

DIETARY PROTEIN SUPPLEMENTATION. FUNDAMENTALS AND EXAMPLES OF PRACTICAL APPLICATION

*J. J. Dreyer*¹

National Food Research Institute (CSIR)
Pretoria, South Africa

SUMMARY

Involving the most expensive of all the nutrients required by the body, the biological utilization of dietary protein remains an important topic, particularly to Third World countries wherein protein supplies are frequently limited. Protein supplementation of cereals and other foodstuffs is often used as a means of increasing protein intake. Supplementation not only increases protein intake but often also changes the physiological usability of the protein component of the resultant mixture.

Employing an updated biological method for assessment of protein nutritive value, the author investigated the nature of the change in protein assimilability as a result of graded changes in the proportions of certain proteins combined pairwise into single mixtures.

Data are presented on the basis of which the digestibility and/or assimilability of the protein component of a given mixture can be estimated. Lastly, the author indicates the need to evaluate high-protein foods and protein supplements in terms of, not only protein content relative to cost, but also of inherent nutritive value and complementary effect.

INTRODUCTION

Protein-energy malnutrition is generally associated with Third World conditions, which are characterized by the consumption of low-protein diets consisting predominantly of cereals. Supplementation of such diets with special food mixtures or with high-protein foods in common use is, therefore, a logical approach to solving the problems caused by the protein-deficient diets.

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1 Head, Biological Evaluation Division, National Food Research Institute of the Council for Scientific and Industrial Research (CSIR), P. O. Box 395, Pretoria 0001, South Africa.

Because protein is known to be the most expensive of human nutrient requirements, protein supplementation should be employed with discretion (1). The amount supplemented should as far as possible be adjusted according to physiological requirements, i.e. to the ability of the supplement to meet these requirements in terms of assimilable or physiologically usable protein.

As was pointed out by Mitchell (2) as early as 1924, the assimilability of the protein in a mixture of protein-bearing foodstuffs is seldom merely the weighted mean of the assimilabilities of the different components combined in the mixture. The fundamental aspects of this issue are as illustrated in Figure 1. Nutritionally, each of the proteins to be combined consists of three fractions: an indigestible fraction; an assimilable fraction (which can be used for synthesis of body proteins), and a fraction which is unassimilable because it is deficient in one or more of the essential amino acids required by the body. It is however, possible that the deficiencies of the unassimilable fraction of protein A in Figure 1 might be the abundances of the unassimilable fraction of protein B, and *vice versa*. When combined, these two fractions could therefore complement each other, causing the assimilability of the protein in the mixture to be *greater than the weighted mean of the values obtained for the two proteins separately*.

UTILIZATION OF PROTEIN MIXTURES

High- & low-quality protein mix

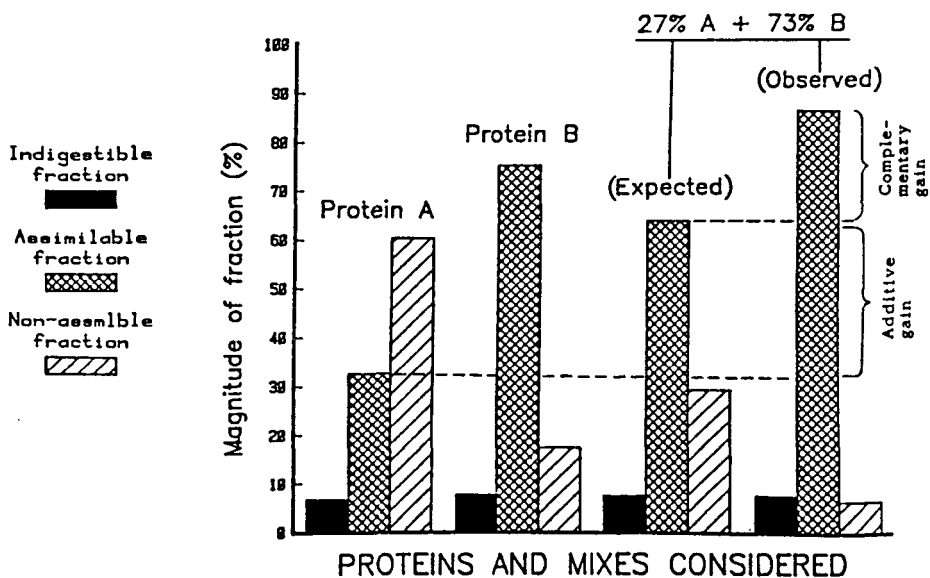


FIGURE 1

Illustration used in explanation of "additive" and "complementary" effects on protein assimilability obtained when two proteins differing in nutritive value are combined in a single mixture

