THE NUTRITIVE VALUE OF EGG PROTEIN AS DETERMINED BY THE NITROGEN BALANCE INDEX (NBI)[1]

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ABSTRACT

A rapid method for evaluating protein quality in humans was tested using whole egg. The technique applied was an adaptation of the nitrogen balance index assay. This short-time experimental method takes only 10-12 days during which small daily increments of the protein under test are fed, while a minimun of 24 days is required by the conventional procedure. Nitrogen balances were run on young adult men receiving different protein levels with an adequate and constant calorie intake. The relation between nitrogen retained and nitrogen absorbed was used to determine the quality of the protein.

The short nitrogen balance index test gave an average value of 0.67, while the conventional method rendered a value of 0.57. These values are not statistically different and are similar to those reported by other laboratories.

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INTRODUCTION

In their experiments with dogs, Allison and Anderson (1) demonstrated that at low levels of nitrogen intake a linear relationship exists between nitrogen balance and nitrogen absorbed. At high levels of protein intake, this relationship becomes curvilinear, well on the positive side of the curve. The slope of the line or the regression coefficient is a measure of the biological value of the protein, which these authors named the Nitrogen Balance Index assay. Application of this technique using short experimental periods of nitrogen intake was employed by Bressani *et al.* (2), with dogs as the experimental animals. Results of these studies indicated that the modified method gave practically the same protein quality values as those obtained with the conventional technique, and suggested that the same results could be accomplished with human subjects.

The purpose of this work, therefore, was to compare results of studies using short periods of time, feeding small increments of protein with the conventional long-period assay to test the possibility of applying the method in humans. It was thought that the use of a simple assay for protein quality evaluation would be most useful in controlling industrial processing products prior to their entering the commercial channels, and that it would also represent significant economic savings in evaluating protein quality in human subjects.

METHODS

Nineteen young men, 24 to 30 years old, weighing from 55 to 68 kg, participated in three experiments. Their physical characteristics are indicated in Table I.

Fresh whole egg was used as the protein source. A large lot of eggs was homogenized before weighing out portions and these were frozen until cooking time. The basal diet included low-protein bread and wheat starch cookies, fresh fruits (pineapple, pears, and apples), honey, marmalade, coffee, margarine, and carbonated beverages. Water and caloric intake were kept constant throughout the test to 1 ml/kcal and 45-50 kcal/kg, respectively. All subjects received a daily vitamin and mineral supplement.

TABLE I

Physical characteristics of the subjects

Subject initials	Age years	Height cm	Weight Initial kg	Weight Final kg	Calculated caloric intake kcal/kg/day
		Short Time Modification			
FS	22	167.6	52.8	53.3	45
EV	26	170.0	68.9	69.1	45
HM	25	168.0	51.5	51.7	45
JC	24	151.6	53.2	53.4	50
SY	22	171.5	58.4	59.1	45
LD	30	170.0	69.5	70.1	45
AC	33	166.0	66.2	66.8	45
LG	23	171.0	58.8	58.4	45
GM	27	163.0	54.3	54.3	50
		Conventional Method			
HM	25	168.0	51.4	50.9	50
LS	25	159.0	54.6	53.5	50
SY	23	171.5	58.5	57.7	42
EM	22	161.7	60.1	61.0	45
AC	33	166.0	65.9	65.6	45
FS	23	167.6	52.5	52.3	45
GM	28	163.0	54.6	55.0	50
WH	21	169.0	57.2	56.3	45
AS	33	168.0	63.5	63.5	45
JE	26	179.0	65.1	64.7	45

A practically nitrogen-free diet was fed for 3 days to the study subjects to determine the endogenous nitrogen losses from urine and fecal collections during the last 2 days. The average intake during this period was 14.5 mg N/kg/day and the diet was administered prior to or after the protein-feeding period, according to variations included in the experimental design. Protein levels were

fed either in an ascending or a descending order; when using the short method each level was fed for one day without allowing time for adaptation, and with the conventional method for six days: three for adaptation to the protein level change, and the other three for quantitative collection of feces and urine.

