Nutrition
Editors: A. Chávez, H. Bourges, S. Basta, Mexico
Publishers: S. Karger, Basel
Separatum (Printed in Switzerland)

Proc. 9th int. Congr. Nutrition, Mexico 1972, vol. 4, pp. 171–175 (Karger, Basel 1975)

Laboratory Evaluation of Protein-Rich Mixtures

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Introduction

Most protein-rich food mixtures are usually formulated by mixing two or more protein-containing foods according to their respective essential amino acid pattern in regard to a reference protein. Formulation of protein-rich mixtures by this method takes into consideration the fact that some proteins are deficient in one or two amino acids which, by calculation, are supplied by a second or third protein source. Although in theory it should be possible to arrive at good quality mixtures following this technique, more often than not the result is generally not the one expected because the availability of the amino acids in the different food proteins is not usually considered. Furthermore, the only consideration given is with respect to the limiting amino acids, but not to excesses which can take place upon mixing, which may reduce the efficiency of utilization of the protein in the mixture.

Furthermore, the components of such mixtures are based on new protein sources or traditional sources which have undergone a certain degree of processing. These factors, which may alter nutritional value, must also be considered. If new protein sources are utilized in the formulation, the possibility that they may contain toxic substances should be considered as well. Finally, if the protein-rich food is to be used as a supplement to poor quality diets, its supplementary effect should be known.

This paper describes briefly the testing which has been carried out on protein-rich foods developed at INCAP or in other institutions or laboratories [1–4].

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Evaluation of Protein Quality

1. Experimental Animals

Evaluation of the nutritive value of protein-rich foods should include the following tests: a) animal growth studies; b) nitrogen balance; c) toxicity tests; d) improvement in protein quality by amino acid or protein supplementation; and e) supplementary effect of the protein-rich food to poor quality diets.

a) Animal Growth Studies: There are various methods available for the determination of the protein quality in protein-rich foods. The method most commonly used is the protein efficiency ratio, carrying out the following recommended techniques and using for comparison a reference protein. Since not all proteins give maximum values at the same protein level in the diet, it is better to test the protein-rich food at various levels of dietary protein. Regression equations of protein intake to weight gain are calculated with the regression coefficient representing the quality of the mixture. Examples are shown in table I.

This method has been utilized with various protein-rich foods and the results obtained have given values which correlate highly with evaluation in children, as will be shown later.

b) Nitrogen Balance: Additional and useful information on the protein quality of the mixture is derived from nitrogen balance studies. These can be carried out directly by nitrogen analysis of food, feces, and urine, or indirectly by carcass analysis. The determination of nitrogen in feces can be used to evaluate apparent protein digestibility. In this case also, the information obtained is more valuable if the test is carried out at various protein levels of intake, particularly at low levels, where there is a straight-line relationship between absorbed and retained nitrogen. By carcass analysis, net protein utilization values are also obtained.

c) Toxicity Tests: As indicated above, toxicity tests are necessary, particularly when the mixture is made from components representing new protein sources which have not been previously used to any extent in human feeding.

These tests are long-term and costly and, therefore, are not often carried out, particularly if the components have already been screened for toxicity.

An alternative is to feed the protein-rich food to various animal species, which often react differently with respect to toxic substances.

d) Supplementary Effect: Protein-rich foods have usually been developed for the purpose uf using them as supplements to diets of low-protein quality and quantity.

Therefore, if this is the case, the supplementary efficiency of the mixture is probably one of the most useful tests which should be conducted. The test is of value in indicating if there is a supplementary effect, and how much of the mixture is needed to bring about an improvement of the diet ingested.

e) Protein Quality Improvement of the Mixture: The sequence of tests indicated above should suggest whether the quality of the mixture is high or if it is low owing to a deficiency of amino acids. If the limiting amino acids are known, their deficiency may be corrected, either by adding them in the synthetic form, if available and of low cost, or by increasing the level of protein source rich in such nutrients.