With a view to stimulating interest in a fundamental approach to the evaluation of protein supplements, data are presented on a series of five trials with pairs of proteins. Each pair of protein sources was tested at various combinations, in such a way that assimilable protein contents and gains due to additive, as well as complementary effects can be estimated from the results for any given combination of the two protein sources.

MATERIAL AND METHODS

The pairs of materials studied were: 1) wheat gluten plus L-lysine; 2) maize protein concentrate plus milk protein; 3) potato protein plus whole hen's egg protein; 4) maize protein concentrate plus PVM; and 5) maize protein concentrate plus a commercial high-protein product, Pro Nutro. The nature of the materials used and the reasons for selecting them for investigation are set out in Table 1. The reason for using maize protein concentrate instead of maize was that the protein content of maize was found to be too low for the preparation of experimental diets of appropriate nitrogen content. It is, however, considered that preparation of the concentrate had no effect on the quality of the maize protein, as it involved no heat treatment or fractionation of the protein.

Protein digestibility and assimilability were determined in assays with weanling rats according to the updated procedures for dietary protein evaluation for monogastric animals proposed by Dreyer and Van der Walt (4). A total of 42 rats were used for each assay, the animals being of the F₂ generation of two inbred strains supplied by the SABS Animal Breeding Centre, Groenkloof, Pretoria.

Nitrogen was determined in test materials, diets and excreta by the Kjeldahl method, with use of a Kjel-Foss apparatus. Moisture determinations on test materials and diets were made by means of a Brabender apparatus, at a drying temperature of 90°C maintained for three hours.

Data processing over and above calculation of protein digestibility and assimilability entailed fitting of regression lines representing (i) linear relationships between digestibility and the change in the ratio in which the two proteins were mixed; and (ii) biphasal relationships between assimilability of the digestible protein and the change in the ratios of the two components of the mixture. Line-fitting operations were performed according to the least-squares method. Points of deflexion in biphasal relationships were calculated by iteration, and data weighting was employed whenever necessary, according to Bartlett's test; there were significant differences in degrees of scattering in the different sets of results used to fit a given statistical model. The reciprocals of variances were used as weighting factors.

RESULTS AND DISCUSSION

The relationship between protein digestibility and the ratios in which the nitrogen from the two protein sources were combined, is given in Table 2 for each pair of products.

TABLE 1

DESCRIPTION OF MATERIALS USED IN PROTEIN SUPPLEMENTATION TRIALS, AND REASONS FOR THE CHOICE OF EACH OF THE VARIOUS COMBINATIONS OF PRODUCTS TESTED*

