BIOCHEMISTRY

Assessment of Relative Nutritive Value of Protein using *Streptococcus zymogenes*

ONE of the outstanding difficulties in assessing the nutritional quality of proteins is the time and expense involved in carrying out methods using animals[1,2]. Attempts have been made to develop convenient alternatives[3–5], but so far these have been shown to yield results valid only for specific cases.

In 1960, Ford[6] proposed a method for the estimation of relative nutritive value of protein based on the growth of *S. zymogenes*. He found the method to be comparable with rat-feeding assays. However, his suggestion that the microbial growth be measured turbidimetrically restricts its use to samples containing little or no cereal because of the effect on light transmission of the substrate. He reported that titration of the acid produced during growth yielded results which were unreliable.

We had sufficient success using Ford's method on samples of low starch content (Table 1) that we experimented with the titration procedure in order to extend its scope.

For titration we used 0·3 N sodium hydroxide with α-naphthol phthalein indicator.

Over a wide range of samples we obtained titres between 2 and 4 ml. for dosages of 0·1–0·7 mg nitrogen. Moreover, the log/log plot of response against dose[7] was rectilinear over the main parts of the graphs. In the case of high-quality protein the rectilinear portion extended between approximately 0·1–0·5 mg nitrogen and that for poorer quality protein up to 0·7 mg nitrogen. We, therefore, modified the method by introducing dosages equivalent to 0·1–0·7 mg nitrogen in steps of 0·1 mg (Fig. 1).

In reading off results, it would seem equally reasonable to obtain relative nutritive value from the response ratio of sample to standard at a given dose or from the dose ratio of standard to sample at a given response. However, equal results are obtained only when the slope of the lines is 45°.

We define relative nutritive value as the percentage of the standard response obtained by the sample at a given dose.

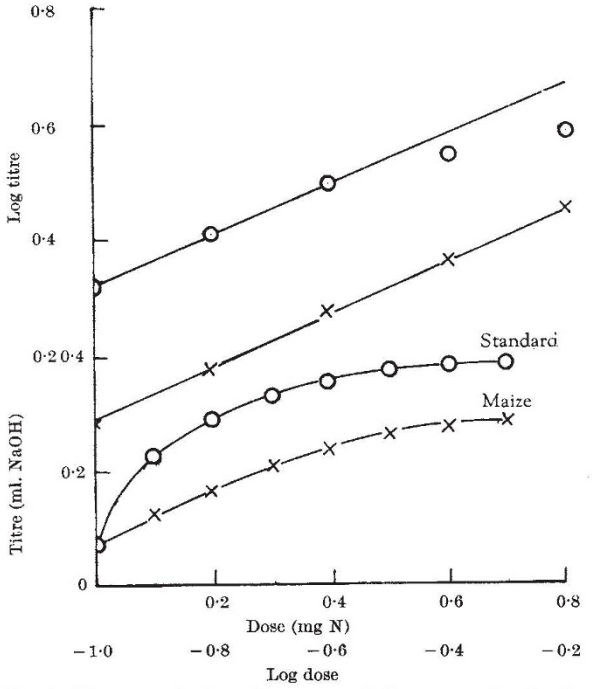

Fig. 1. Measurement of growth response of *S. zymogenes* by titration

Table 1. COMPARISON OF RELATIVE NUTRITIVE VALUE WITH RESULTS OF FEEDING BROILERS ON A COMPOUND PREVIOUSLY SUBJECTED TO VARYING HEAT TREATMENT

Sample	Food conversion factor average of 76 birds (wt. food taken/body-wt.)	Relative nutritive value (turbidimetric)
1	2·70	56
2	2·63	54
3	2·69	49
4	2·76	42
5	2·79	33
6	2·80	27
7	2·89	28

Table 2. RELATIVE NUTRITIVE VALUE OF VARIOUS PROTEINS

Sample	Relative nutritive value	Sample	Relative nutritive value
Whole egg	100	Meat meal	50
Hydrolysed casein	80	White fish meal	60
Whole milk	77	Digestive biscuits	71
Maize	58	Uncooked sausage	75
Soya meal	54	Canned tuna fish	80
Wheat germ	67	Boiled potatoes	62
Oats	67	Amino-acid mixture (ref. 6)	102

Our results for a range of products are recorded in Table 2.

We have used the amino-acid mixture defined for *S. zymogenes* by Ford[6] as a standard under conditions when the unavoidable degradation of casein precluded its use.

We are now using this method in investigations of the effect of processing and storage on protein degradation, the results of which will be reported in due course.

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Mammalian Degradation of (—)-Demethylcotinine

THE degradation of the pyrrolidine ring of (−)-nicotine in a number of mammalian species[1–5] involves the formation of the intermediate, cotinine. This lactam of (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid is in turn demethylated by the dog[6] and the rat[7], but possibly not by man[8], to give the urinary metabolite (−)-demethylcotinine. In the metabolism of (−)-nornicotine, a companion alkaloid to nicotine in many situations, (−)-demethylcotinine, is also formed[9]. Demethylcotinine thus occupies a unique position in the early metabolic oxidation of the two alkaloids and we were led to explore some of the detail in its metabolic degradation. Natural (−)-demethyl 1/*m* cotinine, which was obtained from the metabolism of (−)-cotinine in the dog[6], was used in the investigations.

A group of 12 male albino rats received by oral intubation a total of 5·5 g of (−)-demethylcotinine (250 mg/kg in single oral doses on each of 5 successive days). The urine, which was preserved with sodium fluoride and freezing, was collected as run-off from metabolism cages until 48 h after the last dose. After adjustment to *p*H 5 the combined urine was placed on 'Dowex 50 (H^+)'. The eluate obtained by treating the resin column with 1 N ammonia was continuously extracted with chloroform. This extraction served to remove from the aqueous phase demethylcotinine and some additional Koenig-positive components which have not been identified.

The aqueous solution that remained from the extraction with chloroform was placed on 'Dowex 21*K* (OH^-)', and a Koenig-positive carboxylic acid-containing fraction was obtained by elution with 1 N acetic acid.