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Evaluation of the Nutritive Quality of Proteins by Using Slope-Ratio Assay

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Abstract

Young rats were fed diets containing three different proteins : lactalbumin, soy and peanut protein, each fed at levels from zero to over 35% of the diet over a 2-week time period. Response was measured as the amount of body weight gain in relation to total nitrogen consumption as a measure of dose. The slopes of the regression of the proteins in the linear range were compared to that of the standard lactalbumin. The relative growth index of soy and peanut protein was calculated to be 78.4 and 55.7, respectively. Thus, it is concluded that the slope-ratio assay is a very useful method for the evaluation of the nutritional quality of proteins under well-designed experimental conditions.

Key words : nutritive quality, proteins, slope-ratio assay

INTRODUCTION

In the evaluation of diets and in the establishment of protein requirement, it is considered necessary to take into account both the amount and nutritional quality of dietary protein. There are basically two biologic procedures in common use for the evaluation of the nutritive value of proteins. Biological value (BV) as determined by Mitchell[1] is defined as the "percentage of absorbed nitrogen retained" and is generally considered the method of choice[2]. However, BV can be determined only when the animal is depleted of nitrogen and the intake of protein is low so that the animal is negative balance or near balance. The other method is the protein efficiency ratio (PER) originally proposed by Osborne et al[3]. This method has been criticized[2] since the PER is highly correlated with weight gain, not the characteristic of the protein, but of the rate of weight gain of the animals consuming the diet. The PER is also not a true measure of efficiency since not all the protein is utilized for growth.

The purpose of a biologic assay is to determine the relative potency of a test protein compared with a standard protein. Hegsted and Chang[2] suggested that the slope-ratio assay using weight gain as the response and nitrogen intake as the measure of dose could be most satisfactory for the evaluation of the nutritive value of proteins. In this method, the relative nutritive value or potency of test proteins can be calculated by comparing the slopes of test proteins with that of a standard protein which is known to be of high biological protein.

In the current study, the relative nutritive values of soy and peanut protein were estimated using the growing rat slope-ratio assay.

MATERIALS AND METHODS

Diets

The protein-free diet contained a vitamin mixture, 0.5% ; a salt mixture, 5.0% (Teklad Test Diets, Madison, WI) ; choline chloride (50% solution), 0.4% (U. S. Biochemicals, Cleveland, OH) ; and corn oil, 5.0% cerelose, 89.1% (CPC International, Englewood Cliffs, NJ). The sources of three different proteins widely varying in protein quality ranging from high, intermediate to low quality were as follows : lactalbumin, U. S. Biochemicals, Cleveland, OH ; soy protein, Central Soya Company, Chicago, IL ; and defatted peanut grits, Gold Kist Inc., Lithonia, GA. Protein was added to the diet at the expense of equal weight of cerelose.

