PROTEIN QUALITY OF SPRAY-DRIED WHOLE MILK AND OF CASEIN IN YOUNG ADULT HUMANS USING A SHORT-TERM NITROGEN BALANCE INDEX ASSAY

R. BRESSANI, D. A. NAVARRETE, V. A. L. DE DAQUI, L. G. ELIAS, J. OLIVARES and P. A. LACHANCE

ABSTRACT

Studies were performed to measure the protein quality of spray-dried whole milk and of casein using a short-term nitrogen balance index with adult human subjects. Comparative values were obtained using the conventional nitrogen balance assay technique. The quality of the protein was calculated by regression analysis of all nitrogen absorbed to nitrogen retained. The coefficients of regression for milk when the short-term approach was used were 1.06, 0.97, 0.91 and 0.88. The differences were not statistically significant. With the conventional method, the value obtained was 0.88. The value from the conventional method was not statistically different from those obtained by the short-term variation, which has a tendency to give higher values. The coefficient of regression of nitrogen absorbed to nitrogen retained for casein was 0.65. The results also suggested less variation if the nitrogen balances per protein intake are of two days duration, allowing for more representative fecal collections. The results obtained suggest the short-term nitrogen balance index to be a suitable approach to protein quality evaluation in humans, although we recognize the need to perform additional studies to standardize the technique as much as possible.

INTRODUCTION

PROTEIN QUALITY ASSAYS in human subjects are difficult and costly to carry out mainly because of the time required to run a test. The protein quality assay offering various advantages is the Nitrogen Balance Index (NBI) developed in well standardized adult dogs by Allison and Anderson (1945). This method prescribes the feeding of 3 to 4 levels of protein in the region of the nitrogen intake-nitrogen balance relationship which is a straight line. Unlike the classical biological value assay, the NBI procedure does not require the feeding of a nitrogen-free diet; however, when feeding adult subjects, it is necessary to do so in order to hasten adaptation of the individuals to the low nitrogen intakes used in this assay.

Even though our experience has suggested a very high correlation of protein quality values between animal and human results when the same assay is used in both types of subjects (Bressani et al., 1973; Bressani, 1977), it is desirable to obtain values directly from human subjects. As indicated above, human assays are of a relatively long duration, which makes them costly and subject to increased variability. Nitrogen Balance Index studies in dogs, calculated from 1 and 4-day nitrogen balance data (Bressani et al., 1978), suggested the possibility of shortening the time period on the NBI assay. Although the 1-day calculation gave slightly higher values than the 4-day calculation, they were not statistically different. The results of those studies also indicated the possibility of eliminating from the assay the adaptation period previous to the balance period proper. The modified short-term NBI technique has now been tested in young adult human subjects with relatively good and acceptable results. In the first paper, the short NBI test reported on the protein quality for whole egg (Navarrete et al., 1977), the values were similar to those obtained by the conventional assay as performed in our laboratories as well as when compared to values reported from other laboratories.

In the present report, spray-dried whole milk protein and casein were tested by the short and conventional NBI assay procedure.

Authors Bressani, Navarrete and Elias are with the Division of Agricultural & Food Sciences, Institute of Nutrition of Central America & Panama (INCAP), Calzada Roosevelt, Zone 11, Guatemala City, Guatemala, C.A. Author De Daqui was a postgraduate student of the Center for Higher Studies in Nutrition & Food Sciences/INCAP; and author Olivares a student of the School of Nutrition of INCAP. Author Lachance is Professor of Nutritional Physiology, Rutgers State U., New Brunswick, NJ.

0022-1147/79/0004-1136$02.25/0

Table 1—Basal diets

Breakfast 07:30 hr	Orange drink, Toast, Margarine, Apple jelly, Coffee or tea, Sugar	Grape drink, Toast, Margarine, Pineapple jelly, Coffee or tea, Sugar	Orange drink, Toast, Margarine, Honey, Coffee or tea, Sugar
Snack 09:30 hr	Vanilla cookies, Carbonated drink	Vanilla cookies, Carbonated drink	Vanilla cookies, Carbonated drink
Lunch 12:00 hr	Garlic soup, Toast, Margarine, Grape drink, Pineapple	Celery soup, Toast, Margarine, Orange drink, Pear	Onion soup, Toast, Margarine, Grape drink, Pineapple
Snack 15:00 hr	Applesauce	Apple	Applesauce
Supper 18:00 hr	Onion soup, Toast, Margarine, Orange drink, Lemon pudding	Herb soup, Toast, Margarine, Grape drink, Strawberry pudding	Celery soup, Toast, Margarine, Orange drink, Lemon pudding
Snack 20:00 hr	Vanilla cookies	Apple cookies	Vanilla cookies

MATERIALS & METHODS

Proteins

For the present study, two protein foods were tested. The first was a whole powdered cow's milk manufactured by a Central American dairy industry by spray-drying. This product contained 26.4% protein, 37.7% lactose, 26.0% fat, 6.9% mineral matter and 3.0% moisture. Liquid milk was prepared daily with distilled water and served cold or as cream of tomato soup to the subjects. Aliquots were taken from each batch for nitrogen determinations. The second protein used was ANRC casein, representing a commercial product utilized as a reference protein in experimental animal assays for protein quality (human grade).

