A 28-h Method for Estimating Protein Nutritional Quality[†,‡]

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A rapid method for evaluating protein nutritional quality based on the determination of weight loss in rats after a 4-h fast subsequent to a 24-h feeding is presented. Protein nutritional quality as estimated by the classical method of Miller and Bender was compared with quality estimated by the rapid procedure by evaluating 46 samples from different sources. The results obtained with the proteins tested could be described by a unique regression equation that satisfactorily fitted the experimental data. A direct semiquantitative correspondence of protein quality with high, good, and low nutritional values and the percent weight loss is established. An empirical criterion based on the percent weight loss during the fast period being greater than that for the previous 24 h of the feeding period is also introduced for the evaluation of protein with very low nutritional quality.

Several methods have been proposed for dietary protein quality evaluation (Bodwell, 1977). The food industry needs rapid methods for protein quality evaluation in processing products and process development (Nesheim, 1977). A rapid method (28 h) for estimating animal or plant protein quality has been developed (Farina et al., 1977). This method is based on the weight lost by rats after a 4-h fast subsequent to a 24-h feeding period with the protein diet. The rapid method has not been widely used in the nutritional area probably because of the question of the biological validity of the values generated. Data based on 24-h body weight change during test diet feeding followed by a 4-h fast are limited. However, high correlations were demonstrated by Farina et al. (1977) for five animal and nine vegetable proteins between weight loss and nutritional quality obtained by accepted procedures, such as net protein utilization (NPU) and biological values (BV). Our laboratory collaborated with different food technology groups in determining the nutritional quality of a variety of proteins by the conventional method of Miller and Bender (1955). In parallel, we have been performing the rapid method in some samples to avoid the laborious techniques of the formal quantification of biological values and/or net protein utilization. The results reported in the present work support the concept that the simple test of percent weight loss in rats may be used to evaluate the nutritional quality of proteins in close agreement with classical methods (Miller and Bender, 1955).

MATERIALS AND METHODS

Sprague-Dawley rats of our laboratory of either sex and aged 30–33 days, with an average weight of 60 ± 1 g, were used. Animals were housed individually in screen-bottom cages in a temperature-controlled room at 21 ± 1 °C with a 12-h light–dark cycle.

Determination of Protein Quality. (A) *The rapid method* was performed as described by Farina et al. (1977). Briefly, rats were fed a protein-free diet for 10 days. The animals were weighed, and those that had lost about 25% of their initial weight (about 90% of the rats) were then divided into groups of six rats each. They were then fasted for 4 h and weighed (W_0). The weight lost over this first fasting period was about 0.6–1.0 g. Test diets and water were then offered ad libitum for 24 h, starting at midday. At the end of the feeding period, diets were withdrawn and the animals reweighed (W_1). After a 4-h fast, they were weighed again (W_2) to determine the weight lost over that time ($W_1 - W_2$). Weight loss (WL) was then expressed as the percentage of the weight gained during the 24-h feeding period ($W_1 - W_0$) according to the formula

$$WL\% = (W_1 - W_2)/(W_1 - W_0) \times 100$$

(B) *The classical method* of Bender and Miller (1953) was performed to determine NPU using body water as an index of body nitrogen (Miller and Bender, 1955). Three groups of four rats each were used for each sample of protein during 10 days, and biological values (BV = NPU/digestibility) were calculated according to the method of Miller and Payne (1963).

Diet composition was 4 g of salt mixture (Phillips and Hart salt mixture, ICN Nutrition Biochemical, Cleveland, OH) 2.2 g of vitamin mixture (Vitamin Diet Fortification, ICN Nutrition Biochemical), and 5 g of lipid (corn oil). The protein under study was incorporated to obtain a 10-g level. A mixture of wheat starch and sucrose (1:1) was added so as to complete 100 g of the diet.

Dietary Proteins. Wheat gluten from ICN Nutritional Biochemical was used because the biological values of wheat gluten from Argentine commercial sources were higher than those reported for this protein. Other sources of proteins are described in Table II.

Statistics. The results were expressed for the rapid method as a mean of six rats ± SD and in the classical method as a mean of three groups of four rats each ± SD. The Pearson correlation test was used for correlation analysis.

Other. The nitrogen content of each protein source was determined according to the Kjeldahl method (AOAC, 1980).

