NUTRITIVE EFFICIENCIES OF LACTALBUMIN AND WHEAT GLUTEN AT VERY LOW LEVELS OF INTAKE IN ADULT RATS

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Summary The nutritive values of proteins in relation to their intake levels were evaluated by feeding adult male rats weighing 250 g diets containing 0%, 0.39%, 0.78%, 1.56%, 2.34%, 3.90%, 7.79% and 15.58% lactalbumin or wheat gluten for three weeks. The biological values (BV) of both proteins were high at low levels of protein intake but decreased with increase in protein intake. The BV of wheat gluten was estimated to be about 100 at a level of intake of 1.56% but only 25 at a level of 15.58%. Similarly, the BV of lactalbumin decreased with increase in the protein level, being 67 at a level of 7.79%. The BVs of both proteins at low levels of dietary protein (below 2.34% of lactalbumin or 0.78% of wheat gluten) were apparently more than 100 because urinary N excretion was less than endogenous N. The BVs also decreased with time during the three-week test period. It is concluded that the BV of a protein is not a fixed value but varies with the experimental conditions especially with changes in the amount of intake, and that differences in the qualities of various proteins cannot be compared quantitatively at a single level of protein. The results were briefly discussed in relation to protein requirements.

The utilization of proteins differ according to their amino acid compositions. But the efficiency of utilization of a protein by an animal depends not only on the quality of the protein, but also on the interactions of the protein and the state of the animal. Thus, there are two aspects of protein evaluation, static and dynamic. One is to rank proteins according to their amino acid compositions, digestibilities and availabilities. This aspect is mainly concerned with the attributes of a protein

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in itself and is important in monitoring the qualities of proteins during food processing and in determining the quality of a new protein. The other aspect is concerned with the physiological conditions of the man or animal eating the protein. Thus, in assessing protein requirements accurately, it is important to clarify the utilization of proteins under various experimental conditions.

The utilization of a protein is influenced by many factors, such as the age of the test animal, its nutritional state, its energy intake, the amount of protein consumed, the other components of the diet and the length of experimental period. It has long been recognized that the efficiency of protein utilization is low when the protein supply is abundant, because excess protein cannot be stored in the body and the surplus is used as a source of energy. Therefore, protein quality has usually been measured at a single, low or moderate, test level of protein, which is assumed to be adequate for obtaining constant efficiency. However, several workers reported that the nutritional values of proteins in men and rats change with protein intake, even when supplied at near or below the maintenance levels (1-6). Furthermore, MCLAUGHLAN and NOEL (7) reported that lysine-deficient amino acid mixture gave an erroneously high biological value (BV) when estimated at relatively low nitrogen levels. The utilizations of proteins at very low levels of intake have not been studied extensively in relation to protein requirement. Therefore, we examined the nutritive values of lactalbumin and wheat gluten to adult rats in terms of protein level and the length of the feeding period.

**MATERIALS AND METHODS**

Adult male rats of the Sprague-Dawley strain were fed a control diet containing 11.69% lactalbumin for 7 to 10 days until they weighed about 250 g (Table 1).

| Composition of control diet. |  |
|-----------------------------|--|
| Lactalbumin                 | 150\(^a\) |
| α-Cornstarch                | 478 |
| Sucrose                     | 239 |
| Corn oil                    | 50 |
| Salt mixture\(^g\)          | 50 |
| Vitamin mixture\(^g\)       | 10 |
| Chocola A\(^d\)             | 0.5 |
| Choline Cl                  | 2 |
| Cellulose                   | 20.5 |
| **Total**                   | **1,000.0** |

\(^a\) Protein content, 11.69% (N × 6.25).
\(^b\) Value for Chocola A is in ml. Other values are in grams.
\(^g\) From Tanabe Amino Acid Research Foundation, Tokyo, Japan.
\(^d\) From Eisai Co., Ltd., Tokyo, Japan. The preparation contains 30,000 I. U. vitamin A and 75 μg vitamin D\(_3\) per ml.