The granular texture and insolubility of the casein presented difficulties in the preparation of an acceptable product for the subjects to eat. A pudding recipe was developed with fruit juices and artificial colors which had good palatability. The formulation used

Table 2—Apparent nitrogen balance of young men fed whole milk—short-term, Experiment 1-A

Protein level (g/kg)	Intake	Urine	Fecal	Absorbed	Retained
	(mg N/kg/day)				
0.0	10.8 ± 0.5	57.7 ± 0.2	22.8 ± 0.5	−11.4 ± 0.9	−72.0 ± 0.3
0.1	26.0 ± 0.5	42.6 ± 3.4	35.3 ± 13.0	− 9.2 ± 13.1	−55.7 ± 10.2
0.2	43.4 ± 1.6	45.9 ± 7.6	25.7 ± 2.5	19.7 ± 4.2	−26.2 ± 4.0
0.3	56.7 ± 0.4	56.5 ± 3.5	28.1 ± 1.2	28.6 ± 1.4	−29.6 ± 3.4
0.4	74.7 ± 1.7	52.8 ± 6.3	15.4 ± 4.3	59.3 ± 4.5	6.4 ± 5.5
0.3	58.7 ± 1.7	46.1 ± 5.7	27.2 ± 9.5	32.2 ± 10.2	−16.3 ± 12.3
0.2	41.7 ± 0.1	40.9 ± 2.3	19.5 ± 4.2	22.2 ± 4.8	−17.6 ± 5.1
0.1	26.2 ± 0.4	48.9 ± 4.1	23.7 ± 4.5	2.5 ± 5.0	−47.7 ± 0.4
0.0	10.3 ± 0.6	43.6 ± 3.6	17.1 ± 7.5	−19.4 ± 10.3	−62.2 ± 9.6

contained 15 parts margarine, 36 parts sugar, 12 parts cornstarch, 0.5 parts salt, 15 parts casein, 150 ml of fruit juice and 50 ml of water. The pudding prepared by controlled cooking was distributed in known amounts and refrigerated until used. Samples were taken for nitrogen determination.

Other experimental information

Meals were prepared in the experimental kitchen and served in the dining room of the Agricultural & Food Sciences Division of INCAP. Standardized recipes were used for the food preparations. Several menus were prepared to make the study less monotonous. An example of the menus is presented in Table 1. Fresh fruits (pineapple, pears, apples, melon or peaches) were included to help intestinal peristalsis and to make the diet more appealing. The ingredients used for cooking were wheat starch, DPP (Dietetic Paygel—P wheat starch, General Mills Chemical Inc., Minneapolis, MN 55435), cornstarch, shortening, margarine, honey, applesauce, marmalade, coffee, tea, iodized salt, seasonings and baking powder. The small amount of nitrogen from these food sources was not substracted from intakes or urinary excretions since they were constant at the different levels of protein intake.

The protein bread and cookies were prepared from the starch wheat DDP; cornstarch was used for pudding and soups.

Distilled water was used for food preparation. The subjects received 40–50 kcal/kg/day from the basal diet and the protein tested. This intake was sufficient to maintain body weight. Water intake was kept constant for each individual. A daily supplement of vitamins (UNICAP-T, Compañía Farmacéutica Up-John S.A., km. 14½ Carretera Roosevelt, Guatemala, Guatemala, C.A.) and minerals was given to the subjects. Each tablet contained: vitamin A (synthetic), 1.5 mg; Vit. D, 12.5 mcg; thiamin mononitrate, 10 mg; riboflavin, 10 mg; sodium ascorbate, 300 mg; niacin, 100 mg; piridoxine hydrochloride, 2 mg; calcium pantothenate, 20 mg; Vit. B_{12}, 4 mcg; copper sulfate, 3.92 mg; iron sulfate, 31.3 mg; potassium iodine 0.196 mg; calcium carbonate, 125 mg; manganous sulfate, 3.08 mg; magnesium sulfate, 29.7 mg and potassium sulfate, 11.142 mg.

Milk studies

Short-term NBI. A total of 12 individuals, divided into two groups, were used. Group A was made up of 5 subjects, and Group B, consisted of 7 individuals. The subjects in group A were between 22 and 33 yr of age, weighing between 48.8 and 70.3 kg, and between 150 cm and 167 cm tall. The test lasted a total of 11 days. They were fed a nitrogen-free diet as described before (Navarrete et al., 1977) for 3 days, followed by daily increases in protein intake from 0.1, 0.2, 0.3 and 0.4 g/kg/body weight per day. After the highest point of protein intake was reached, the same levels were fed in a decreasing order so as to end the experiment with the nitrogen-free diet again. Fecal and urine collections were made every 24 hr.

