# A new biological method for estimating food protein nutritive value

BY SHOSHANA MOKADY, S. VIOLA AND G. ZIMMERMANN*

*Department of Food and Bio-Technology, Technion, Israel Institute of Technology, Haifa, Israel*

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1. A new method has been devised for the nutritional evaluation of food protein quality. The method is analogous to the classical determination of net protein utilization (NPU). The suggested new criterion, the protein utilization by the liver (LPU), expresses the amount of food nitrogen 'retained' in the liver as a percentage of the food nitrogen intake.

2. Five different foods, casein, soya-bean protein isolate, maize gluten, wheat gluten, cottonseed meal alone or with supplements of amino acids, a total of thirteen samples, were tested for LPU and NPU in groups of six rats. The correlation coefficient between values for LPU and NPU for all seventy-eight rats was +0·85 and was highly significant.

Many detailed reviews of conventional methods of protein evaluation are found in the literature (Allison, 1949, 1959, 1964; Frost, 1959; Rippon, 1959; Campbell, 1963). The body nitrogen-balance methods (Thomas, 1909; Mitchell, 1923–4) for protein evaluation are laborious and time consuming. Shorter methods (Bender & Miller, 1953; Miller & Bender, 1955; Miller 1963) have been proposed, in which the ratio of body N to body water is determined and used to calculate the N content of the body. The accuracy of this method has been questioned (Stucki & Harper, 1962; Pellett, 1967).

N-balance determinations on single organs instead of the whole body could contribute valuable information, but only a few papers concerning protein evaluation based on changes in single organs have been published. One publication (Allison, 1964) showed that the susceptibility of different organs to changes in the quality of nutritional proteins is in the order: blood > liver > muscle > kidney > brain. Guggenheim (1964) tried to use as criterion average daily gain of liver N which he expected to be correlated with dietary protein quality, but it did not fulfil expectations.

Henry, Kosterlitz & Quenouille (1953) described a liver N method based on gain in liver N per 100 g initial body-weight. The correlation with N balance methods was generally good, provided the nutritive value of the protein was not greater than that of casein; with this method the variance was large and dependent on the length of the experiment (Henry, Cormack & Kosterlitz, 1961).

The objective of this paper is to propose a new short method for protein evaluation, protein utilization by the liver (LPU), based on estimation of the changes in N content of the liver in relation to the N consumption; the method introduces a control group to account for N consumption for maintenance.

* Professor Zimmerman died on 21 August 1968.

## EXPERIMENTAL

### *Materials and methods*

Weanling rats of the Charles River C.D. strain were divided into groups of six, three males and three females, each group having the same average weight. The rats were kept in individual cages in an air-conditioned room (24°, 50–60% relative humidity) for 10 days.

The diets, designed according to the Association of Official Agricultural Chemists (1965), contained 10% protein and were offered *ad lib.* Water intake was unrestricted.

The proteins studied were: vitamin-free casein—90% protein (N × 6·3); commercial soya-bean protein isolate—75% protein (N × 5·7); maize gluten—45% protein (N × 6·25); wheat gluten—75% protein (N × 5·7); cottonseed meal I, II and III, 45, 34·5 and 33·5% protein (N × 5·3) respectively.

All diets tested contained either these proteins alone or supplemented with the respective limiting amino acid or acids. Those animals not receiving diets supplemented with essential amino acids received a mixture of non-essential amino acids (DL-alanine 12%, L-glutamine 54·5%, glycine 12%, L-proline 10·5%, DL-serine 11%) to compensate for the addition of the essential amino acids. The control groups were given protein-free diets.

At the end of the experimental periods the rats were weighed and killed, their livers removed and weighed and food consumption was determined for each rat. The carcasses were dried for 48 h at 105° and were homogenized in an electric coffee grinder (Braun AG Mx333). The N contents of samples of the homogenate were determined by the Kjeldahl method. Livers were homogenized with four parts of water in a glass tissue grinder with a teflon pestle (Arthur H. Thomas Comp.). Liver N was determined in samples of the homogenates: 1 ml of homogenate was digested with 1 ml concentrated sulphuric acid and the N was determined in samples according to Conway's (1947) method.

### *Calculations*

The individual values for N content of carcasses and livers for all animals in each group were averaged.

In order to arrive at the estimate for the N retention of the experimental animals, the mean N content of the carcasses of control rats fed on a protein-free diet, which had had the same initial weight as the experimental animals, was subtracted from the individual body N content of the experimental animals at the end of 10 days.

An analogous procedure was followed for estimation of N retention by the livers.

LPU was calculated from the equation:

$$\text{LPU} = (L - L_c)/I \times 100,$$

where $L$ = liver N content (g) of experimental rat, $L_c$ = estimate of liver N content (g) of control rat with the same initial weight after 10 days of protein-free feeding and $I$ = N (g) consumed by experimental rat.

The NPU was calculated according to Miller & Bender (1955) on individual rats from the equation:

$$\text{NPU} = (B - B_c)/I \times 100,$$

where $B$ = carcass N content (g) of experimental rat, $B_c$ = estimate of carcass N content (g) of control rat with the same initial weight after 10 days of protein-free feeding and $I$ = N consumed by experimental rat.