\(^3\) From Sigma Chemical Co., St. Louis, Mo., U. S. A.
Then groups of 6 rats were given diets containing the following percentages of lactalbumin or wheat gluten: 0.39, 0.78, 1.56, 2.34, 3.90 and 7.79. In addition, one group was given a 15.58% wheat gluten diet, and another group of 7 rats was given a protein-free diet to determine endogenous nitrogen output for calculations of the BVs of the proteins. The graded levels of protein in the diets were obtained by adding protein in place of an equal weight of carbohydrate mixture (two parts of α-cornstarch and one part of sucrose) so that the diets were approximately iso-caloric. Each diet was made into a dough with half its weight of water so that it could be picked up easily when it was spilt, and so that it would not contaminate urine in the metabolic cages. Experimental diets were given ad libitum, and food consumption and body weight were measured daily for 3 weeks. Urine and feces were collected separately for the last three days every week and their nitrogen contents were analyzed by the semi-micro Kjeldahl method. The nitrogen contents of the proteins were also analyzed by this method and protein contents were calculated as N \times 6.25. Except during metabolic studies, animals were housed individually in wire-bottom suspended cages in a room maintained at 23±1°C with alternate 12-hour periods of light and dark.

RESULTS

Changes in food intake and body weight

Table 2 shows that the food intake of rats on a protein-free diet was least. The intakes of lactalbumin and wheat gluten diets increased with increase in the

| Protein level (%) | Lactalbumin | Wheat gluten |
|-------------------|-------------|--------------|
|                   | Food intake (g/day) | N intake (mg/day) | Food intake (g/day) | N intake (mg/day) |
| 0                 | 10.8±1.5a   | 0            | —             | —            |
| 0.39              | 12.9±1.3    | 8.0±0.8      | 12.5±1.8     | 7.8±1.1     |
| 0.78              | 13.4±1.6    | 16.6±1.9     | 15.4±1.7     | 19.2±2.1    |
| 1.56              | 15.2±1.7    | 37.9±4.1     | 16.0±1.0     | 39.8±2.4    |
| 2.34              | 16.7±2.7    | 62.3±10.1    | 18.3±1.2     | 68.3±4.5    |
| 3.90              | 18.7±1.2    | 116.4±7.7    | 14.9±1.2     | 92.8±7.5    |
| 7.79              | 17.5±2.1    | 217.9±26.1   | 18.1±1.1     | 225.8±13.6  |
| 15.58             | —           | —            | 20.1±1.6     | 501.5±40.3  |

* Mean of 6 to 7 rats ± SD.

protein content of the diet and there was no significant difference in food intakes of rats on lactalbumin and wheat gluten diets of equal protein contents. For some unknown reason, rats on 3.90% wheat gluten diet ate significantly less food than rats on a 2.34% or 7.79% wheat gluten diet. As shown in Fig. 1, the growth of

From Wako Pure Chemical Industries, Ltd., Osaka, Japan.
Fig. 1. Change in body weight in response to N intake. The regression equations calculated from change in body weight ($Y$: g/21 days) as a function of N intake ($X$: g/21 days) were as follows:

Lactalbumin, $Y = 40.5X - 66.3$ (0% to 3.90%)
Wheat gluten, $Y = 39.1X - 64.1$ (0% to 2.34%)
$Y = 7.3X - 18.1$ (2.34, 7.79 & 15.58%)

The relation between the NPR and protein level

The relation between the net protein ratio (NPR) and protein levels of above 1.56% in week 3 is shown in Fig. 2; the NPRs for 0.39% and 0.78% protein are not shown because they were so scattered. The average NPRs of lactalbumin were $6.38 \pm 1.00$ and $6.43 \pm 0.92$ at levels of 2.34% and 3.90%, respectively, and were significantly reduced to $4.52 \pm 0.17$ at a level of 7.79% ($p<0.001$). The average NPR of wheat gluten was $6.06 \pm 1.02$ at a dietary level of 2.34% and this was not significantly different from that of lactalbumin at the same level of protein. On increasing the level of wheat gluten, however, the NPR decreased sharply to $1.81 \pm 0.15$ with 15.58% protein. Similar values to NPR can be obtained from the slopes of the lines in Fig. 1. The ratios of weight gain to protein intake calculated as $N \times 6.25$, were 6.48 with below 3.90% lactalbumin, and 6.26 with below 2.34% and 1.17 with above 2.34% wheat gluten.
Correlation between $N$ intake and $N$ balance