2. Children

a) Nitrogen Balance: Protein-rich mixtures are then evaluated in healthy children if the mixture is intended for their supplementary feeding. The method commonly used is the nitrogen balance technique, carried out at a

Table I. Regression equations of protein intake to weight gain in weanling rats and nitrogen absorbed to nitrogen balance in children

Protein mixture	Regression equation	
	rats	children
INCAP No. 9	Y = — 11.86 + 1.96 X	— 29.4 + 0.49 X
INCAP No. 14	Y = — 15.18 + 2.26 X	— 37.2 + 0.62 X
INCAP No. 15	Y = — 9.80 + 2.13 X	— 36.7 + 0.47 X
Skim milk	Y = — 9.86 + 3.49 X	— 40.8 + 0.73 X
Cassava-soya	Y = — 9.50 + 2.99 X	— 39.8 + 0.67 X
IRL	Y = — 12.88 + 2.68 X	— 30.7 + 0.58 X

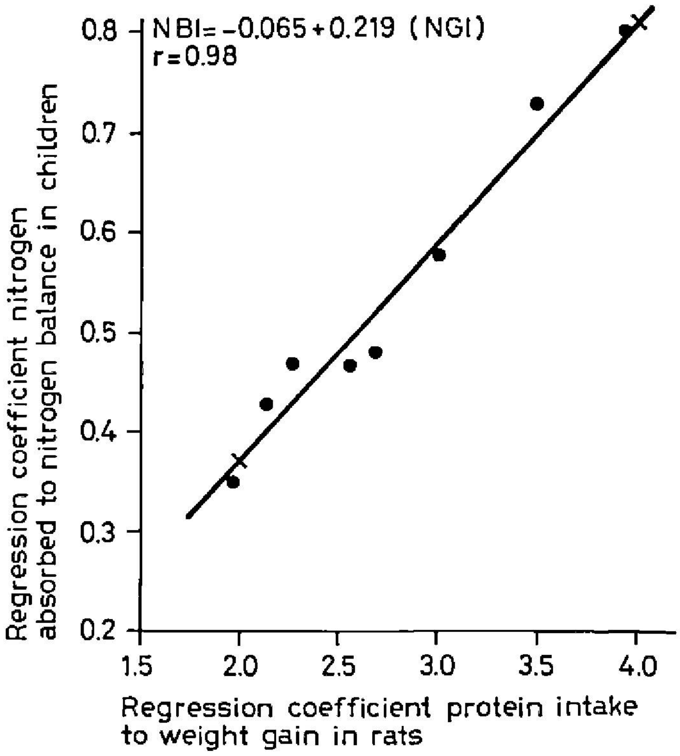

Fig. 1. Relationship between nitrogen growth index in rats and nitrogen balance index in children.

level of nitrogen intake between 150 and 250 mg/kg/day. Milk protein should be used as reference protein.

Although such a test will give some information on the protein quality of the mixture, a preferred technique in our laboratories is to feed the protein at levels of intake of 0.5, 0.75, 1.00, 1.25, and 1.50 g protein/kg/day. The coefficient of regression of nitrogen absorbed to nitrogen balance is an index of the protein quality of the mixture (table I). The relation of this coefficient to that of milk protein fed at equal levels gives the relative nutritive value of the mixture. This technique also provides data on minimum protein intake for nitrogen equilibrium, protein digestibility, and biologic value. Results obtained by the method correlate highly with results in experimental animals, as shown in figure 1.

b) Supplementary Effect: As indicated previously, if the mixture is to be used as a supplement to poor quality protein diets, the supplementary effect of the mixture should also be evaluated in children.

To obtain such information, the child is fed the basic diet to which a protein of reference, such as milk, is added in 1 or 2 levels, keeping total intake of nitrogen close to 250 mg/kg/day. This would be followed by adding the equivalent levels of the protein mixture.

The result, however, may be due to both an increase in protein intake and, probably, to quality improvement. To elucidate this, non-protein nitrogen added in equivalent amounts is fed, together with the basic diet.

Conclusion

Even though the testing steps indicated may not all be necessary, protein-rich mixtures should be tested to insure that they can or at least have the potential to increase the quality of diets consumed by large population groups in developing countries, particularly by children.

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