by DAPA, ^{15}N, ^{35}S and finally the amino acid (a.a.) profile approach.

The a.a. profile technique was proposed by Evans et al. (1975) and is the only method currently available that can provide simultaneous estimates of the proportions of bacteria, protozoa, feed and endogenous components in digesta. The technique has, however, received little use. This may be partly because many laboratories do not have ready access to computer technology that allows the necessary calculation to be made by the methods originally proposed. Other limitations are imposed by the need to obtain pure samples for analysis of the constituents that are to be identified in digesta and by analytical errors associated with obtaining specific a.a. profiles. These considerationsare discussed in this paper.

THEORY OF TECHNIQUE

The composite a.a. profile in duodenal or abomasal digesta can be considered to be a mixture of amino acids derived from a number of digesta constituents, e.g. unfermented dietary, bacterial, protozoa1 and endogenous materials - each with a different specific a.a. profile. Evans et al. (1975) used a computer program to simulate the mixing of the known a.a. profiles of individual digesta constituents in different proportions in order to produce a profile (containing 15 a.a.) similar to that in composite duodenal digesta. An organized search was carried out for the minimum value of the objective function:

$$\sum_{n=1}^{15} (\text{profile actual} - \text{profile calculated})^2.$$

Computer programs suitable for making this calculation are not readily available in most biological laboratories and this may explain, in part, why the original procedure has not been more widely adopted. However, a discrete solution giving the fraction of each constituent in the composite digesta can be obtained by a direct rather than an iterative method, as follows:

---

*Department of Biochemistry and Nutrition, University of New England, Armidale, N.S.W. 2351.

+Present Address : School of Agriculture, Riverina C.A.E., Wagga Wagga, N.S.W. 2650.

Let $x_1, x_2 \ldots x_c$ represent, respectively, the mass of dry matter (DM) in unfermented bacteria, protozoa . . . etc. per unit DM in abomasal digesta. Let $X_{1,i}, X_{2,i} \ldots X_{n,i}$ represent the concentrations of marker i per unit DM in bacteria protozoa, ...etc., and Di be the concentration of marker per unit DM in abomasal digesta.
Thus,

$$Di = x_1 X_{1,i} + x_2 X_{2,i} + \ldots\ldots + x_c X_{c,i}$$

for i = 1, N, where N = number of markers (e.g. amino acids) used, and C = number of constituents. If N = C, then the unique solution for x_i, when i = 1, C, is obtained by solving N simultaneous equations in C unknowns. If there were no errors in the numerical values, a unique solution would still be possible when N > C but as experimental errors inevitably occur, this is not possible in practice. It is still possible, however, to 'solve' the N equations with a least-squares approach, i.e. by multiple regression using matrix algebra. Multiple regression models are in common use in biological laboratories, and the technology is usually readily available.

The markers used in this approach can be specific, i.e. some $X_{c,i}$ values are zero, or non-specific, i.e. all $X_{c,i}$ values are non-zero. Knowledge of the error associated with the analysis of each marker is important in determining whether it should be included in the analysis. It is the error associated with marker measurement and not the relative difference between marker concentrations in different components that determines a marker's usefulness. If marker error is not known (and assumed constant) all markers should be included in the analysis. If not all markers are used, the optimum index of amino acids should scale each amino acid according to its independent (uncorrelated) ability to distinguish between feed, bacterial and protozoa1 components, assuming all markers are measured with similar accuracy. Discriminant analysis can achieve this goal (Cottle 1980).

RESCALING

If all constituents are included in the analysis, $\sum_{i=1}^{N} xi = 1$. When this does not occur (because there are missing constituents or experimental errors) the resulting estimates of Xi can be rescaled to sum to 1. Alternatively an extra equation with Xi = 1, Di = 1 can be added. This can also be done, if required, when there are only C-l markers available.

The estimation of the amounts of individual constituents in abomasal or duodenal digesta using either the least-squares procedure or the method of Evans et al. (1975) relies on the correct values being included for $X_{c,i}$. One may wish to separate the digesta in terms of total organic matter or total N. If the $X_{c,i}$ values are in terms of g marker/g markers analysed, then the estimated Xi values must be rescaled, i.e. expressed as proportions of total organic matter or total N. The correct Xi values are:

$$\frac{X_1}{SF_c}.T, \frac{X_2}{SF_2}.T, \ldots\ldots \frac{X_c}{SF_c}.T$$

Where SF_c = total mass of all markers in component C per unit DM of component C and $T = \dfrac{1}{\sum_{n=1}^{c} \frac{X_n}{SF_n}}$

This rescaling process will be needed, for example, if marker concentrations are expressed as g amino acid/g total amino acids analyzed, and when nitrogenous substances other than marker amino acids are present in the component being identified and measured. This point has not been recognized by other workers, including Offer et al. (1976).