The relations between $N$ intake and $N$ balance in weeks 1 and 3 are shown in Figs. 3-A and 3-B, respectively. A rectilinear relation was observed in week 1 for lactalbumin at all protein levels tested, including zero protein. In contrast, the relation for wheat gluten gave a curved line with an inflection at about 50 mg of $N$ intake in week 1. In week 3 the relation was still linear with zero to 3.90%...
lactalbumin, but it became curved with above 3.90% protein. Rectilinear regression lines relating N balance to N intake were calculated from results with between zero and 3.90% lactalbumin and between 2.34% and 15.58% wheat gluten in week 3. The slopes of these lines, representing the approximate efficiencies of utilization of these proteins at around their maintenance levels, were 0.827 and 0.186 for lactalbumin and wheat gluten, respectively. The maintenance requirement of N, estimated from the regression lines in week 3, were 0.24 g/kg/day for lactalbumin and 0.55 g/kg/day for wheat gluten.

**Urinary N excretion at low levels of protein intake**

Although urinary N excretion generally rises with increase in protein intake, it remained essentially constant in rats on 0.39, 0.78, 1.56 and 2.34% lactalbumin diets (Table 3-A). Furthermore, rats on these low levels of lactalbumin or on 0.78% wheat gluten diet excreted significantly less nitrogen than rats on a protein-free diet. Apparently the BVs of more than 100 in the limited ranges of protein intake shown in Fig. 4 and Table 4 were due to the nitrogen-sparing effect of feeding small amounts of proteins. The urinary N of rats on 3.90% lactalbumin diet or 1.56% wheat gluten diet was as high as that of the protein-free diet group, but above these levels of protein it significantly exceeded the endogenous output ($p < 0.01$).

**Relation of BV to N intake**

The BVs of lactalbumin and wheat gluten calculated from the N balance data

Table 3-A. Urinary and fecal N excretions in weeks 1 and 3 in rats fed graded levels of lactalbumin diets.

| Protein level (%) | Intake N (mg/day) | Urinary N (mg/day) | Fecal N (mg/day) |
|-------------------|-------------------|--------------------|------------------|
| 0                 | —                 | 56.6±6.6           | 17.1±3.7         |
| 0.39              | 8.6±1.2           | 53.0±11.7          | 23.6±5.4         |
| 0.78              | 18.8±1.4          | 56.8±9.8           | 22.3±2.4         |
| 1.56              | 41.4±4.8          | 55.0±14.0          | 29.7±2.5         |
| 2.34              | 61.1±6.0          | 50.8±8.2           | 29.0±5.0         |
| 3.90              | 116.3±11.3        | 52.6±3.3           | 32.9±4.7         |
| 7.79              | 219.5±21.3        | 74.9±14.7          | 33.6±4.1         |

Week 3

| Protein level (%) | Intake N (mg/day) | Urinary N (mg/day) | Fecal N (mg/day) |
|-------------------|-------------------|--------------------|------------------|
| 0                 | —                 | 36.8±2.1$^{b}$    | 16.7±2.3         |
| 0.39              | 8.0±0.9           | 27.8±4.9$^{*b}$   | 19.5±3.6         |
| 0.78              | 17.3±2.8          | 24.1±2.2$^{*b}$   | 19.5±3.4         |
| 1.56              | 37.6±3.8          | 25.5±3.8$^{*b}$   | 24.2±4.3         |
| 2.34              | 65.4±9.0          | 26.5±1.0$^{*b}$   | 29.6±5.2         |
| 3.90              | 116.3±7.3         | 37.6±3.6$^{a}$    | 32.3±3.7         |
| 7.79              | 221.3±25.4        | 103.5±12.5$^{*b}$ | 37.7±4.5         |

$^a$ Significantly different from endogenous level ($p < 0.01$).