The subjects in group B were between 23 and 29 yr of age, weighing between 52 and 58 kg and varying in height between 155 to 170 cm. The seven subjects were divided at random among two sub-groups: B-1, consisting of 3 individuals, and B-2 consisting of 4. This was necessary in order to be able to compare the results obtained when fecal collections were from 24 hr with those of 48 hr at the same level of nitrogen intake, in an attempt to minimize the problems or difficulties in getting representative fecal samples. All subjects were fed with the nitrogen-free diet for 3 days, with collection of feces and urine on the last 2 days. The subjects in group B-1 were fed every day an increasing protein intake from milk from 0.1 to 0.6 g/kg/body weight/day. In this group, 24-hr collections of urine and feces were made. Group B-2 received the same levels of protein after the nitrogen-free diet period; however, the intake was changed from 0.2 to 0.4 to 0.6g protein per kg/day every 2 days, so as to obtain 48-hr fecal and urine collections.

Conventional NBI. In order to test the reliability of the short-term variation of the NBI assay, it was necessary to run a conventional assay using the same protein under similar experimental conditions, so as to have the length of each level of protein feeding as the only variable. In this study 12 subjects participated, varying in age from 21 to 35 yr, in weight from 49.3 to 63.6 kg and in height from 164–172 cm.

The subjects were divided into 2 groups of six (I and II) according to their body weight. Group I received levels of 0.2, 0.4 and 0.6g protein/kg/body weight/day, while Group II received 0.3, 0.5 and 0.7g of protein/kg/body weight/day. All subjects were fed a nitrogen-free diet for 3 days at the beginning of the experiment. For both groups, each level of protein was given for 6 days. The first 3 days on each level were considered as adaptation period, followed by a 3-day balance period. There was a daily collection of urine and feces. Charcoal and carmine were used to facilitate the separation of stools and the pigments were alternated and fed every 3 days. At the end of each 6-day period of feeding, a blood sample was taken for analysis of the nonessential (NE) to essential (E) amino acid ratios in plasma.

Casein study

In this case, using the short-term NBI assay, seven subjects were fed daily decreasing intakes of protein from 0.6 g/kg/body weight/day to 0.1g, and ending with 2 days on a protein-free diet. Fecal and urine collections were taken every 24 hr.

RESULTS

Milk studies

Experiment No. 1: Short-term. The results of Experiment 1-A are summarized in Table 2. When protein intake was increased from a close to a zero value, nitrogen balance became less negative, reaching a positive value when intake was 74.7 mg N/kg/day. Nitrogen retentions then decreased as nitrogen intake was taken back to zero. In general, urinary nitrogen excretion followed nitrogen intake; however, the same is not true for fecal nitrogen. This was felt to be due to the problems involved in collecting 24-hr samples.

Nitrogen absorbed increased with intake and it was 59.3 mg/kg/day, with the highest nitrogen intake of 74.7 mg/kg/day.

The coefficient of regression was calculated between the nitrogen retained and the nitrogen absorbed, using all the data from the ascending and descending sequences of nitrogen feeding. The results are shown in Figure 1. The NBI value of 1.06 obtained in the ascending period may have been the result of a prolonged depletion in contrast to 0.88 in the descending period when the subjects had only 3 days of zero protein intake. Allison and Anderson (1945) indicated that NBI values above one resulted when the individuals were protein-depleted before feeding the protein under test.

The nitrogen balance data (Experiment 1-B) on the study comparing 24-hr and 48-hr fecal collections are found in Table 3.

In group B-1 with daily increases in protein intake, urinary excretion increased with the protein intake, reaching the highest level of 71.2 mg N/kg with the 0.6g protein intake. On the other hand, group B-2 presented almost constant urinary nitrogen excretion. The results indicate that less variation was found in fecal nitrogen excretion in the 2-day protein fed group as compared with the 1-day, probably because fecal separation was considerably improved for