Pair No.	Nature and origin of materials	Reasons for selection of combination
	<p><i>Gluten + L-lysine.</i> Laboratory grade (BHD) wheat gluten. N content on air-dry basis = 12.4^o/o. Analytical grade (Merck) L-lysine hydrochloride. Concentrations of lysine nitrogen in total nitrogen contents of mixtures (°/o): 0, 1.88, 3.69, 5.43 and 7.12 .</p>	<p>To show the effect of supplementation with a single essential amino acid of a protein grossly deficient in that amino acid.</p>
	<p><i>Milk protein + maize protein concentrate.</i> High-grade, defatted, spray-dried milk powder supplied by the Animal and Dairy Research Institute, Irene. N content on air-dry basis = 5.8^o/o. Maize protein concentrate prepared by the Fermentation Technology Division of the N Food R I through amylase digestion of the starchy component of sifted granulated maize meal and subsequent filtration of the digest. Total protein loss is estimated at only about 4^o/o. Nitrogen content on air-dry-basis = 2.77^o/o. The concentrations of milk nitrogen in the total nitrogen contents of the combinations investigated were: 0, 20, 30, 40, 60, 80 and 100^o/o.</p>	<p>Used as an example of combining a high-quality protein (milk) with a poor-quality protein (cereal) resulting in mutual complementation of protein nutritive value.</p>
	<p><i>Egg protein + potato protein.</i> Fresh, whole, hen's eggs were boiled, minced, freeze-dried and ground by mortar and pestle. N content on air-dry basis = 5.18^o/o. Fresh potatoes were cooked in boiling water, peeled, minced, freeze-dried and ground in a pindisc mill. N content on air-dry basis = 1.818^o/o. Concentrations of egg-protein nitrogen in the nitrogen contents of the mixtures investigated were: 28.2, 40, 60, 80 and 100^o/o.</p>	<p>It has been claimed (3) that, when combined in a specific ratio, a mixture of potato and egg protein can be obtained which is superior to egg protein in protein quality. A product having this property would be very useful in the feeding of subjects suffering from kidney disease or from phynylketonuria.</p>

Table 1 (Cont.)

Pair No.	Nature and origin of materials	Reasons for selection of combination
4	<i>PVM + maize protein concentrate.</i> A commercial sample of the P(rotein) V(itamin) M(inerals) supplementary food mixture developed at the N. Nutr. R. I. in the sixties. Nitrogen content on air-dry basis = 7.19 ^o /o. The maize protein concentrate was as given for pair 2. Concentrations of the PVM nitrogen in the nitrogen contents of the mixture were: 0, 50, 67, 83 and 100 ^o /o.	Because PVM was developed at the CSIR and is used by the Dept. of Health and Welfare for distribution to needy children, its protein supplementary value is of special interest.
5	<i>"Pro Nutro" + maize protein concentrate.</i> A 4-kg quantity of this commercial food mixture (the so-called "regular" version) was purchased at a supermarket and ground in a pindisc mill. Nitrogen content on air-dry basis = 3.24 ^o /o. The maize protein concentrate was as given for pair 2. Concentrations of nitrogen from the Pro Nutro in the nitrogen contents of the mixtures were: 0, 50, 67, 83 and 100 ^o /o.	Because of the wide use of this commercial product and of earlier indications that protein quality as well as complementary effect is mediocre.
*	The choice of products was for the purpose of comparison and not because of either approval or disapproval of any of the materials investigated.	

TABLE 2

DATA SHOWING THE CHANGE IN THE DIGESTIBILITY OF THE PROTEIN CONTENTS OF MIXTURES OF CERTAIN PAIRS OF PROTEINS DUE TO CHANGE IN THE RATIO IN WHICH THE TWO SEPARATE COMPONENTS OF EACH MIXTURE WERE COMBINED*

Pair of products combined	Product of increasing concentration in the nitrogen mixture (= X in equation)	'a' in equation, i. e. digestibility when X = zero, i. e. digestibility of the un-supplemented protein in the other member of the pair	'b' in equation, i. e. change in digestibility (Y) with change in X	r = correlation coefficient	P = probability of no relationship between Y and X
1. Gluten + L-lysine	L-lysine nitrogen (o/o)	97.6	0.043	0.834	<0.05
2. Maize + milk	Milk nitrogen (o/o)	93.2	-0.0067	-0.67	<0.05
3. Potato + egg	Egg nitrogen (o/o)	80.7	0.107	0.964	<0.01
4. Maize + PVM	PVM nitrogen (o/o)	91.1	-0.034	-0.972	<0.01
5. Maize + Pro Nutro	Pro Nutro nitrogen (o/o)	91.4	-0.086	-0.999	<0.001

* In all cases the change can be described on the basis of a simple linear relationship of the type: $Y = a + bX$, where Y = digestibility and b = rate of change in Y due to an increase in X, where X = o/o of N in total N mixture from one of the two components.

From previous experience, as well as that gained in the present series of trials, it was clear that the above relationship is linear in every case. This means that there is no interaction with respect to digestibility among different proteins when proteins are combined in a single dietary mixture. The digestibility of a mixture of proteins from different sources is therefore the weighted mean of the digestibility figures obtained when the proteins are tested separately.