RESULTS

Food Intake and Body Weight Changes during Feeding and Fasting Periods. In Table I are given the mean values of each group of rats under experimentation to show (i) the calculation of WL% ± SD and (ii) the increase of ±SD as a function of the decrease in protein nutritional quality. This possibility was not considered in the original paper of Farina et al. (1977). The analysis

Table I. Determination of Nutritional Values with the Rapid Method[a]

protein	$(W_1 - W_0)$, g	$(W_1 - W_2)$, g	WL%[c]	food intake, g/24 h
defatted egg	6.5 ± 0.4	2.2 ± 0.2	33.7 ± 2.9	8.9 ± 0.6
sunflower isolate	3.0 ± 0.6	1.4 ± 0.2	49.8 ± 6.8	8.2 ± 0.4
wheat gluten[b]	0.9 ± 0.2	1.0 ± 0.3	110.5 ± 24.3	6.9 ± 0.9

[a] Values are means ± SD. [b] ICN Nutrition Biochemical. [c] WL $= W_1 - W_2/W_1 - W_0$.

of the individual values allows us to calculate the statistical significance of the difference between two dietary proteins under assay, as will be shown in the accompanying paper (Sammán et al., 1993). As can be seen in Table I, the body weight gain and the weight loss during the fasting period were clearly different when the rats were fed high, intermediate, or low balanced proteins such as egg, sunflower isolate, and wheat gluten, respectively. This behavior is the basic phenomenon of the rapid method. In the case of wheat gluten the weight loss was higher than the weight gained during the previous 24 h of feeding.

Comparison between the Rapid and Classical Methods. Table II shows the nutritional values obtained with the rapid method (Farina et al., 1977) and the method of Miller and Bender (1955) for several protein preparations, protein supplemented with limiting amino acids, and different protein complementation. In both methods a 10% protein level was used. The semilogarithmic plot of the BV and NPU values vs the percentual weight loss (WL%; Figure 1) gave straight lines described by the following equations obtained by regression analysis:
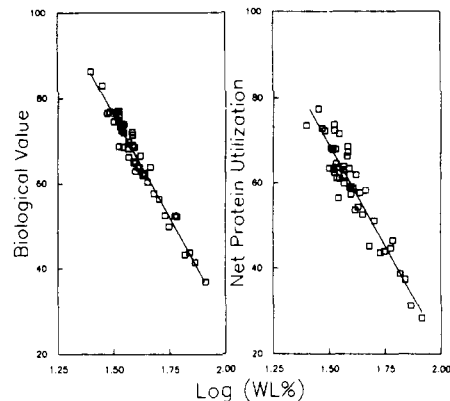
$$BV = 219.5 - 95.6 \log (WL\%) \qquad (1)$$

$$NPU = 211.8 - 95.1 \log (WL\%) \qquad (2)$$

The Pearson correlation coefficients (r^2) obtained for VB and NPU are 0.96 and 0.89, respectively, indicating that 4 and 11% are the percentages of the total variation in VB and NPU not due to WL%. The dispersions of the individual values around the straight line are 2.19 and 3.92 for VB and NPU, respectively. The values of 0.82 (VB) and 1.48 (NPU) were found for half of the maximum amplitude of the 95% joint confidence intervals.

The data obtained by Farina et al. (1977) were fitted by equations similar in form to eqs 1 and 2, although with different parameters (slope and y-axis intercept). The fitting procedure of Farina et al. (1977) was also dependent on the origin of the protein, i.e., plant or animal. In our case, the equation that fitted the experimental data was independent of the protein source. Furthermore, equations similar to eqs 1 and 2 were found when the data of Farina et al. (1977) from plant and animal proteins were pooled (except the sample of wheat gluten with log WL% = 2.04, see below) and replotted and the fitting procedure performed again: BV = 241.1 − 107.6 log (WL%), r^2 = 0.78; NPU = 204 − 89.9 log (WL%), r^2 = 0.5184. It appears that the low correlation coefficients (r^2) observed by Farina et al. (1977) are mainly due to (i) the small amount of data processed and (ii) the diet protein range used (from 7.1 to 23.8%). In the present work, only one protein concentration (10%) was used.