Short Method

Two groups of 5 (Group A) and 4 (Group B) subjects participated in the short trial. Those in Group A received the depletion diet for 3 days followed by increasing protein levels to 0.4, 0.5, 0.6 and 0.5 g/kg/day. Nitrogen balance was calculated on daily collections of food, feces and urine.

Subjects in Group B were placed for 4 days on a high-protein diet of whole egg, receiving 0.7 g/kg/day, with collection of biological material on the last 2 days. Thereafter, the protein level was decreased daily to 0.6, 0.5, and 0.4, measuring daily nitrogen balance; finally, a low-nitrogen diet was fed for 3 days measuring nitrogen balance on the pooled fecal and urine samples.

Conventional Method

The assay was run in the conventional manner with 3 days of adaptation and 3 days of collection of biological material per level of protein intake.

The 10 men participating in the study were divided into two groups, G and H. All subjects received a low-nitrogen diet for 3 days at the beginning of the experiment. Protein was then fed in ascending levels, from 0.2 to 0.4 to 0.6 mg/kg/day; these were increased every 6 days for subjects in Group G, while those in Group H received 0.3, 0.5 and 0.7 g protein/kg body weight/day, also changing levels every 6 days.

RESULTS

Short Method

The nitrogen balance data of Groups A and B are presented

in Table II. For Group A, nitrogen balance increased from a negative value of −59.9 mg to a positive value of 25.4 mg as nitrogen intake was increased on a daily-basis from 14.6 to 107.2 mg N per kg, per day. The last level fed, which originally was to be 0.7 g protein/kg/day, actually was 0.5 g/kg/day. It is of interest to point out that the nitrogen excretion in the urine and feces was similar to that found when the 0.5 g protein level was fed previously. With respect to Group G, nitrogen balance decreased from a positive value of 1.6 to a negative value of −65.6 mg as protein intake decreased from an intake of 122.1 mg to one of 12.8 mg N/kg/day.

Fecal nitrogen at each protein level was also similar in both groups. Absorbed nitrogen was parallel to nitrogen intake in the ascending or descending period as the experimental time progressed. In the case of Group A, positive nitrogen retention was achieved with 0.4 g protein in the ascending phase, while in Group B, positive nitrogen balance was maintained up to the 0.5 g protein intake.

Regression equations were calculated for both groups in the descending and ascending phase of the experiment. The nitrogen balance index is presented in Fig. 1: Group A gave a value of 1.02 in the ascending phase, while Group B showed a value of 0.67 when protein was fed in increasing amounts.

Conventional Method

The nitrogen balance data are shown in Table III. In both groups nitrogen retention changed from a strongly negative value to a positive one as nitrogen intake increased from about 14 to 114 mg for Group G, and from 14.2 to 129.6 mg/kg/day for Group H.

Nitrogen retention was proportional to N intake, changing to positive at 0.4 g protein/kg/day for Group G who had lower nitrogen excretion values than Group H when the low nitrogen diet was fed. In Group H positive nitrogen balance was observed when feeding 0.7 g protein/kg/day. Using the data from both groups (G and H), the regression equation calculated between nitrogen retained and nitrogen absorbed gives an NBI index of 0.57. The individual regression coefficient for both groups was 0.59.

Table II

Apparent nitrogen balance in young men fed whole egg (Short Method)