$^b$ Significantly different from the value in week 1 ($p < 0.01$).
### Table 3-B. Urinary and fecal N excretions in weeks 1 and 3 in rats fed graded levels of wheat gluten diets.

| Protein level (%) | Intake N (mg/day) | Urinary N (mg/day) | Fecal N (mg/day) |
|-------------------|-------------------|--------------------|-----------------|
| 0                 | —                 | 56.6±6.6           | 17.1±3.7        |
| 0.39              | 8.2±1.1           | 60.2±10.1          | 18.5±6.9        |
| 0.78              | 17.3±2.9          | 42.1±5.8<sup>a</sup> | 22.2±2.4       |
| 1.56              | 42.7±3.5          | 49.5±5.8           | 23.7±1.9        |
| 2.34              | 78.5±7.2          | 65.0±5.8           | 29.0±5.0        |
| 3.90              | 97.6±17.0         | 78.0±6.4<sup>a</sup> | 23.2±5.9       |
| 7.79              | 238.8±22.9        | 174.8±19.6<sup>a</sup> | 37.0±5.8       |

* Week 3

| Protein level (%) | Intake N (mg/day) | Urinary N (mg/day) | Fecal N (mg/day) |
|-------------------|-------------------|--------------------|-----------------|
| 0                 | —                 | 36.8±2.1<sup>b</sup> | 16.7±2.3        |
| 0.39              | 7.8±1.1           | 32.8±5.2<sup>b</sup> | 20.0±4.3        |
| 0.78              | 16.5±2.3          | 26.7±5.3<sup>a,b</sup> | 16.8±3.8       |
| 1.56              | 33.9±3.0          | 37.4±5.3<sup>b</sup> | 21.7±2.7        |
| 2.34              | 56.8±4.5          | 48.8±8.1<sup>a,b</sup> | 22.6±3.9       |
| 3.90              | 91.2±5.6          | 78.3±5.6<sup>a</sup> | 23.1±3.0        |
| 7.79              | 215.9±25.3        | 164.9±17.0<sup>a</sup> | 36.2±5.5       |

<sup>a</sup> Significantly different from endogenous level (p<0.01).

<sup>b</sup> Significantly different from the value in week 1 (p<0.01).

### Table 4. Effects of protein level and length of experimental period on the biological value.

| Protein level (%) | Lactalbumin | Wheat gluten |
|-------------------|-------------|--------------|
|                   | Week 1      | Week 2      | Week 3      |
|                   | 1           | 2           | 3           |
| 0.39              | 151±88<sup>a,b</sup> | 231±85      | 233±27      |
| 0.78              | 146±62      | 152±20      | 192±35      |
| 1.56              | 127±18      | 154±28      | 137±15      |
| 2.34              | 112±18      | 134±7       | 119±4       |
| 3.90              | 104±4       | 103±3       | 99±4        |
| 7.79              | 91±8        | 78±6**      | 67±5***     |

<sup>a</sup> Mean of 6 to 7 rats ± SD.

<sup>b</sup> BVs over 100 are apparent.

Asterisks indicate significant differences from values in week 1. (*: p<0.05, **: p<0.01, ***: p<0.001)
The BVs of both proteins decreased curvilinearly with increase in protein intake. INOUÉ et al. (3) empirically expressed these relationships between N intake and BV for egg protein or wheat gluten in men by a fractional equation. As shown in Fig. 4 and Table 4, the BVs calculated for limited intakes of wheat gluten and lactalbumin were apparently more than 100 because animals receiving small amounts of these proteins excreted less than the endogenous level of nitrogen in the urine (Table 3). The difference in quality of the two proteins was obvious with above 2.34% protein, where the BYs of wheat gluten were distinctly lower than those of lactalbumin. At a level of 7.79% protein or more, lactalbumin could not be utilized with 100% efficiency.

**Effect of the length of the experimental period on the BV**

The BVs of the two proteins varied not only with the level of protein but also with the experimental period (Table 4). At higher levels of lactalbumin and wheat gluten, BVs in week 1 were higher than those in week 3, while no definite changes were observed at lower levels of intake. For instance, the BV of lactalbumin at a concentration of 7.79% protein was 91 in week 1 but only 67 in week 3, and similarly the BV of wheat gluten at a concentration of 15.58% protein fell from 31 to 25.