Limitations in the Application of the Rapid Method. Values of WL% ≥100 (log WL% ≥ 2) imply greater weight loss over the 4-h fasting period than weight gained in the previous 24-h feeding period. According to eqs 1 and 2 these values correspond to BV ≤ 28.3 and NPU ≤ 21.3, respectively. In our experience, when the biological

Figure 1. Relationship between nutritional protein quality and log WL%. Data of BV and NPU determined by the classical method were plotted as a function of log of WL%. The values of BV, NPU, and WL% are from Table II. Pearson correlation coefficients (r^2) obtained for BV and NPU were 0.96 and 0.89, respectively.

qualities of the proteins tested were close to these BV and NPU values, the WL% values were higher than 100 or, as in the case of bean flour and calabash flour, the rats lose weight during the 24-h feeding period (Table III). These samples were not included in the data of Figure 1.

DISCUSSION

The main result obtained in the present work is that the nutritional quality of a protein can be rapidly and semiquantitatively evaluated by a method based on percent weight loss in rats subjected to consecutive feeding and fasting periods. A strong indication of the applicability of the method proposed is shown in Table II. Similar values of BV and NPU, according to eqs 1 and 2, were obtained with respect to the BV and NPU values determined by classic method (Miller and Bender, 1955). The method proposed for evaluating protein quality presents the following advantages when compared with the classical approach: (i) it is simple and substantially faster than the existing methods, provided one has available a supply of rodents that have been depleted of protein for the prescribed 10 days; (ii) only small amounts of sample are required for feeding trials. Comparatively, 4–6 vs 50–72 g of protein are required by the rapid and classical methods, respectively. These are highly attractive features for food technologists.

Application of the WL% Values in the Evaluation of Nutritional Quality of Proteins. The experimental data shown in Table II and Figure 1 could be expressed as BV or NPU values by applying eqs 1 and 2 (see text). Most of the protein sources tested showed values of BV that were within a relatively narrow range; there are relatively few estimates of BV below 55 or above 80 (Figure 1). This may appear to be somewhat unsatisfactory because one can question if there is sufficient power to determine what the true uncertainty is in estimates of the quality of a protein with high or low BV. Additionally, the SDs for WL% are larger than those for BV determined by the classical method (Table II). However, the SD for WL% is calculated from six individual values, whereas the SDs for BV and NPU are determined from means of three groups of four rats each; therefore, these SD values are not strictly comparative. The minimal number of animals by group or number of groups that would be required to discriminate between nutritional differences by the rapid method is under study.

The degree of repeatability of the rapid method for BV was investigated on several independent lots (n) of six

Table II. Values of Nutritional Quality of Proteins Obtained by Rapid and Classical Methods[a]