Protein level g/kg	Nitrogen				
	Intake	Urinary	Fecal	Absorbed	Retained
			mg/kg/day		
			Group A		
0.0 (3 days)	14.6 ± 0.5	57.5 ± 5.7	17.1 ± 1.9	-1.6 ± 2.1	-59.9 ± 5.4
0.4	77.2 ± 0.6	41.2 ± 2.1	18.0 ± 1.6	59.2 ± 1.2	18.0 ± 2.8
0.5	91.2 ± 0.6	44.2 ± 2.5	24.2 ± 3.2	57.0 ± 3.0	22.8 ± 2.6
0.6	107.2 ± 0.6	60.0 ± 3.2	21.8 ± 5.3	81.8 ± 5.5	25.4 ± 7.2
0.5	92.0 ± 0.4	46.8 ± 12.6	25.5 ± 2.5	66.0 ± 2.0	19.7 ± 20.5
			Group B		
0.7 (3 days)	122.1 ± 0.4	94.1 ± 6.8	26.2 ± 2.5	95.7 ± 2.0	1.6 ± 6.8
0.6	106.5 ± 1.2	83.2 ± 10.1	19.0 ± 5.1	87.5 ± 5.9	4.2 ± 9.9
0.5	92.0 ± 1.1	73.0 ± 16.7	18.5 ± 4.6	73.5 ± 4.1	0.5 ± 14.0
0.4	76.2 ± 0.8	78.0 ± 7.3	22.2 ± 1.7	54.0 ± 1.9	-24.0 ± 7.1
0.0	12.8 ± 0.7	56.5 ± 7.9	20.9 ± 2.1	-8.2 ± 1.9	65.6 ± 9.5

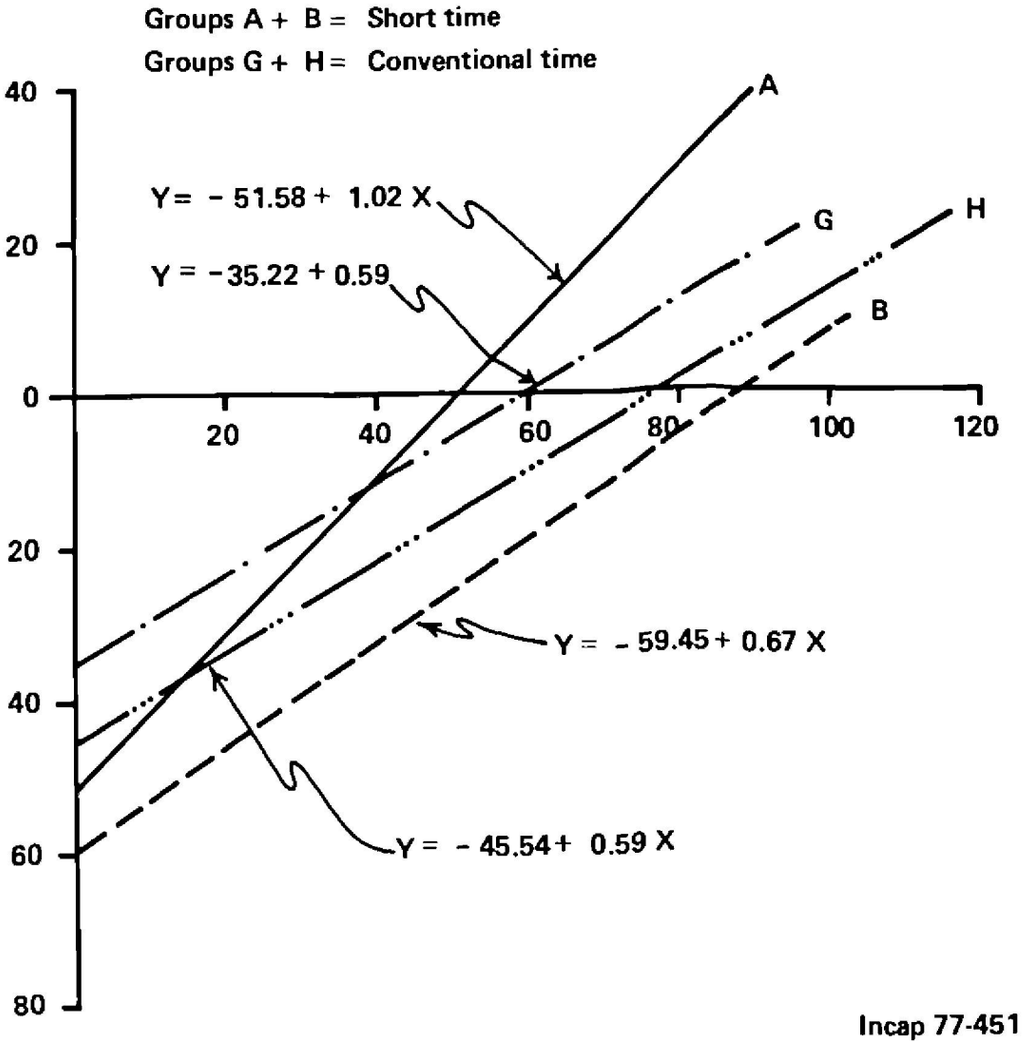

Fig. 1. Nitrogen balance index of egg protein.