To prove this point, the results obtained with PVM and maize protein should suffice. The figures obtained for these products separately were 88.0/o and 91.30/o, respectively. The comparison of results obtained with those predicted from the values obtained for the two products separately is as follows:

- i) The 50:50 ratio: obtained = 89.0/o, predicted = 89.70/o.
- ii) The 33:67 ratio: obtained = 88.40/o, predicted = 90.20/o.
- iii) The 17:83 ratio: obtained = 88.50/o, predicted = 90.70/o.

The data listed in Table 2 can therefore be used confidently for prediction of the digestibility of the protein mixture for any combination of any two of the products investigated. On the basis of these data we can estimate, for example, the digestibility of the various "proteins" in un-supplemented form as gluten = 97.6; lysine = ca. 100.0/o; milk protein = 92.50/o; potato protein = 80.70/o; egg protein = 91.40/o; PVM protein = 87.70/o; Pro Nutro protein = 82.80/o; and maize protein (average of 3 values) = 91.90/o.

The effects of combining pairs of proteins from different sources on biological utilization of the absorbable fraction of the dietary protein (the assimilability of the absorbable fraction) are shown in Figures 2 to 6. The broken lines in these Figures represent the changes due merely to the increase in the level of one protein of a specific quality and simultaneous decrease in the level of another protein of another quality. The broken lines, therefore, show the relationships to be expected had there been no interaction in respect of nutritive value between the two proteins when combined in a single mixture.

Because, with the exception of the lysine, the supplements were generally of higher quality than the supplemented proteins, the broken lines mostly have positive slopes, the latter varying in magnitude according to the quality of the protein supplement.

It can be seen, however, that most of the data obtained suggest assimilability figures which exceed the values suggested by the straight-line relationship represented by the broken lines. The above difference indicates the effect of an essential amino acid complementation varying in nature and magnitude according to the types and quantities of the materials combined to obtain a particular series of mixtures.

One of the most informative sets of results is that obtained in the maize + milk protein series (Figure 2). This case represents supplementation of a poor-quality cereal protein with a high-quality animal protein, having both an additive and a complementary effect; the broken line has a steep, positive slope, and there is a substantial difference (indicated by the shaded area) between values expected and values obtained. It can also be seen that, in this case, mutual complementation

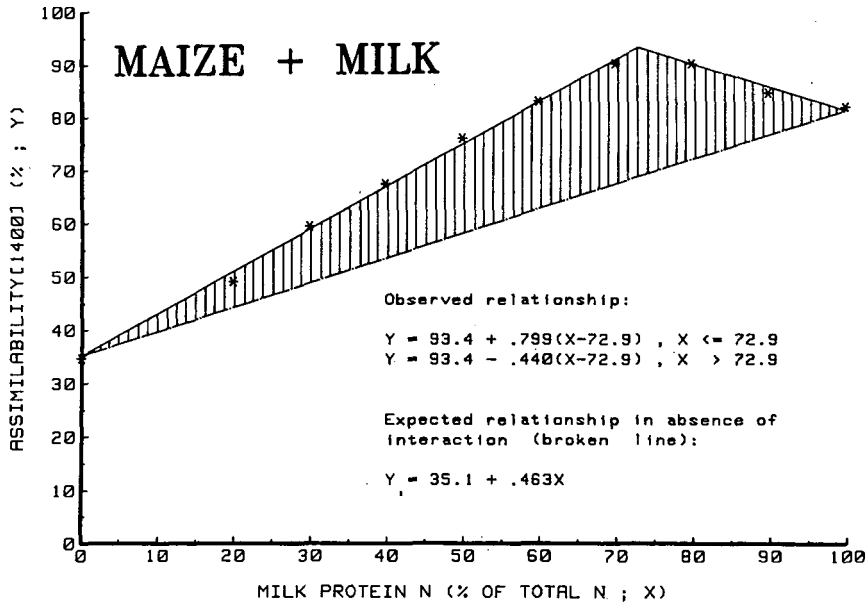


FIGURE 2

Effect on protein assimilability of combining maize and milk proteins. The shaded area indicates gains due to a complementary effect and the broken line the values to be expected in absence of a complementary effect

is obtained: maize protein quality is improved through addition of milk protein, and milk protein is improved through the addition of maize protein. There is also a point of optimal complementation at about maize N:milk N = 27.1:72.9. At the optimal N:N ratio a protein mixture is obtained of an assimilability equal to that of egg protein. At this point, the gain due to complementation is about 36% of what would be obtained in an assimilable protein, had there been no complementation or had the two proteins been fed separately in comparable amounts.

