# WHAT DETERMINES THE AMINO ACID COMPOSITION OF RUMEN MICROBES?

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## Introduction

The amino acid composition of the microbial fraction residing in and leaving the rumen is remarkably constant (Hespell, 1981). Such is the case even following major changes in the diet of the animal. The underlying determinant of the protein complement of any cell is DNA. Recent molecular evidence from individual sequenced genes reveals the non-random nature of codon usage by cells, and the correlation of codon choice with the fractional guanine + cytosine (G.C) content of the DNA (see Bernardi and Bernardi, 1986).

In this paper the amino acid composition of whole microbial cells is shown to be a function of the fractional adenine + thymine content of the DNA (A.T, the complement of G.C) and hence invariant for any species. The results indicate that the major groups of rumen microorganisms - bacteria, fungi and protozoa - have unique amino acid profiles that appear, in the case of fungi and protozoa, to reflect extreme DNA base composition.

The ascomycete fungus *Aspergillus nidulans* strain *bi*A-1 was grown on conventional complete medium. *Neocallimastix* sp. LM-2 was grown as described by Brownlee (1989). Automated amino acid analysis of acid hydrolysed fungi was conducted on a Biotronik LC5001. Using the molar ratio of those amino acids with frequencies significantly correlated with bacterial genomic G.C content (Elton, 1973), a parameter "$R_i$" has been calculated to characterise the amino acid composition of microorganisms:

$$R_i = \frac{\text{Ile + Lys + Phe + Tyr + AspX + Ser}}{\text{Arg + Ala + Gly + Pro}}$$

(aspartate and asparagine are summed as AspX).

Estimates of $R_i$ were made for rumen microorganisms from published amino acid compositions: individual rumen bacteria (Purser and Buechler, 1966), pooled rumen bacteria (*Ibid*, Hespell, 1981 and various other sources), individually cultured rumen fungi and fractionated rumen protozoa (contact author for a full list of published sources).

## Results

The relationship between $R_i$ and genomic A.T content for various well characterised non-rumen microorganisms is shown in figure 1. Data were taken from the literature for bacteria, yeasts, a unicellular alga and a protozoan. The amino acid composition of the filamentous fungus *A. nidulans* was obtained at this laboratory. The polynomial curve fitted to only the bacterial A.T data (X) by a least squares curve fitting program was:

$$R_i = -3.348 + 24.952X - 47.21X^2 + 30.814X^3.$$

The $R_i$ values for rumen microorganisms are presented in figure 2 superimposed on the fitted curve from figure 1.

## Discussion

The constancy of the rumen microbial amino acid omposition can be understood in the light of the results presented in figure 2. The majority of rumen bacteria have genomic G.C contents that would put them on the flattened portion of the curve, i.e. 60-35% G.C (40-65% A.T). Thus even changes in the bacterial population structure would not result in appreciable shifts in the amino acid profile. The rumen fungal and protozoal populations, however, can be differentiated from the bacteria and from each other in having the coding fraction of their DNA apparently richer in A.T, the protozoa extremely so. Confirming this trend, the anaerobic rumen fungus Neocallimastix has recently been shown to possess a remarkably A.T rich genome (Brownlee, 1989). No information is available regarding the genomic composition of rumen protozoa although the related holotrich protozoan *Tetrahymena* has a comparatively A.T rich genome (75% A.T, data point on figure 1).

It has been generally observed that rumen protozoal (and fungal) fractions tend to have a higher content of lysine, isoleucine and phenylalanine (essential amino acids for the animal) and