The most satisfactory way of expressing individual amino acid results, within a group of markers, is as g of amino acid/g DM of component. When this is not measured or reported, the results may be expressed as g of amino acid N/100g of total N accounted for by all the amino acids (measured in all components) in that profile. This may involve recalculations of data, so that the same array of amino acids is represented in each component of the digesta. When the regression model is solved for data in this form, rescaling is required to obtain the actual proportions of total N or DM present.

DISCUSSION

One of the main limitations of the a.a. profile method is that the usual techniques for estimating a.a. composition, e.g. ion-exchange chromatography, are subject to considerable error. Least-squares analyses of profiles are very sensitive to changes in the measured concentration of individual a.a. in the profiles (Cottle 1980). In addition, because feed, microbial or endogenous residues cannot readily be isolated from digesta for a.a. analysis, these marker profiles will often need to be obtained by analyzing the dietary materials and isolated ruminal microbes, or taken from the literature.

Many workers have found that the a.a. profiles of undergraded feed protein leaving the rumen are different from those of the protein in the diet. The a.a. profile of the feed can be weighted by a factor representing the proportion of each a.a. that escapes degradation (from an average value from the literature) followed by the appropriate rescaling of the profile. However, the proportion of each a.a. resistant to degradation appears to vary widely between diets (see McMeniman et al. 1976, Tamminga et al. 1979).

There is also some controversy as to whether the a.a. profiles of bacteria are constant, regardless of the diet fed to the animal. Bacteria and protozoa have different profiles and different protein contents. An analysis of variance of profiles are not different when results are obtained in different laboratories and under different conditions. Profiles of a.a. in protozoa1 protein analysed in different laboratories were also not significantly different. Average a.a. profiles of ruminal bacteria and protozoa from the literature have to be used when only profiles for feed and duodenal digesta are measured (Cottle 1980).

If endogenous proteins entering the rumen are extensively fermented, then omasal and abomasal secretions will be the principal endogenous components in abomasal digesta. Offer et al. (1975) used the a.a. profile of pepsinogen to identify endogenous material, but albumin and γ, β and a globulins from the plasma are also present (Harrop 1974). The total endogenous contribution appears to be only 2–10% of digesta flow (Harrison et al. 1979). This represents a flow of ca. 2-3.5 g N/d in sheep. The endogenous profile could change under different conditions, e.g. increased turnover of albumin occurs when there is gastrointestinal helminth infection (Steel 1978).

EVALUATION OF TECHNIQUE

The procedures outlined were applied to data from this and other laboratories for which independent estimates of the proportion of microbial and dietary material in digesta had been obtained by single-marker methods (Cottle 1980). The main conclusions of this study were as follows:

i) using the a.a. technique the variability between animals on a similar diet is large but no greater than that obtained using other specific markers. The use of an average duodenal digesta profile appeared to give results closest to single marker results, and use of average bacterial and protozoa1 a.a. profiles

from the literature rather than from the experiment under examination did not appreciably affect the results;

ii) the numbers of components that could be included in the analysis depended on the individual variation in data in each study. The estimated proportions of each component in digesta changed substantially when different sets of components were included in the analysis. The results obtained with some diets, particularly when different components had rather similar profiles, did not result in well-constrained solutions;

iii) the estimated proportions of microbial N in digesta N calculated for different diets within a study were ranked in a similar order by the a.a. profile technique and single-marker techniques.

iv) the endogenous profile appeared to be most closely represented by the profile of albumin (Pigman and Moschera 1973).

v) when groups of only five a.a. were used as markers, rather than complete a.a. profiles the analysis was very sensitive to the amino acids included.

The confidence that can be attached to any particular solution can be partially determined from the variability accounted for by the model solution, i.e. r^2 of the regression. However, cognizance should be taken of the precision of analysis of the profiles of individual components included in the model, and the extent to which these profiles are likely to be representative of the components of the digesta. As the a.a. markers are non-specific the analysis can be very sensitive to errors in the a.a. profiles (experimental, sampling and analytical).

REFERENCES

COTTLE, D.J. (1980). Ph. D. Thesis, University of New England.

EVANS, R.A., AXFORD, A.F.E. and OFFER, N.W. (1975). Proc. Nutr. Soc. 34:64A.

HARRISON, D.G., BEEVER, D.E. and OSBOURN, D.F. (1979). Br. J. Nutr. 41:521.

HARROP, G.J.F. (1974). J. Agric. Sci. (Camb). 83:249.

LING, J.R. and BUTTERY, P.J. (1978). Br. J. Nutr. 39:165.

OFFER, N.W., EVANS, R.A. and AXFORD, R.F.E. (1976). J. Agric. Sci.(Camb).87:567.

PIGMAN, W. and MOSCHERA, J. (1973). In "The Biology of the Cervix", p.143, editors, K.J. Blandau and K. Moghissi. (University of Chicago Press: Chicago),

SIDDONS, R.C., BEEVER, D.E., NOLAN, J.V., McALLAN, A.B. and MACRAE, J.C. (1979). Ann. Rech. Vet. 10:286.

STEEL, J.W. (1978). In "Recent Advances in Animal Nutrition", p. 98, editor D.J. Farrell. (University of New England:Armidale).