The gluten + lysine pair (Figure 3) shows certain points of correspondence with the maize + milk pair. This combination can also be considered a case of mutual complementation because the point of optimal assimilability exceeds both of the values suggested by the data for the two components separately; it can be said that the lysine complements the gluten, and the gluten complements the lysine. In comparison with the maize + milk combination, lysine did, however, not have an additive effect, because it represents the case of a single amino acid which, when fed alone or in excess of what is needed to complement the protein in

question, has an assimilability of zero. As is shown in the Figure, additions exceeding the optimal supplementation level, therefore have a "diluting" or assimilability-diminishing effect. The ability of lysine to improve the assimilability of the gluten is therefore limited, but it is nevertheless impressive in the sense that supplementation at a lysine nitrogen level of less than 30% caused assimilability to rise from 34 to 57%.

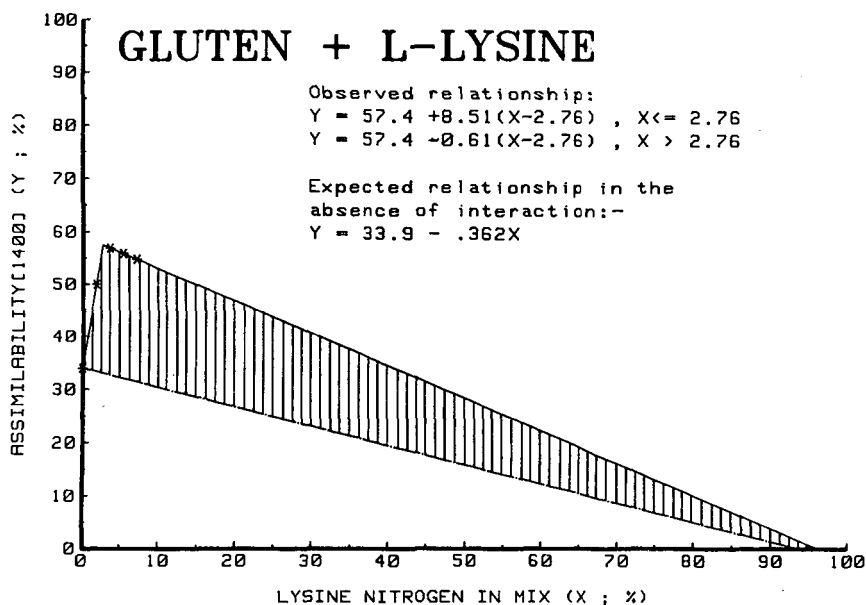


FIGURE 3

Effect on protein assimilability of combining gluten and L-lysine. The shaded area indicates gains due to a complementary effect and the broken line the values to be expected in absence of a complementary effect

Egg protein supplementation of potato added to, and also complemented potato protein in terms of assimilability (Figure 4). The match, however, appears to be a one-sided affair, as potato protein did not improve egg protein assimilability. The results therefore do not support Kofrányi's claim (3) that there is a combination of egg and potato protein which is more assimilable than egg protein alone. It is, however, interesting to note that the point of optimal N:N ratio of 53% egg nitrogen in the mixture obtained in the present study, corresponds closely to Kofrányi's 47% as obtained in trials with human subjects.