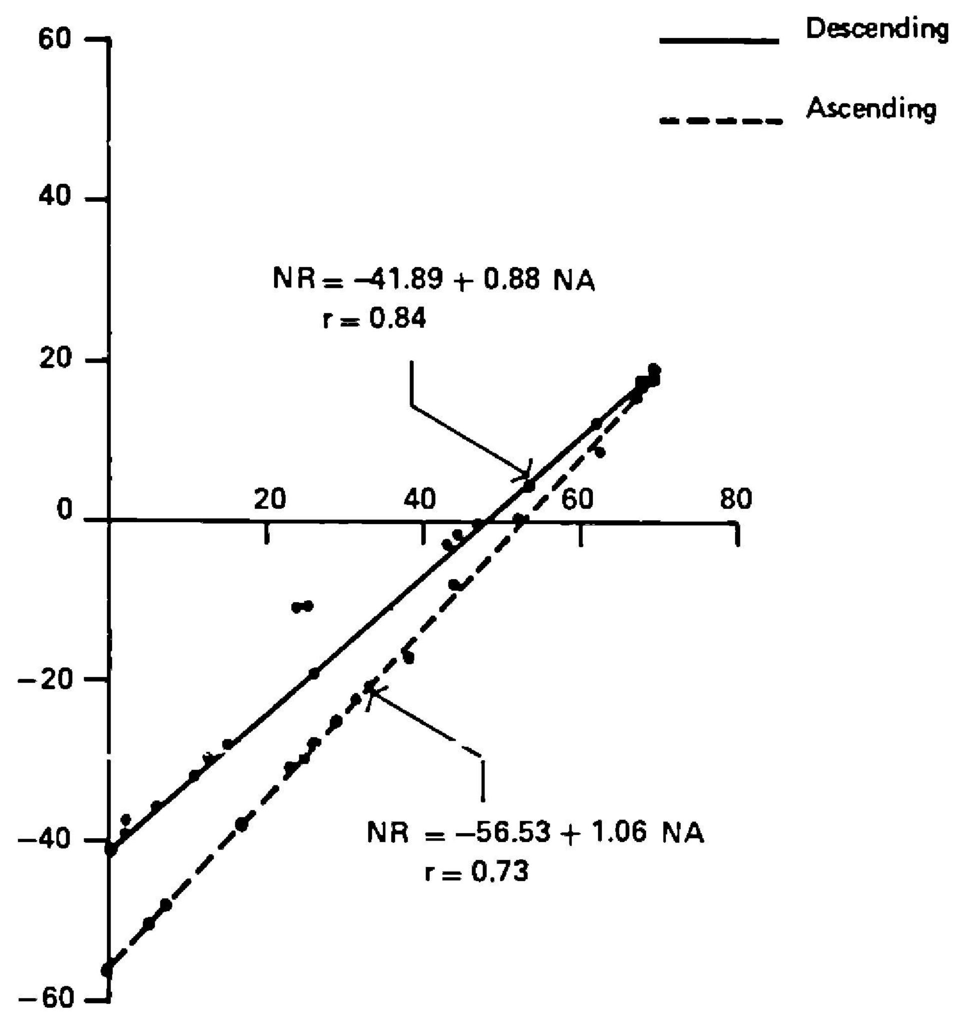

Fig. 1—Nitrogen balance index of spray-dried whole milk as determined by the short method with ascending and descending levels of protein intake.

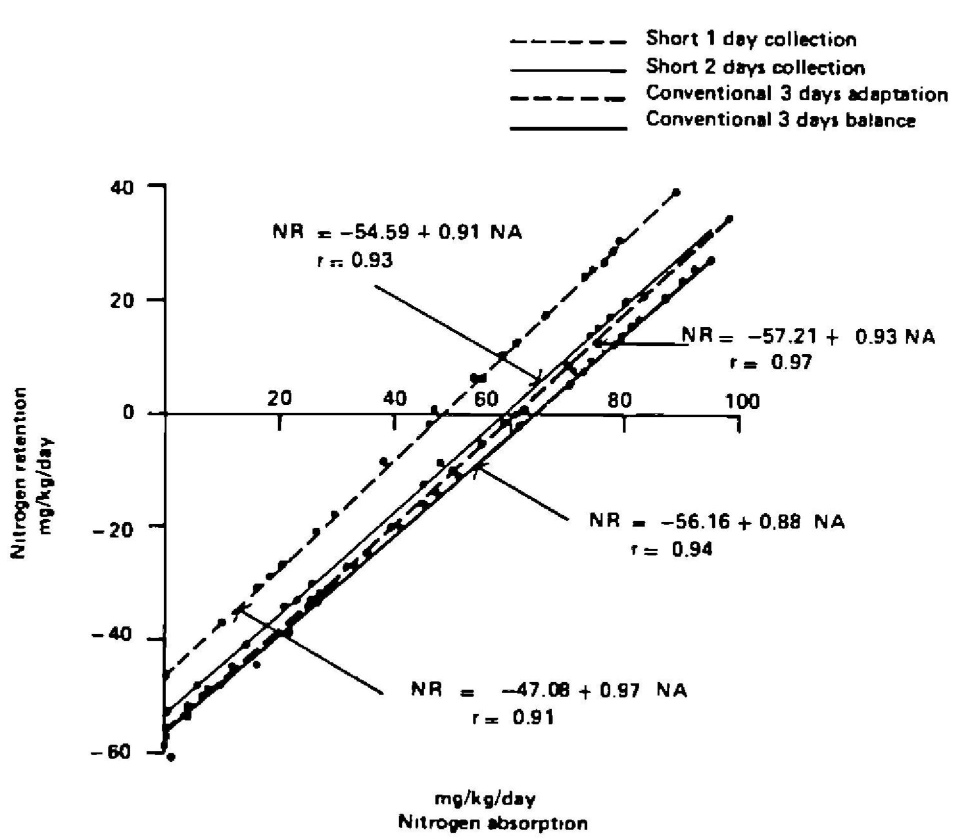

Fig. 2—NBI of whole milk.

group B-2. For group B-1, nitrogen retention was negative up to the 0.4g protein level of intake, reaching a positive balance with 0.5g protein. It was observed that negative balances occurred with the 0.6g protein intake mainly due to the large urinary excretion of one of the subjects. In group B-2, nitrogen retention was initially negative, changing into positive when intake was over 04g protein. Nitrogen absorption increased parallel to the protein intake. The regression equation between all-nitrogen retained and all-nitrogen absorbed for both groups was calculated and is presented in Figure 2. The slope of the line or the NBI was 0.91 for group B-1 and 0.97 for group B-2, values that are similar to the one obtained in the earlier experiment using milk.