protein	classical method BV	NPU	digestibility, %	rapid method WL%	food intake, g/24 h
animal origin					
casein (ICN)	74.0 ± 1.4	71.7 ± 1.1	96.9 ± 0.7	35.1 ± 3.9	8.1 ± 0.4
casein (Sancor)	69.2 ± 2.0	67.4 ± 1.8	96.4 ± 1.1	38.4 ± 8.8	8.1 ± 0.5
meat (boiled, freeze-dried)	71.6 ± 2.4	68.7 ± 2.3	95.9 ± 0.2	38.3 ± 4.3	8.4 ± 0.3
sardine in oil (canned, *S. maris*)	77.1 ± 5.2	67.9 ± 4.8	87.9 ± 1.6	32.9 ± 5.1	13.2 ± 0.8
anchovies, freeze-dried	77.1 ± 1.6	73.9 ± 0.1	95.8 ± 2.1	33.2 ± 3.8	9.2 ± 0.6
sardine in oil (canned)[b]	68.7 ± 1.0	63.4 ± 0.9	92.3 ± 0.4	39.1 ± 5.6	12.6 ± 0.9
sardine in oil (canned)[b]	62.4 ± 2.5	57.7 ± 2.0	92.6 ± 0.7	43.5 ± 6.0	11.0 ± 0.8
sardine in oil (canned)[b]	72.0 ± 1.0	66.4 ± 1.0	91.8 ± 1.3	38.2 ± 6.0	10.6 ± 1.5
sardine in oil (canned)[b]	62.9 ± 2.9	59.1 ± 1.8	93.9 ± 1.8	39.6 ± 4.9	12.1 ± 0.6
fish meal cooky	72.0 ± 1.7	61.2 ± 1.2	95.9 ± 0.9	35.1 ± 4.3	8.4 ± 0.5
cows' milk powder (Sancor)	86.3 ± 1.5	73.6 ± 0.9	85.3 ± 0.8	25.0 ± 3.6	8.7 ± 0.5
caseinate of calcium	66.6 ± 1.4	62.0 ± 1.3	93.1 ± 1.5	41.7 ± 10.8	8.9 ± 1.3
cheese whey HPLD[c]	83.0 ± 1.8	77.5 ± 1.1	93.3 ± 0.4	28.2 ± 3.3	8.2 ± 0.5
cheese whey HPHD[c]	76.0 ± 2.6	72.5 ± 2.9	95.4 ± 0.3	33.3 ± 3.9	9.4 ± 0.6
vegetable origin					
wheat grits	63.8 ± 5.9	53.6 ± 4.7	84.0 ± 2.6	41.3 ± 7.6	7.4 ± 1.2
vermicelli[d]	64.0 ± 1.3	58.8 ± 1.4	91.7 ± 2.0	40.5 ± 7.3	11.1 ± 1.0
vermicelli[e]	66.3 ± 5.3	61.8 ± 3.4	93.1 ± 1.0	36.8 ± 7.1	10.6 ± 0.8
vermicelli[f]	68.9 ± 2.9	62.6 ± 1.5	90.8 ± 1.7	33.3 ± 5.8	8.7 ± 0.6
vermicelli[g]	76.8 ± 1.2	72.4 ± 1.2	94.2 ± 0.6	30.2 ± 5.9	8.0 ± 0.4
wheat meal	62.9 ± 2.7	58.3 ± 1.5	84.2 ± 0.3	46.1 ± 5.4	7.4 ± 0.8
wheat meal[h]	76.6 ± 2.8	72.9 ± 1.0	95.9 ± 0.7	29.5 ± 4.7	7.8 ± 0.8
bread (commercial, dried 60 °C)	52.5 ± 1.9	44.6 ± 1.1	85.1 ± 0.8	59.6 ± 12.8	7.8 ± 0.8
bread	60.4 ± 1.7	52.5 ± 0.9	86.9 ± 0.7	44.7 ± 7.8	6.3 ± 0.3
bread[i]	70.0 ± 2.3	63.9 ± 0.9	91.4 ± 0.8	36.8 ± 7.2	7.6 ± 0.8
bread[j]	73.4 ± 1.9	68.1 ± 0.8	92.8 ± 0.2	33.8 ± 7.7	7.6 ± 0.2
flax isolate	62.0 ± 0.7	54.3 ± 0.4	87.6 ± 1.5	42.7 ± 6.4	8.0 ± 0.6
flax meal	49.8 ± 1.1	43.9 ± 0.6	88.1 ± 0.5	56.0 ± 9.4	7.5 ± 1.3
sunflower meal	57.7 ± 2.2	45.1 ± 1.5	78.3 ± 1.4	47.9 ± 5.2	8.0 ± 0.7
soybean meal (integral)	36.9 ± 0.4	28.3 ± 0.1	76.7 ± 1.3	81.9 ± 16.5	7.4 ± 0.8
soybean meal (husker)	41.5 ± 0.4	31.2 ± 0.5	75.2 ± 0.5	73.2 ± 8.3	8.3 ± 1.1
soybean meal (inactivated partially)	56.4 ± 2.4	51.1 ± 0.1	90.6 ± 1.8	50.5 ± 4.6	8.1 ± 0.7
soybean milk spray-dried	68.4 ± 1.9	63.1 ± 2.0	92.2 ± 0.8	36.7 ± 3.3	8.9 ± 0.9
soybean milk jam[k]	74.9 ± 2.8	63.3 ± 3.7	84.5 ± 1.0	33.1 ± 7.4	9.2 ± 0.4
soybean (autoclaved, 10 min)	74.1 ± 2.5	68.3 ± 1.5	89.1 ± 1.1	34.5 ± 2.9	9.0 ± 0.6
soybean (predigested, defatted)[l]	68.7 ± 3.1	56.5 ± 1.0	82.7 ± 1.4	34.9 ± 5.4	7.9 ± 0.4
soybean (predigested)[l]	65.3 ± 1.1	57.3 ± 1.0	87.8 ± 0.1	39.6 ± 3.4	8.4 ± 0.4
soybean with lactic fermentation[l]	76.6 ± 2.4	68.3 ± 2.0	89.1 ± 1.1	32.2 ± 3.4	9.5 ± 0.6
rice 70%, soybean 30%	68.2 ± 2.2	60.0 ± 1.7	87.8 ± 0.5	36.8 ± 5.3	8.0 ± 0.6
rice 60%, soybean 40%	72.6 ± 3.2	61.2 ± 1.4	84.3 ± 1.5	34.2 ± 4.8	7.8 ± 0.5
rice 50%, soybean 50%	74.6 ± 1.4	63.5 ± 1.1	85.0 ± 1.0	31.7 ± 3.9	7.8 ± 0.6
corn 85%, soybean 15%	74.0 ± 4.4	64.6 ± 4.0	87.3 ± 1.2	34.0 ± 6.2	10.3 ± 1.8
Lucerne leaf protein concentrated	43.3 ± 1.9	38.7 ± 0.9	89.4 ± 0.7	65.9 ± 7.2	7.6 ± 1.1
Lucerne leaf protein concentrated plus Lys 0.6%, Met 0.4%	65.0 ± 1.8	59.0 ± 1.2	90.7 ± 0.9	39.0 ± 4.2	8.3 ± 0.7
wheat gluten (unpure)[m]	52.3 ± 2.2	46.5 ± 1.2	88.9 ± 0.8	60.9 ± 6.7	7.1 ± 0.7
zein plus Trp 0.2%, Lys 1.5%	43.8 ± 3.2	37.4 ± 1.7	85.4 ± 0.7	69.2 ± 8.2	7.0 ± 0.8
miscellaneous					
bakers' yeast (commercial)	52.7 ± 3.5	43.6 ± 2.0	83.0 ± 1.5	53.7 ± 7.6	7.9 ± 0.8