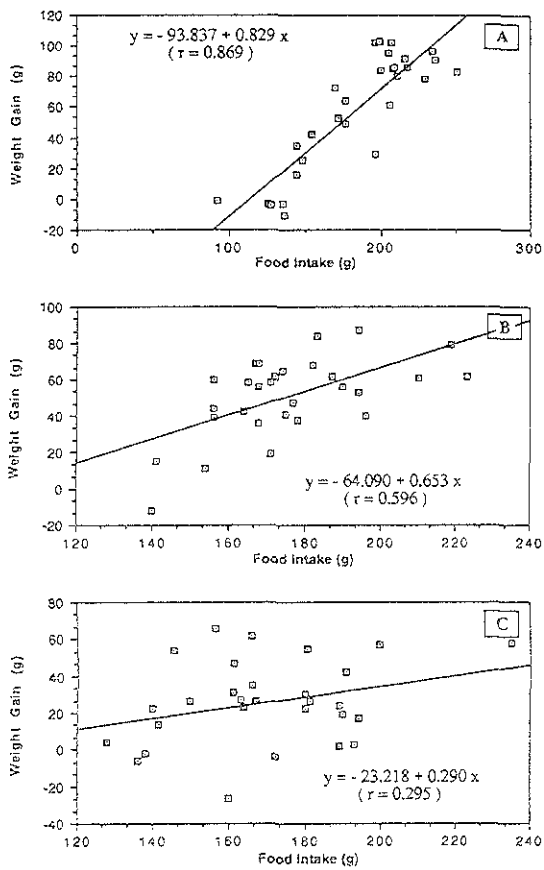

Fig. 1. Relationship between total food intake and weight gain of rats fed different protein diet. A : lactalbumin, B : soy protein, C : peanut protein.

Animals

Young male rats (64~72g, Holtzman Co., Madison, WI) were fed a 10% casein diet supplemented with 0.3% of L-methionine for 3 days prior to treatment group assignment. Three different proteins of varying protein quality (lactalbumin, soy and peanut protein) were observed in this study. A total of 95 rats were weighed and divided into 19 groups of similar weight, 5 rats per group. The average weight of the rats in each group was 69±3.3g. The rats were individually housed in stainless steel cages in a room with controlled temperature (23℃) and relative humidity (60%) as well as 12hr cycles of light and dark. One group received a protein-free diet and 6 groups received 6 different levels of lactalbumin ranging from 0.49% to 2.86% nitrogen ; another 6 groups, soy protein containing 1.03% through 3.65% nitrogen ; and the last 6 groups were fed a peanut protein diet whose nitrogen content varied from 0.74% to 2.20 % nitrogen. The protein levels were selected to cover the widest possible but the highest level below that which allowed maximum growth. Food and water were provided ad libitum for 14 days. Food consumption and weight gain were measured three times a week for each protein. Total nitrogen intake was calculated for each animal from the nitrogen content of the diet and the amount of food consumed.

RESULTS AND DISCUSSION

Table 1 shows daily food intake, daily weight gain and food efficiency ratio for rats fed the protein free

Table 1. Daily food intake, daily weight gain and the food efficiency ratio (FER) for rats fed the protein free diet[a)]

Total food intake (g)	Total weight gain(g)	Daily food intake(g/day)	Daily weight gain	Food efficiency ratio[b)]
149 ± 24.7	−15.6[c)]±0.55	10.70±1.970	−1.11±0.035	−0.107±0.018

[a)] All values represent the mean ± SD of five values. Rats were fed protein free diet over a 2 week time period.
[b)] Food efficiency ratio= Daily weight gain ÷ Daily food intake.
[c)] Negative values represent loss in weight.

Table 2. Dietary nitrogen content, total food intake, total nitrogen intake, and total weight gain of rats fed diets containing no protein, lactalbumin, soy protein and peanut protein[a]

Protein fed	Dietary nitrogen content (%)	Total food intake (g)	Total weight gain (g)	Total nitrogen intake (g)
Protein free	0.00	149.8±24.7	−15.6±0.5	0.00
Lactalbumin	0.49	123.0±16.1	−4.4±3.4	0.60±0.07
	1.30	158.0±22.0	26.3±6.2	1.62±0.23
	1.44	176.8±16.7	53.8±7.9	2.55±0.24
	1.97	228.2±14.3	85.8±5.9	4.49±0.28
	2.50	208.4± 5.2	88.6±7.7	5.21±0.13
	2.86	201.2±21.1	92.8±10.9	5.75±0.60
Soy protein	1.03	151.5±12.5	14.3±3.1	1.56±0.13
	1.50	174.5±13.3	38.8±1.7	2.61±0.20
	1.75	176.2±14.6	48.4±5.3	3.08±0.57
	2.07	168.4± 8.5	60.0±4.2	3.48±0.18
	2.80	202.4±18.8	65.8±6.7	5.66±0.65
	3.65	176.8±10.3	74.2±9.6	6.45±0.38
Peanut protein	0.74	151.8±15.3	−9.8±9.7	1.13±0.11
	1.23	168.9±28.3	7.7±6.3	2.07±0.35
	1.50	170.0±20.9	23.4±2.6	2.55±0.31
	1.72	166.8± 6.8	27.6±4.9	2.87±0.12
	2.00	190.3±26.7	53.2±7.5	3.81±0.53
	2.20	165.9±13.6	46.2±15.5	3.65±0.30