Experiment No. 2: Conventional method. The results of this study are presented in Table 4. The nitrogen balance data calculated from the adaptation periods are presented in Table 5 and were analyzed by regression analysis. As in the early experiments, urinary and fecal nitrogen increased with protein intake. Nitrogen balance was reached at the 0.4g protein level. Absorbed nitrogen changed from 29.8 and 41.6 mg N/kg/day at the low levels of intake to 86.5 and 101.8g N/kg/day with the higher levels of protein.

The regression equation shown in Figure 2 between all nitrogen absorbed and retained for the adaptation period was NR = −57.21 + 0.93 NA and for the balance period was NR = −56.16 + 0.88 NA; the coefficients of correlation were 0.97 and 0.94, respectively.

The NBI index of 0.88 is lower, but not statistically different from the 0.93 value. In comparing the values obtained by the short and the conventional methods, no statistical differences were found, even though the short method gives higher values perhaps as a result of the short periods of fecal collection and the lack of an adaptation period.

Other investigators have reported figures lower than the ones reported here: Summer and Murlin (1938) found a value of 62, while Martin and Robinson (1922) reported a biological value of 53 for whole milk.

Studies carried out at INCAP (Bressani et al., 1972) using the method of Allison and Anderson (1945) in the conventional manner, and with infants, gave a NBI value of 69. Evidence exists from animal studies that a higher biological value is obtained with adult than with young subjects, particularly when the intake is low (Bressani, 1977).

Bricker et al. (1945) determined the biological value of milk using a similar technique with a regression approach between nitrogen intake and nitrogen balance expressed per basal calorie, and found a value of 74. Table 6 summarizes the NEAA/EAA ratio on the two groups. It can be seen that the ratio decreases as protein intake increases. Values approaching 1 have been taken to indicate better protein nutriture.

Casein study

Table 7 presents the average values of the nitrogen balance data for the casein experiment. Urinary nitrogen excretion decreased with the intake; the higher value was 79.9 mg/kg/day at the 0.6g protein intake. Fecal excretion remained relatively constant. The retention of nitrogen was initially positive, turning negative as the level was reduced below an intake of 0.6 g/kg/day.

The regression equation between nitrogen retention and the absorbed nitrogen was calculated and is presented in Figure 3. The coefficient of correlation was 0.90. The biological value, expressed as the "b" value, was 65%, a close figure to the 68 value obtained by Hawley et al. (1948). Forbes et al. (1958), using rats in a similar approach to the nitrogen balance index, obtained a biological value of 62%. On the other hand, Allison and Anderson (1945) reported a value of 82% with adult protein-depleted dogs using the nitrogen balance index method. Similarly, Bressani et al.

(1978), using dogs and the conventional and short method for nitrogen balance index, reported values of 0.78 and 0.74, respectively. In the present experiment the subjects were not depleted in the early phase as it was done in other studies. The nitrogen-free diet was given at the end of the experimental period. To reach nitrogen balance, an intake of 102 mg/kg/day was necessary.

DISCUSSION

THE RESULTS of the present investigation support the contention as previously indicated for dogs (Bressani et al., 1978) and human subjects (Navarrete et al., 1977) that the experimental period to assess the quality of proteins could be shortened significantly without affecting the numerical value representing protein quality. The results reported in the present study showed that the protein quality of milk proteins as determined by the NBI short-term varied from 0.91–0.97 as compared to a value of 0.88 for the conventional test. The difference, however, was not statistically significant. In previous studies (Bressani et al., 1978; Navarrete et al., 1977) it was indicated that the short-term variation had a tendency to give slightly higher values than those obtained from the conventional method, and this is also true in the present report for milk proteins. The reason for this difference is that on longer term studies, when feeding a higher protein intake to be maintained for a time, there is at first a high retention which decreases to some extent with respect to time. The reverse takes place when going from a high to a lower intake. This, therefore, affects the

Table 3—Nitrogen balance of young adult men fed whole milk—short-term, Experiment 1-B