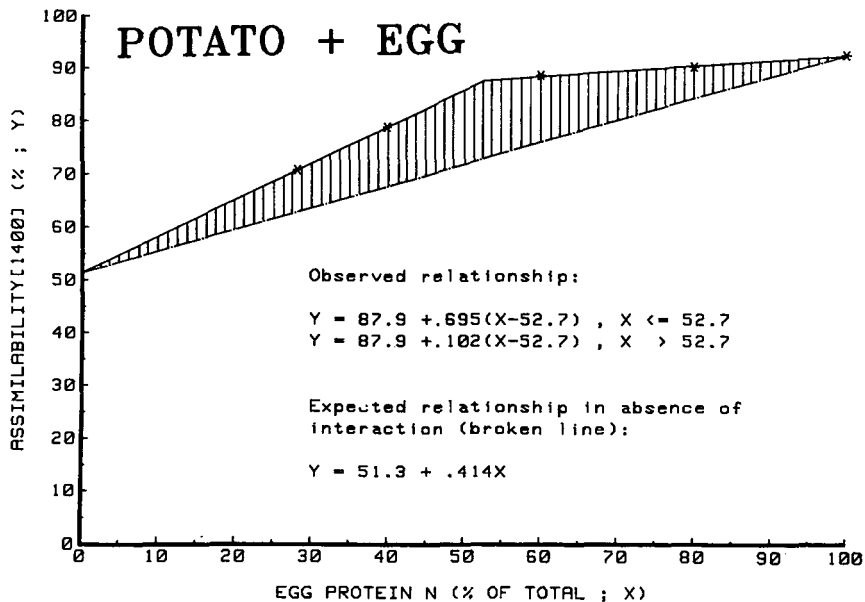


FIGURE 4

Effect on protein assimilability of combining potato and egg protein. The shaded area indicates gains due to a complementary effect and the broken line the values to be expected in absence of a complementary effect

The assimilability of the protein in the CSIR's PVM supplementary food mixture was found to be approximately the same as that of milk (Figure 5). These proteins were, therefore, equal in additive effect, but the complementary effect on maize protein of PVM was, less than that of milk protein. The average complementary gain represented by the shaded area in Figure 4 is about 55% that represented by the corresponding area in Figure 2.

The results summarized in Figure 6 (maize + Pro Nutro protein) exemplify a combination in which the additive effect was small (on account of the relatively low assimilability figure obtained for the supplement *per se*), while no significant complementary effect could be detected. Absence of a complementary effect was probably due to prior inclusion of cereal protein in the production of the supplement in question.

Equations given in Table 2 on digestibility, and those recorded in the graphs on assimilability (Figures 2-6), can obviously be used to estimate all the various parameters pertaining to protein value (digestibility, assimilability, net utilization (NPU) and assimilable protein contents (NPV))

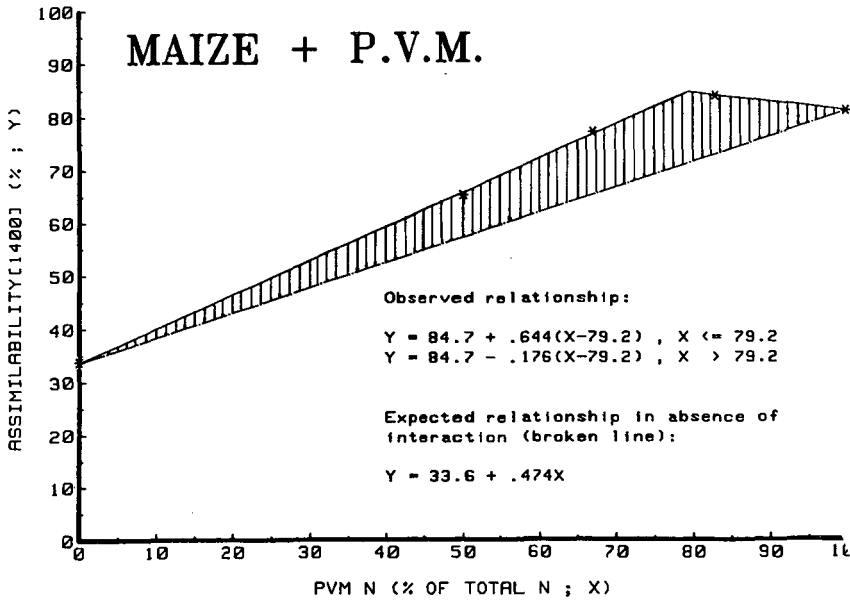


FIGURE 5

Effect on protein assimilability of combining maize and PVM protein. The shaded area indicates gains due to a complementary effect and the broken line the values to be expected in absence of a complementary effect