[a] All values represent the mean ± SD of five values, except for the rats fed 0.74% nitrogen peanut protein diet in which one rat was removed due to death. Rats were fed individual diet over a 2 week time period.

diet over a 2-week period. Although rats daily consumed a diet containing all the essential nutrients except protein on an average of 10.7g, these rats lost 1.11g per day, indicating that protein is the essential nutrient for the growth in rats. Thus, the protein-free diet showed a food efficiency ratio of −0.107 on an average.

A summary of intake-response data is given in Table 2. Group means are presented; however, individual values were used in the subsequent plotting and calculations. Generally, food intake reached a peak at dietary nitrogen level below that needed to maximize body weight gain. This indicates that rats overeat when dietary nitrogen intakes approaches the requirement[4]. Efficiency of protein utilization as measured by weight gain increased continuously with increasing dietary nitrogen levels. When food intake was plotted against total weight gain of rats fed lactalbumin, soy and peanut protein diet for 14 days, there was a positive correlation between the two (Fig. 1). The highest correlation between total food intake and total weight gain was observed for rats fed the lactalbumin diet (r=0.869) followed in decreasing order by soy (r=0.595) and peanut protein diet (r=0.295). It is noteworthy that most of rats consumed less peanut protein diet as compared to lactalbumin and soy protein diet.

The nitrogen dose-response curves with total weight gain as the response are shown in Fig. 2. The curve fit was accomplished by the second order polynomial regression. The asymptotic or sigmoid nature of these curves is apparent, particularly for lactalbumin and soy protein diet. Inspection of these curves indicates, however, that the central portions may be completely or nearly linear. Whether the response curve obtained with peanut protein diet is typical of very poor quality protein[5] or only representative of some unusual occurrence in this study needs further study.

In the slope-ratio assay, the potency is proportional to the slopes of the regression lines relating dose and response[2]. Regression analyses were done utilizing the points which appear to be in the linear range shown in Fig. 2. Some of the points which deviate sharply from the linearity were excluded

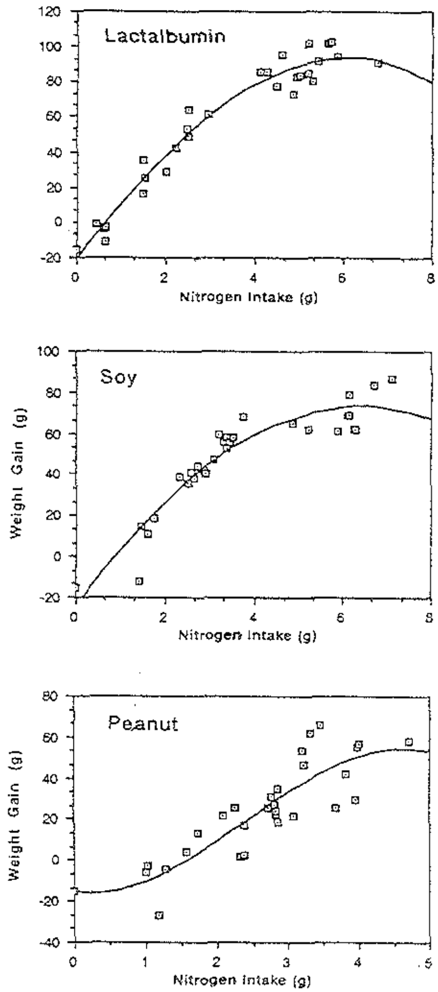

Fig. 2. Relationship between total nitrogen intake and weight gain of rats fed different protein diets. A : lactalbumin, B : soy protein, C : peanut protein.