Protein level (g/kg)	Intake	Urine	Fecal	Absorbed	Retained
	(mg N/kg/day)				
Group B-1					
0.0	14.9 ± 0.4	59.6 ± 3.6	34.4 ± 1.5	−19.3 ± 1.5	−78.0 ± 3.2
0.1	28.6 ± 0.5	45.4 ± 6.0	21.4 ± 4.3	7.2 ± 3.9	−38.1 ± 9.5
0.2	46.9 ± 0.7	50.5 ± 5.8	22.8 ± 2.4	24.6 ± 1.6	−26.0 ± 11.6
0.3	61.0 ± 0.5	50.8 ± 4.7	37.4 ± 23.1	38.6 ± 8.4	−12.2 ± 7.0
0.4	76.6 ± 0.7	58.2 ± 7.1	25.8 ± 3.4	51.0 ± 4.1	− 7.2 ± 10.5
0.5	96.1 ± 0.8	61.4 ± 5.2	18.5 ± 2.2	77.6 ± 1.8	16.2 ± 3.4
0.6	98.5 ± 0.6	71.2 ± 12.7	34.6 ± 6.5	64.0 ± 6.1	− 7.2 ± 17.4
Group B-2					
0.0	15.1 ± 0.4	54.7 ± 6.9	25.3 ± 4.0	−10.4 ± 4.1	−66.1 ± 9.0
0.2	42.8 ± 1.8	45.4 ± 2.0	23.3 ± 2.4	19.4 ± 2.6	−26.0 ± 2.4
0.4	76.8 ± 0.4	45.3 ± 2.6	25.2 ± 3.2	51.8 ± 3.1	7.3 ± 3.5
0.6	101.7 ± 1.2	53.9 ± 2.5	25.2 ± 2.1	76.5 ± 2.2	22.6 ± 3.1

Table 4—Nitrogen balance of young adult men fed whole milk—conventional method

Protein level (g/kg)	Intake	Urine	Fecal	Absorbed	Retained
	(mg N/kg/day)				
Group I					
0.0	17.3 ± 0.6	58.3 ± 2.3	25.8 ± 2.2	−8.5 ± 2.2	−66.8 ± 3.2
0.2	47.5 ± 0.4	45.6 ± 3.4	17.6 ± 1.9	29.8 ± 1.6	−15.8 ± 3.5
0.4	82.3 ± 0.5	59.0 ± 2.0	19.8 ± 3.0	62.6 ± 3.0	4.2 ± 5.7
0.6	112.3 ± 0.4	75.5 ± 2.1	25.8 ± 3.2	86.5 ± 3.1	11.0 ± 1.5
Group II					
0.0	17.3 ± 0.7	64.3 ± 3.8	21.0 ± 4.0	− 4.4 ± 4.9	−67.6 ± 5.6
0.3	64.5 ± 0.6	56.5 ± 4.1	22.8 ± 1.7	41.6 ± 1.9	−14.8 ± 4.8
0.5	99.6 ± 0.6	67.1 ± 4.5	21.0 ± 2.8	78.6 ± 2.8	11.5 ± 5.7
0.7	128.8 ± 0.7	83.6 ± 5.8	26.8 ± 3.6	101.8 ± 3.6	18.1 ± 3.3

Table 5—Nitrogen balance of young adult men fed spray-dried whole milk—adaptation period, conventional method

Protein level (g/kg)	Intake	Urine	Fecal	Absorbed	Retained
	(mg N/kg/day)				
Group I					
0.0	17.3 ± 0.6	58.3 ± 2.3	25.8 ± 2.2	− 8.5 ± 2.2	−66.8 ± 3.2
0.2	46.5 ± 0.4	51.5 ± 1.9	15.5 ± 2.1	31.0 ± 2.0	−20.5 ± 2.9
0.4	81.1 ± 0.6	53.3 ± 2.2	23.1 ± 2.2	58.0 ± 1.7	5.6 ± 2.3
0.6	113.0 ± 0.3	70.3 ± 1.8	21.1 ± 3.0	91.8 ± 2.7	21.5 ± 3.2
Group II					
0.0	17.3 ± 0.7	64.3 ± 3.9	21.0 ± 4.0	− 4.4 ± 4.9	−67.6 ± 5.6
0.3	63.1 ± 0.4	57.6 ± 1.6	22.0 ± 5.1	41.1 ± 4.7	−16.0 ± 4.6
0.5	98.0 ± 0.5	64.1 ± 3.8	21.1 ± 2.9	76.8 ± 2.5	12.6 ± 3.3
0.7	129.8 ± 0.5	82.6 ± 4.9	27.5 ± 1.9	102.5 ± 1.7	19.8 ± 4.7