at any given combination of the two products in question. Such data furnish a rational basis for evaluation of protein supplements relative to the cost, which should involve consideration not only of the protein content, but also of its inherent nutritive value (the additive effect) and the prospect of a favorable interaction between the supplementary and supplemented protein (the complementary effect). It is scientifically unsound to compare protein supplements solely on the basis of cost per unit protein content since inherent protein assimilability and complementary effect can vary substantially from one combination of proteins to another.

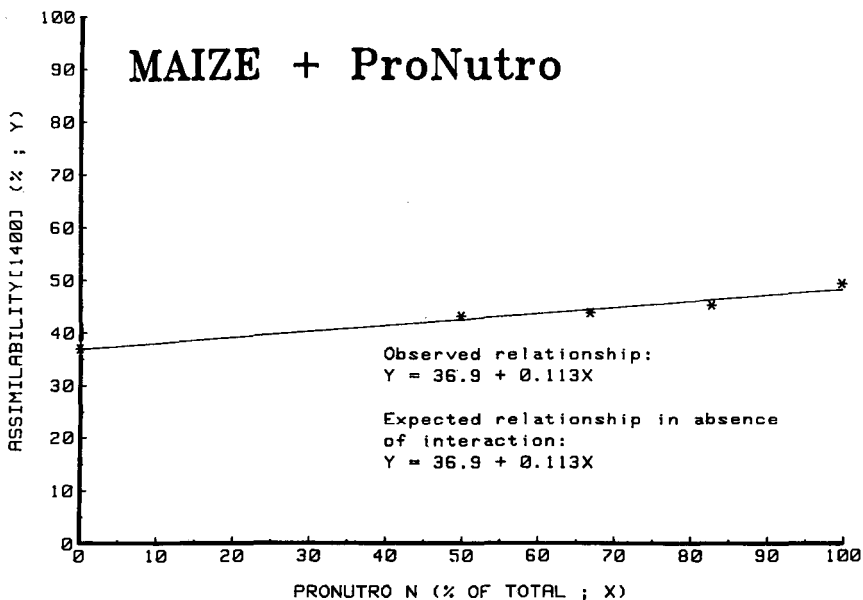


FIGURE 6

Effect on protein assimilability of combining maize and Pro Nutro protein. The data represent a case in which there was no complementary effect and only a small additive effect in terms of protein quality

ACKNOWLEDGEMENTS

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RESUMEN

SUPLEMENTACION DE LA PROTEINA DIETARIA: CONSIDERACIONES FUNDAMENTALES Y EJEMPLOS DE APLICACION PRACTICA

La utilización biológica de la proteína dietaria, el más costoso de los nutrientes requeridos por el organismo, sigue siendo un tópico de importancia, particularmente para los países del Tercer Mundo, donde los abastecimientos proteínicos frecuentemente son limitados. Como medio de incrementar la ingesta de proteína, a menudo se utiliza la suplementación con proteína de cereales y otros productos alimenticios. Esa suplementación no sólo aumenta la ingesta de dicho nutriente, sino que a menudo también induce cambios en la utilización fisiológica del componente proteínico de la mezcla resultante.

El autor investigó la naturaleza de esos cambios en cuanto a la asimilabilidad de la proteína, como resultado de cambios escalonados en las proporciones de ciertas proteínas que, en combinaciones por pareja, se incorporan a mezclas simples. Empleó para el caso un método biológico actualizado de evaluación del valor nutricional de la proteína.

Se presentan datos con base en los cuales es factible estimar la digestibilidad y/o asimilabilidad del componente proteínico de una mezcla dada. Por último, el autor señala la necesidad de evaluar alimentos ricos en proteína y suplementos proteínicos, no sólo en términos de su contenido de proteína en relación al costo, si no también del valor nutritivo inherente, y de su efecto complementario.

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