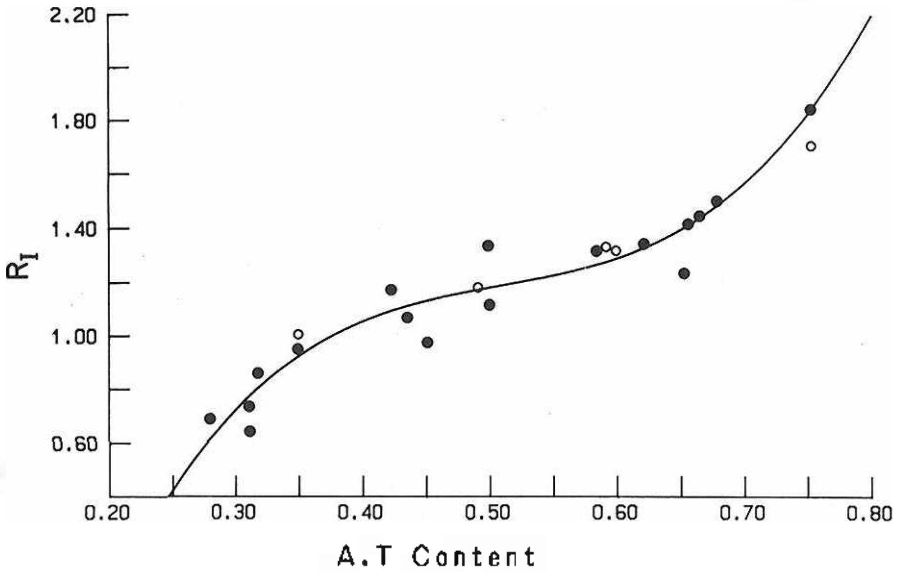

Figure 1. Relationship between amino acid index $R_i$ and A.T content of microorganisms. Closed symbols: bacterial data to which the curve was fitted (r = 0.963); open symbols: eukaryotic microbes including *A. nidulans* (49% A.T), and *Tetrahymena* (75% A.T).

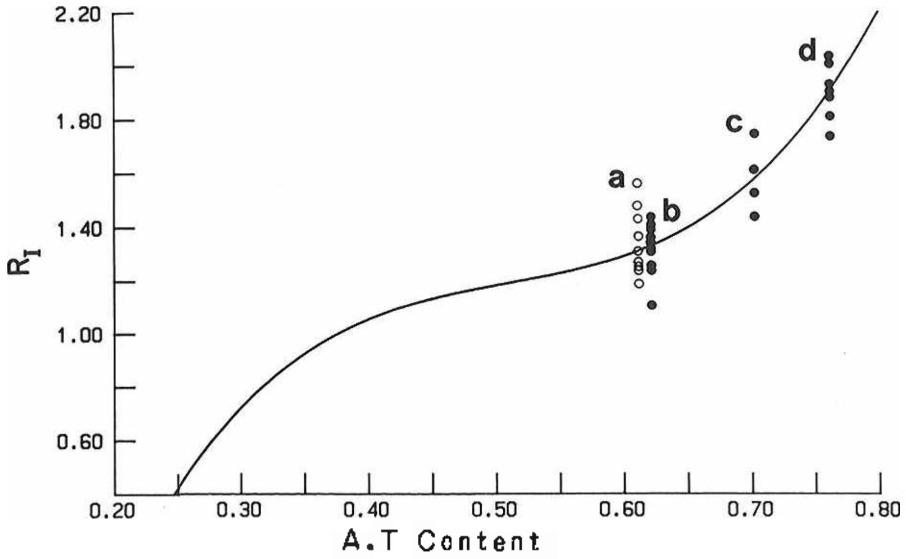

Figure 2. $R_i$ values of rumen microorganisms calculated from published data. Values are plotted with their mean falling on the curve redrawn from Fig. 1: a, individual rumen bacteria (n=22); b, mixed rumen bacteria (n=17); c, anaerobic rumen fungi (n=4 including *Neocallimastix* LM-2 determined here); d, mixed rumen protozoa (n=9).

lower amounts of alanine and arginine than the bacterial fraction. The present results show that these differences in amino acid composition are related to genomic differences between these groups of microorganisms. The reasons for the particular distribution of the rumen microbial population and its nutritional ramifications will be reported in more detail elsewhere.

(**Key Words**: Rumen Microbes, Amino Acids, G.C Content)

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