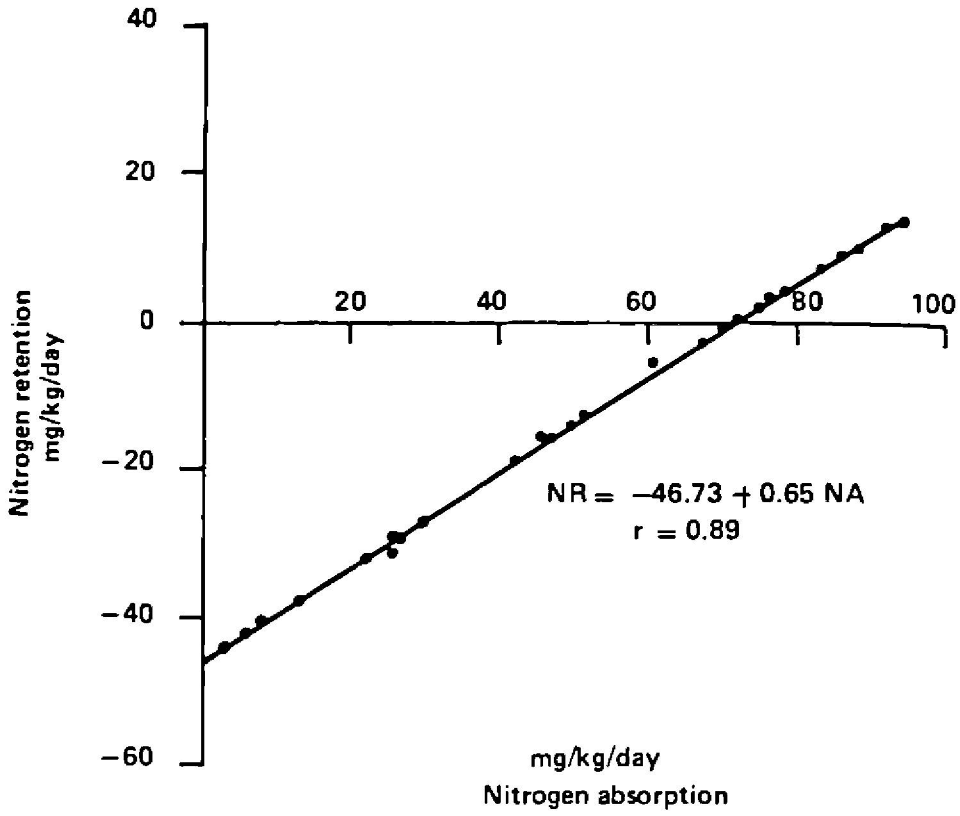

Fig. 3—Nitrogen balance index of casein.

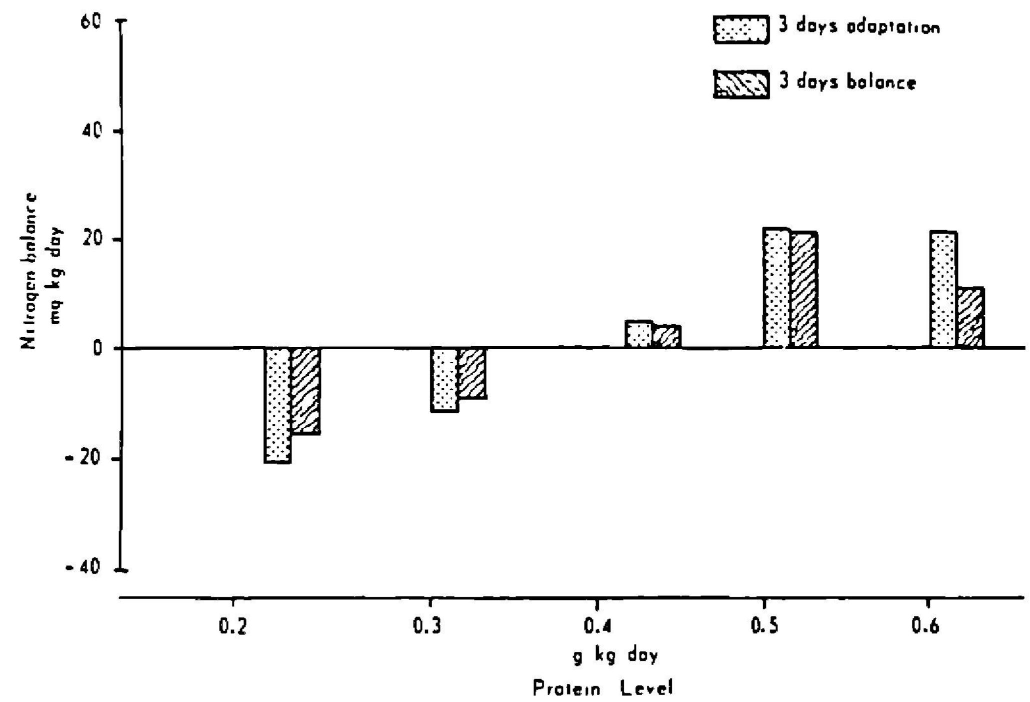

Fig. 4—Nitrogen balance during the specified collection periods.

slope of the nitrogen absorbed to nitrogen retention relationship. The effect is evident when looking at the nitrogen balances in Figure 4, obtained during the 3-day adaptation period and 3-day collection periods in the conventional method. In the negative nitrogen balance area, the adaptation balance period is always more negative than the second 3-day period. On the other hand, on the positive nitrogen balance area, the adaptation balance period is always more positive than the 3-day collection period. Obviously, the slope of the nitrogen balance to nitrogen intake or nitrogen absorbed of the adaptation period is higher (0.93) than the slope during the 3-day balance period (0.88). This effect is

Table 6—NEAA/EAA ratio of subjects fed milk on the conventional NBI method

Protein level (g/kg/day)	NE/E
0.0	2.32 ± 0.28
0.2	2.30 ± 0.39
0.4	1.87 ± 0.20
0.6	1.76 ± 0.38
0.0	2.10 ± 0.26
0.3	1.86 ± 0.28
0.5	1.77 ± 0.26
0.7	1.45 ± 0.19

also true when collection periods are shorter as in the modified NBI technique.