## RESULTS

The values of LPU and NPU for the different proteins are given in Table 1. The relationship between LPU and NPU was calculated by expressing LPU as a percentage of NPU and was found to be of the same order. Diets scored according to LPU and NPU showed in general the same order of sequence. There was only one exception, the LPU for maize gluten + lysine, which was not statistically significant.

Table 1. *Values for protein utilization by the liver (LPU) and net protein utilization (NPU) by rats consuming diets containing a fixed amount of proteins, with and without supplements of essential amino acids**

(Mean values with their standard errors for six individual rats and the relationship between the values)

| | | Wheat gluten | | | Cottonseed meal | | |
|---|---|---|---|---|---|---|---|
| | Casein | − | +lysine | +lysine and threonine | I | II | III |
| LPU | 4·7 | 1·25 | 2·9 | 4·35 | 1·8 | 2·4 | 2·6 |
| SE | 0·09 | 0·09 | 0·11 | 0·11 | 0·13 | 0·09 | 0·09 |
| NPU | 70·0 | 23·1 | 39·3 | 57·9 | 21·0 | 31·7 | 42·1 |
| SE | 1·2 | 2·7 | 2·7 | 0·8 | 2·5 | 2·2 | 1·5 |
| LPU as % of NPU | 6·7 | 5·4 | 7·3 | 7·5 | 8·5 | 7·5 | 6·1 |

| | | Maize gluten | | | Soya-bean protein | |
|---|---|---|---|---|---|---|
| | Casein | − | +lysine | +lysine and tryptophan | − | +methionine |
| LPU | 5·0 | 2·0 | 1·5 | 4·6 | 2·8 | 4·0 |
| SE | 0·15 | 0·35 | 0·25 | 0·27 | 0·27 | 0·44 |
| NPU | 70·6 | 30 | 40 | 59·0 | 45·7 | 58·4 |
| SE | 2·7 | 1·9 | 3·6 | 2·4 | 2·9 | 1·5 |
| LPU as % of NPU | 7·0 | 6·6 | — | 7·8 | 6·1 | 6·8 |

* Per 100 g diet: for wheat gluten 700 mg lysine, 250 mg threonine; for maize gluten 630 mg lysine, 60 mg tryptophan; for soya-bean protein 100 mg methionine.

The correlation between the values of LPU and NPU was calculated for all seventy-eight individuals and gave a highly significant coefficient of +0·85. The regression analysis, represented in Fig. 1, shows that most of the points lie between the lines of 95% confidence limits.

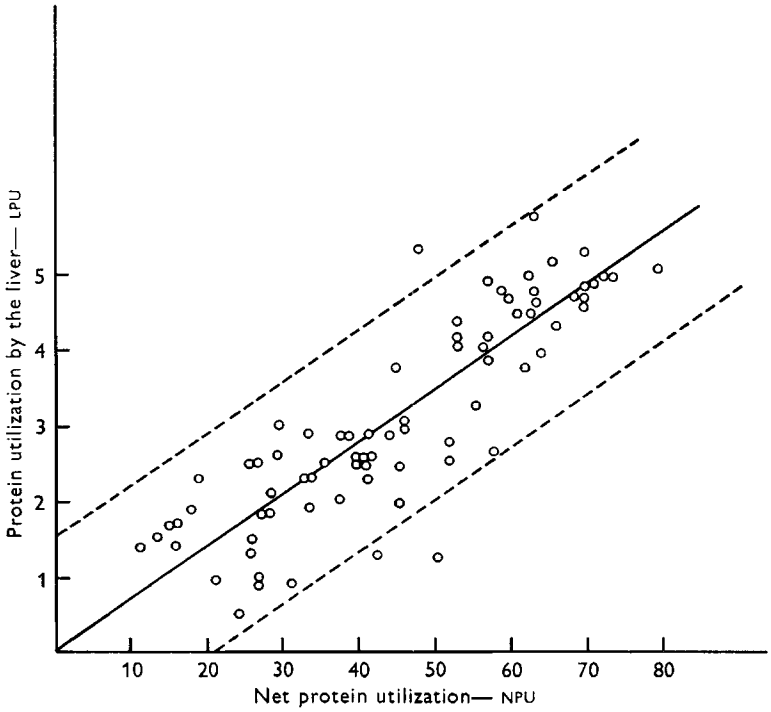


Fig. 1. The correlation between NPU and LPU: the regression line (—) with the lines of 95% confidence (---).

## DISCUSSION

The method discussed here permits the nutritional evaluation of dietary protein by estimating the N retained in the liver related to N intake.

The method proposed is analogous to the conventional NPU method, in which the retention of N is related to the N intake, and the protein needs for maintenance are assessed with the help of a control group. As can be seen from the results there was a highly significant correlation between the new method and the conventional one. The regression line with the lines of 95% confidence limits can be used to predict values of LPU from known values of NPU (Brownlee, 1953).

Because the determinations are done with single organs it is possible to deal with many analyses at a time. The homogenizing procedure is very simple and gives a very uniform material, much more uniform than any homogenate of a whole carcass. This means that N determinations done on samples of liver homogenates are much more representative. In addition, microdeterminations of N in liver samples are more convenient and rapid than the classical N determination by the Kjeldahl method in samples of carcasses, which are necessarily of much greater weight.

As against the shortened method proposed by Miller & Bender (1955), in which the body N is calculated from the water content, the LPU method is based on values derived directly from N determinations. Values for individual rats are determined easily and thus statistical analysis of results is feasible.

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