[a] Classical method: mean of three groups of three rats each ± SD. Rapid method mean of six rats ± SD. [b] Canned sardine in oil from Cascabel factory sterilized at different time–temperature ratios. [c] Cheese whey: from coagulation of milk to make cheese, concentrated by ultrafiltration, and dried at different temperature. HPLD: High protein (55–70%) low denaturalization (60 °C, 30 min), % protein 58.6. MPHD: Medium protein (40–55%), high denaturalization (80 °C, 30 min), % protein 49.1. [d] Elaborated with 94% wheat grits and 6% cheese whey MPHD, mixture containing 5.3% protein. [e] Elaborated with 91% wheat grits and 9% cheese whey MPHD, mixture containing 18.3% protein. [f] Elaborated with 59% wheat grits and 41% cheese whey MPHD, mixture containing 33.4% protein. [g] Elaborated with 92% wheat grits and 8% cheese whey HPLC, mixture containing 18.0% protein. [h] Wheat meal supplemented with 6% cheese whey LDHP. [i] Elaborated with wheat meal supplemented with 4% cheese whey HPLD. [j] Elaborated with wheat meal supplemented with 6% cheese whey HPLD. [k] Sweetmeal or candy prepared with soybean milk and cheese whey (Hernandez et al., 1981a). [l] Hernandez et al. (1981b). [m] Wheat gluten from Argentine commercial source is not totally pure (Samman and Farias, 1981).

animals each one fed with three different proteins, i.e., casein, sunflower isolate, and soybean. The results obtained (expressed as mean values ± SD) were as follows: (i) casein, 69.0 ± 2.4 (*n* = 7); (ii) sunflower isolate, 66.1 ± 1.7 (*n* = 4); (iii) soybean, 70.1 ± 1.6 (*n* = 5).

A direct discernment of the nutritional quality of a protein using the WL% values is justified by the close relation between biological quality (evaluated by BV or NPU) and log (WL%) (Figure 1). As can be seen in Figure 1, when the weight loss during the fasting period is about 50% of the weight gained during the previous feeding period (WL% = 50; log WL% = 1.69), the NPU is about

50. This value would be the lower limit for a protein with good biological quality.

Figure 2 shows a simple and practical way of informing food technologists about the nutritional quality of a protein analyzed by the rapid method, assigning high, good, and low nutritional quality (NQ) to proteins with WL% values lower than 35, in the range from 35 to 50, and higher than 50, respectively. Figure 2 shows the corresponding values of VB and NPU. Although the distinction between high, good, and low nutritional quality is arbitrary, it is the usual way of reporting the results of evaluating nutritional protein quality of food for nonacademic purposes.