from the computation. The regression lines for lactalbumin, soy and peanut proteins are shown in Fig. 3A, B and C respectively. Analysis of the slope-ratio assay gave the linear regression line represented by the equation Y=a+bx for each protein, Y being the response (total weight gain) and x, the total amount of N consumed by each rat. The ratio of the slope (b) for each test protein to that of the standard with high protein nutritive quality, in this case, lactalbumin, is a measure of its relative nutritive value (RNV). The data presented in Table 3 suggest that the most appropriate relative nutritive values for soy and peanut proteins, assuming lactalbumin has a relative value of 100, are 78.4 and 55.7 respectively. The value obtained for soy protein shows a somewhat higher value than that reported by Hegsted et al.[6] for full fat soya flour (59~64% RNV). In another report, Hegsted and Chang[2] found that the soy protein had a RNV of 34. Thus, it appears that the RNV value for soy protein can be substantially variable depending on variety, maturity, processing, etc. and also on the variations in the assay design, such as the number of animals per group, the number of levels of protein tested, and the length of time allowed for assay, etc[6]. On the other hand, RNV for peanut protein shows a very close agreement with the reported value of 57[7]. The reported biological values (BV) and net protein utilization (NPU) are included in Table 3 for comparison with RNV. Consideration should be taken that the BV of poor quality proteins appears to overestimate the nutritive value[2]. Both the BV and NPU show similar magnitude as RNV when they are expressed in relative terms, and give the same decreasing order of efficiency as RNV for the three test proteins. Thus, the adequacies of the slope-ratio method for the evaluation of protein quality are shared by the measurement of BV and NPU.

Table 3. Slope-ratio assay. Characteristics of the regression equation Y = a+bx for lactalbumin, soy and peanut protein

	lactalbumin	Soy Protein	Peanut Protein
Intercept(a)	−19.705	−19.365	−20.215
Slope(b)	28.421	22.208	15.817
Correlation coefficient(r)	0.971	0.971	0.816
SD of Y about regression line	6.539	3.548	7.274
Relative nutritive value or potency(%)	100	78.4	55.7
Biological value[a] (BV)	85[8]	66.4[9]	56[10]
Net protein utilization(NPU)[a]	89.1-94.7[11]	63.3[9]	39.4[5]

[a] Literature values.

The correlation coefficient (r) values for the

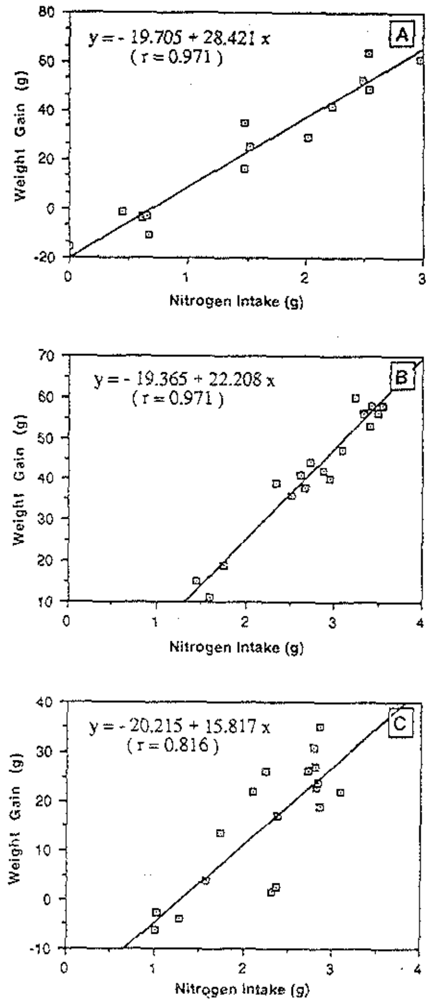

Fig. 3. Regression lines for lactalbumin, soy and peanut protein diet calculated through the points which appear to be in the linear range shown in Fig. 2.

regression lines are relatively high for all three proteins (Table 3, Fig. 3). Appreciable departure from linearity near the intercept can occur when the calculated intercept is lower than that found for the protein-free group. Also, at high levels of protein intake, the rate of growth, i. e., the efficiency of dietary protein utilization varies greatly among individual animals fed the same diet as shown in Fig. 2. Thus, it was necessary to eliminate the points which are above or below the linear curve.