In the present study it was also established that a 48-hr fecal and urine collection period is superior to a 24-hr collection period, particularly for feces, enabling more representative samples and better demarcations of feces. Therefore, it is recommended to run 2-day balances per protein level as an improvement in the methodology as first proposed (Bressani et al., 1978; Navarrete et al., 1977). Even with this modification, the short-term NBI is more economical than the conventional technique.

One way to have better comparative values would be to express the protein quality value as a percentage of a reference protein, as is done with other protein quality evaluation procedures. If for example milk protein is used with a value of 0.95 as reference, casein would have a relative value to milk of 68%.

The protein quality values obtained fall within the range reported by other workers (Summer and Murlin, 1938; Martin and Robinson, 1922; Bricker et al., 1945; Hawley et al., 1948; Forbes et al., 1958) supporting the validity of the modification. Finally, the nitrogen absorbed values for nitrogen equilibrium for milk using the short-term method were 48–60 and for the conventional method, 64 mg/kg/day. For casein, the corresponding value was 102 mg/kg/day.

Additional studies are under way using other protein

—*Continued on page 1149*

Table 7—Apparent nitrogen balance in young men fed casein

Protein level (g/kg)	Intake	Urine	Fecal	Absorbed	Retained
	(mg N/kg/day)				
0.6 (2 days)	102.8 ± 1.4	79.9 ± 3.0	20.2 ± 1.6	82.8 ± 2.2	5.3 ± 4.4
0.5	86.1 ± 0.3	71.6 ± 3.3	20.7 ± 3.5	65.4 ± 3.5	− 6.0 ± 4.7
0.4	63.7 ± 0.2	62.7 ± 2.4	19.3 ± 2.6	44.4 ± 2.6	−18.3 ± 3.9
0.3	57.3 ± 0.3	55.7 ± 5.6	21.2 ± 3.0	36.2 ± 2.9	−18.7 ± 6.9
0.2	38.7 ± 0.2	50.3 ± 1.4	17.0 ± 1.8	21.7 ± 1.7	−28.6 ± 2.7
0.1	22.4 ± 0.2	49.6 ± 2.4	18.2 ± 0.8	4.3 ± 0.9	−31.2 ± 15.7
0.0 (2 days)	8.8 ± 0.2	45.5 ± 1.3	20.8 ± 2.0	−11.9 ± 1.9	−57.6 ± 2.9

sources to evaluate the validity and practical significance of the method and to learn also of ways to improve it.

REFERENCES

Allison, J.B. and Anderson, J.A. 1945. The relation between absorbed nitrogen, nitrogen balance, and biological value of proteins in adult dogs. J. Nutr. 29: 413.

Bressani, R. 1977. Human assays and applications. Ch. 5. In "Evaluation of Proteins for Humans," p. 81, Ed. Bodwell, C.E. The Avi Pub. Company Inc., Westport, CT.

Bressani, R., Elías, L.G., Olivares, J. and Navarrete, D.A. 1978. Comparación del índice de balance de nitrógeno de 3 proteínas calculado de períodos de balance de nitrógeno diarios o de 4 días Arch. Latinoamer. Nutr. 28: 318.

Bressani, R., Viteri, F. and Elías, L.G. 1973. The usefulness of results with human beings in standardizing rat assays. Ch. 19. In "Proteins in Human Nutrition," p. 293, Ed. Porter, J.W.G. and Rolls, B.A. Academic Press, New York.

Bressani, R., Viteri, F., Wilson, D. and Alvarado, J. 1972. The quality of various animal and vegetable proteins with a note on the endogenous and fecal nitrogen excretion of children. Arch. Latinoamer. Nutr. 22: 227.

Bricker, M., Mitchell, H.H. and Kinsman, G.M. 1945. The protein requirements of adult human subjects in terms of the protein contained in individual foods and food combinations. J. Nutr. 30: 269.

Forbes, R.M., Vaughan, L. and Yohe, M. 1958. Dependence of biological value on protein concentration in the diet of the growing rat. J. Nutr. 64: 291.

Hawley, E.E., Murlin, J.R., Nasset, E.S. and Szymanski, T.A. 1948. Biological values of six partially purified proteins. J. Nutr. 36: 153.

Martin, C.U. and Robinson, R. 1922. The minimum nitrogen expenditure of man and the biological value of various proteins for human nutrition. Biochem. J. 16: 407.

Navarrete, D., Daqui, V. Roque de, Elías, L.G., Lachance, P.A. and Bressani, R. 1977. The nutritive value of egg protein as determined by the nitrogen balance index (NBI). Nutr. Rep. Internat. 16: 695.

Summer, E.E. and Murlin, J.R. 1938. The biological value of milk and egg protein in human subjects. J. Nutr. 16: 141.

Ms received 7/13/78; revised 1/28/79; accepted 2/7/79.

Preliminary data were presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, IL, June 8—12, 1975.