Table III. Weight Values and NPU and Digestibility Values of Proteins with Very Low Nutritional Quality Obtained by Rapid and Classical Methods, Respectively[a]

protein	$(W_1 - W_0)$, g	$(W_1 - W_2)$, g	NPU	digestibility, %	food intake, g/24 h
zein	0.5 ± 1.0	1.3 ± 0.6	28.3 ± 1.5	85.3 ± 0.1	5.3 ± 1.1
calabash flour	weight loss during 24-h feeding period		27.9 ± 5.0	87.3 ± 2.3	5.4 ± 2.0
bean flour	weight loss during 24-h feeding period		weight loss during 10 days of assay		3.3 ± 0.5
peanut flour	0.3 ± 0.6	1.9 ± 1.0	26.1 ± 1.4	91.7 ± 0.4	4.2 ± 0.8
miller corn	0.8 ± 0.7	1.6 ± 0.9	30.4 ± 3.0	85.9 ± 2.0	6.4 ± 1.2
wheat gluten (ICN)	0.9 ± 0.2	1.0 ± 0.3	30.8 ± 1.2	90.8 ± 1.0	6.9 ± 0.9

[a] Weight changes (mean ± SD for six rats) were determined by the rapid method; NPU and digestibility values (mean ± SD for three groups of four rats each) were determined by the classical method as described under Materials and Methods.
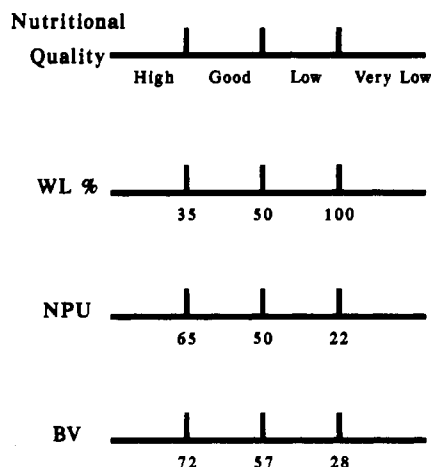
Figure 2. Association between nutritional quality (NQ) of proteins and WL%, NPU, and BV indices. The ranges of NPU and BV were calculated using eqs 1 and 2.

Table IV. Agreement between WL% and Classical Methods for Protein Classification[a]

(A) Biological Values (BV)

	WL%		
classical method	high	good	low
high	16	1	0
good	2	18	0
low	0	0	9

(B) Net Protein Utilization (NPU)

	WL%		
classical method	high	good	low
high	11	3	0
good	7	15	1
low	0	1	9

[a] The data are from Table II and were classified according to the range of nutritional quality given in Figure 2.

To show the agreement between the rapid and classical methods (Miller and Bender, 1955) for protein classification, the evaluated proteins from Table II were grouped into high, good, and low nutritional quality according to the criteria given in Figure 2. From 46 samples analyzed for BV, 43 of them could be grouped, into high (16), good (18), and low (9) quality (Table IVA). The agreement was measured as described by Cohen (Bislop et al., 1976) corresponding to values of $K = 0.91$ (BV) and 0.60 (NPU) with asymptotic variances of 0.003 and 0.007, respectively.

The observation pointed out in Table III gives an additional empirical association between values of WL% higher than 100 and protein of very low nutritional quality. This fact is also included in Figure 2.

From Figure 1, attention should be paid to the fact that two different nutritional parameters such as NPU and biological values are similarly correlated with log (WL%), since the biological values include the digestibility de-

termination which will be particular for each sample (BV = NPU/digestibility). A possible explanation is that the majority of samples (43 of 46) showed a variation of digestibility values in a narrow range (96–83) (Table II), thus giving biological values higher than NPU values in a uniform fashion. Nevertheless, Figure 1 and Table IV show higher r^2 and K values for BV than for NPU, suggesting that the digestibility of the protein affects or is indirectly evaluated by the WL% parameter used in the determination of the nutritional protein quality.

Physiological Basis of the WL% Method and Other Parameters Influencing Its Application. It has been stated that excess of amino acids will accumulate in tissues in protein-depleted rats, provided a dietary protein imbalance occurs. During fasting, following the feeding period, the increased water loss observed in imbalanced animals may be related to the excretion of the excess of amino acids (Sanahuja and Rio, 1968). From a physiological point of view, this is the main assumption of the rapid method.

Previously, the dependence of WL% on protein concentration in the diet was studied (Farina et al., 1984). Both protein concentration and protein quality influenced the values of WL% obtained. As BV decreased, WL% became progressively more dependent on protein concentration. A protein concentration in the diet of about 10% is adequate because the method is sensitive enough at this concentration, which can be achieved even from plant sources usually characterized by low protein contents.