The data and analyses presented in this study show that accurate and inherently valid growth assays of the nutritive values of proteins are feasible under well-designed and well-controlled study. Hegsted and Chang[2] suggested that the RNV of sloperatio assays appear to be most useful when the regression lines are substantially linear over a considerable range and this also fulfills the prime consideration for this assay.

REFERENCES

1. Mitchell, H. H. : A method of determining the biological value of protein. *J. Biol. Chem.*, **58**, 873 (1924)
2. Hegsted, D. M. and Chang, Y. O. : Protein utilization in growing rats. I. Relative growth index as a bioassay procedure. *J. Nutr.*, **85**, 159(1965)
3. Osborne, T. B., Mendel, L. B. and Ferry, E. L. : A method of expressing numerically the growth-promoting value of proteins. *J. Biol. Chem.*, **37**, 233 (1919)
4. Finke. M. D., DeFoliart, G. R. and Benevenga, N. J. : Use of a four-parameter logistic model to evaluate the protein quality mixtures of mormon cricket meal and corn gluten meal in rats. *J. Nutr.*, **117**, 1740 (1987)
5. Summers, J. D. and Fisher, H. : Net protein values for growing chickens as determined by carcass analysis : Exploration of the method. *J. Nutr.*, **75**, 435 (1961)
6. Hegsted, D. M., Neff, R. and Worcester, J. : Determination of the relative nutritive value of proteins. Factors affecting precision and validity. *J. Agric. Food Chem.*, **16**, 190 (1968)
7. Phillips, R. D. : Modification of the saturation kinetics model to produce a more versatile protein quality assay. *J. Nutr.*, **112**, 468 (1982)
8. Pearson, W. N. and Darby, W. J. : *Protein nutrition*, In *"Ann. Rev. of Biochemistry"*, Annual Reviews, Inc., Palo Alto, p.325 (1961)
9. Chalupa, W. and Fisher, H. : Comparative protein evaluation studies by carcass retention and nitrogen balance method. *J. Nutr.*, **81**, 139 (1963)
10. Mauron, J. : *Nutritional evaluation of proteins by enzymatic method.*, In *"Evaluation of Novel Protein Products"*, Bender, A. E., Kihlberg, R., Lofquist, B. and Munck, L. (eds.), Werner-Gren Center International Symposium series 14, Pergamon Press, Oxford (1970)
11. Skid, A. K. and Hegsted, D. M. : Evaluation of dietary protein quality in adult rats. *J. Nutr.*, **99**, 474 (1969)

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경사비율 방법에 의한 단백질의 영양가 평가

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요 약

서로 다른 3가지 단백질인 락타 알부민, 대두 및 땅콩 단백질의 영양가를 평가하기 위하여 각 단백질의 함량(0−35%)을 달리한 사료를 어린쥐에게 2주간에 걸쳐 먹였다. 섭량으로 전체 질소 소비량을 측정하여 이에 대한 체중 증가로 반응을 측정하였다. 직선 부위에 있는 단백질의 회귀분석에 대한 경사를 표준단백질인 락타 알부민의 경사와 비교한 결과 대두 및 땅콩 단백질의 상대적 성장지수는 각각 78.4 및 55.7로 나타났다. 본 실험의 결과 경사 비율방법은 잘 계획된 실험 조건하에서 단백질의 영양가를 평가하는데 매우 유용한 방법이라고 사료된다.