DISCUSSION

The results of our study, as judged by the regression coefficients found, suggest that the short time modification of the conventional nitrogen balance index method for assaying protein quality, renders similar values to those obtained by the conventional assay. As compared to the latter method, the values obtained with the short method tend to be slightly higher, a finding that was also reported by Bressani *et al.* (2) in their studies with dogs during the developmental phase of the modifications introduced.

This small difference is to be expected, since the short time procedure does not include an adaptation period to a new protein level feeding as the conventional assay prescribes. During an in-

Table III

Apparent nitrogen balance in young men fed whole egg (Conventional Method)

Protein level g/k g	Nitrogen				
	Intake	Urinary	Fecal	Absorbed	Retained
			mg/kg/day		
			Group G		
0.0 (2 days)	14.4 ± 0.5	37.7 ± 1.0	23.1 ± 2.5	- 8.7 ± 2.3	- 46.5 ± 2.8
0.2 (3 days)	48.3 ± 0.7	37.6 ± 2.4	21.1 ± 1.5	27.2 ± 1.5	- 10.8 ± 3.0
0.4 (3 days)	83.0 ± 0.8	56.5 ± 6.5	19.2 ± 2.5	63.8 ± 1.7	7.2 ± 6.7
0.6 (3 days)	113.6 ± 0.7	78.0 ± 3.0	24.2 ± 2.3	89.4 ± 1.8	11.4 ± 3.8
			Group H		
0.2 (2 days)	14.2 ± 0.5	44.2 ± 3.4	30.3 ± 2.7	16.1 ± 2.9	−60.2 ± 4.2
0.3 (3 days)	64.2 ± 0.6	49.1 ± 3.3	25.3 ± 2.4	38.9 ± 2.4	−10.0 ± 4.0
0.5 (3 days)	99.0 ± 0.8	83.2 ± 17.6	18.3 ± 2.6	80.6 ± 2.0	− 2.0 ± 19.2
0.7) 3 days)	129.6 ± 0.7	93.3 ± 6.9	19.5 ± 1.7	110.1 ± 2.3	16.8 ± 5.7

creasing protein change, the first effect, if measured, is the attainment of a higher nitrogen balance which decreases with time to a more or less constant value, provided experimental conditions are maintained.

With the short time modification assay, Group A gave an NBI value of 1.02, while that of Group B was 0.67. This is a large difference, which may mean that the method is not acceptable. However, as indicated by Allison and Anderson (1), high values and certainly those above 1, indicate that the subjects have been highly protein-depleted. This is the real explanation for the different values obtained. Subjects in Group A were fed a low-nitrogen diet for 3 days after they had been fed decreasing nitrogen levels: from 0.7 to practically 0 for at least 7 days. The nitrogen balance values show that they were protein depleted and that those values changed from a negative one of −59.9 to a very positive value of 18.0 when fed 77.2 mg N/kg/day.

In the case of the conventional technique, both groups gave a nitrogen balance index value of 0.59, even though nitrogen balances were not equivalent to nitrogen intake. These results support the fact that multiple point assay techniques are valuable for the determination of protein quality.

The regression equations which showed coefficients equivalent to biological values of 67, 59 and 59 were parallel and not statistically different. Furthermore, the values reported in the present paper do not differ greatly from those found in the literature. Young *et al.* (3) found a biological value of 72, while Summer and Murlin (4) reported one of 65. Inoue *et al.* (5) notified coefficients equivalent to biological values of 57 with excess calories, and 36 with maintenance calories.

The rapid NBI modified conventional method, has been used in assays of other proteins which will be the subject of future communications.

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