The influence of dietary fat concentration on the determination of protein nutritional quality was studied with the rapid method (Sammán and Farias, 1981). A 5% fat supplement in the basal diet was employed. Some foods supply an additional amount of fat to the diet. For example, an egg, in which the protein/fat ratio is as high as 1.2, increases the dietary fat supplement up to about 10%. In soybean this ratio is 0.5, an increase of 5%. The fat content in the experimental diet ranged from 5 to 15%. Using this fat concentration range, we reported that neither corn oil, hydrogenated fat supplementation (which has a very different essential fatty acids content) nor fat-free diet influences the biological values obtained by the rapid method. Moreover, when the protein tested was previously defatted, the results were very similar. Hydroperoxides as well as their products of decomposition could increase during food processing. In this connection, we also showed that the high content of peroxides in the fat supplementation appears to have no effect on the biological values determined following a 24-h feeding period (Sammán and Farias, 1981).

Nutritional Quality Index. On the basis of the data presented, the rapid method developed in this work may be used as a screening procedure if one wishes a rapid, semiquantitative measurement of the major impact of a particular food process on protein quality. Protein quality is proposed to be classified into one of four grades (Figure 2), the upper and lower limits of which may be precisely

enough evaluated through the rapid method reported here. The application of the nutritional quality (NQ) index to technological process is shown in the accompanying paper (Sammán et al., 1993).

ABBREVIATIONS USED

BV, biological values; NPU, net protein utilization; NQ, nutritional quality; W_0, initial weight (in g); W_1, weight after the 24-h feeding period (in g); W_2, weight after the 4-h fast (in g); $(W_1 - W_0)$, weight gain during the 24-h feeding period; $(W_1 - W_2)$, weight loss over 4-h fast; WL, weight loss.

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LITERATURE CITED

AOAC. *Official Methods of Analysis*, 13th ed.; Horwithz, W., Ed.; AOAC: Washington, DC, 1980.

Bender, A. E.; Miller, D. S. A new brief method of estimating net protein value. *Biochem. J.* **1953**, *53*, VII–VIII.

Bislop, Y. M. M.; Frienberg, S. E.; Holland, P. W. *Discrete Multivariate Analysis. Theory and Practice*, 2nd ed.; MIT Press: Cambridge, MA, 1976.

Bodwell, C. E. Problems associated with the development and application of rapid methods of assessing protein quality. *Nutr. Rep. Int.* **1977**, *16*, 163–168.

Farina, R.; Rio, M. E.; Sanahuja, J. C. A rapid method for the evaluation of protein quality. *Nutr. Rep. Int.* **1977**, *16*, 293–303.

Farina, R.; Rio, M. E.; Sanahuja, J. C. A rapid method for the evaluation of protein quality: effect of protein concentration. *Nutr. Rep. Int.* **1984**, *20*, 693–699.

Hernandez, J. L.; Adris, J.; de Rank, E. F.; Farias, R. N.; Sammán, N. Soybean milk jam. *Aliment. Latinoam.* **1981a**, *132*, 70–71.

Hernandez, J. L.; Adris, J.; de Rank, E. F.; Farias, R. N.; Sammán, N. Predigested soybeans. *J. Am. Oil Chem. Soc.* **1981b**, *58*, 510–511.

Miller, D. S.; Bender, A. E. The determination of the net utilization of protein by a shortened method. *Br. J. Nutr.* **1955**, *9*, 382–388.

Miller, D. S.; Payne, P. R. A theory of protein metabolism. *Theor. Biol.* **1963**, *5*, 398–403.

Nesheim, R. O. Food industry requirements for rapid methods of protein quality assessment. *Nutr. Rep. Int.* **1977**, *16*, 157–162.

Sammán, N.; Farias, R. N. Effect of dietary lipids on evaluation of protein quality during 24 hour feeding period. *Nutr. Rep. Int.* **1981**, *29*, 411–418.

Sammán, N.; Molina, D. E.; Perotti de Galvez, N. I.; Calleri, D. A.; Farias, R. N. Application of a 28 hour-method for monitoring nutritional protein quality changes during single-cell protein processing. *J. Agric. Food Chem.* **1993**, following paper in this issue.

Sanahuja, J. C.; Rio, M. E. Effect of imbalanced diets containing natural proteins on appetite and body composition in the rat. *J. Nutr.* **1968**, *95*, 295–302.