**DISCUSSION**

SAID and HEGSTED (4) demonstrated that the regression line relating to change in body water with protein intake in adult rats fed wheat gluten diet at concentrations of 3.20% to 9.20% protein was rectilinear but that on extrapolation this line did not intersect the dosage level at zero. To explain this they suggested that the
relation was curvilinear at low protein concentration, but did not test this. In the present study, we paid special attention to utilization of wheat gluten at low levels of intake. Confirming the results and suggestion of Said and Hegsted, we found that the dose-response curve for wheat gluten between zero and 15.58% protein intake could not be represented in terms of body weight or N balance by a single straight line as shown in Figs. 1 and 3-B. The curves had points of inflection. The point of inflection was around 2.34% with wheat gluten, whereas it was above 3.90% with lactalbumin. With neither protein was utilization constant with up to 9% protein, the level at which protein quality is usually assayed (8). Similarly, BERDANIER et al. (6) observed a decreased utilization of casein and soy protein with increased protein intake in the Thomas-Mitchell method (9) of determining the BV. However, recalculating the BV using non-urea nitrogen value for the endogenous nitrogen, they showed an independence of BV from nitrogen intake.

YANEZ and MCLAUGHLAN (5) evaluated the quality of lysine-deficient proteins at two levels of intake and showed that NPR values were much higher when determined at the 5% level than at the 10% level. They also found that addition of lysine to wheat gluten diet gave higher NPR values at the 10% level of protein than at the 5% level. From these results they concluded that the wheat gluten diet was satisfactory for maintenance purposes but inadequate for growth and they suggested that amino acid requirements for maintenance and for growth were different in rats. However, INOUE et al. (3) reported that even in adult men whose body weight was in a steady state wheat gluten was utilized as well as egg protein at levels of below 0.2 g/kg of protein intake. Therefore, the efficient utilization of wheat gluten at low levels of intake observed in the present study did not seem to be due to a difference in the lysine requirements for maintenance and growth. SAID and HEGSTED (10) demonstrated that rats conserved lysine and leucine more efficiently than other essential amino acids. The metabolism of lysine is very specific and its degradation is rather slow, particularly in lysine-deficient animals (11); the amount of lysine in the body relative to the amounts of other amino acids may be sufficient for protein synthesis when protein intake is very low.

At protein intakes of below 1.56%, lactalbumin and wheat gluten were both almost fully utilized and no difference could be detected in their qualities. Thus the lysine content of wheat gluten did not seem to be limiting at this low level of protein although lysine was the first limiting amino acid when higher levels of protein were fed. At very low levels of dietary protein, the BVs of both wheat gluten and lactalbumin were more than 100, apparently because when the dietary level of proteins was low urinary N excretion exceeded the endogenous level. Nitrogen-sparing effects were also observed with 3.5% egg protein diet (12) and methionine-supplemented protein-free diet (13). YOSHIDA and MORITOKI (14) demonstrated that addition of methionine and threonine to a protein-free diet reduced urinary N excretion more than addition of methionine alone. YOKOGOSHI and YOSHIDA (15) further reported that the nitrogen-sparing action of methionine plus threonine was
observed even when as little as 0.0188% of each amino acid was added to protein-free diet. However, 1.30% zein diet, which is deficient in lysine but contains more methionine and threonine than wheat gluten, did not have nitrogen-sparing action (unpublished data). The nitrogen-sparing effect observed in this study requires further investigation.

The difference in quality between lactalbumin and wheat gluten could be seen at protein levels of above 1.56%: utilization of wheat gluten decreased markedly to a BV of 25 at 15.58% protein determined in week 3. In contrast, lactalbumin was utilized with 100% efficiency a level of up to 3.90% protein under the present experimental conditions, but its BV was only 67 at a concentration of 7.79% protein, estimated in week 3. These results show that even good quality protein, such as lactalbumin, is only utilized with 100% efficiency under specific conditions, such as during recovery from depletion, at low levels of protein intake or in rapidly growing animals. The results also show that no protein can be used at a safe level of intake for adult man as a reference of 100% efficiency of usage.

The BV was also affected by the length of the experimental period: the BVs of both lactalbumin and wheat gluten decreased with time during the feeding period (Table 4). This tendency can be seen from the data on N balance in Figs. 3-A and 3-B. Comparison of the relations of N balance and N intake in weeks 1 and 3 showed that N balance in week 3 tended to approach zero from both positive and negative balances. For instance, with 7.79% lactalbumin, N intake did not change but the urinary and fecal N outputs increased with time and so the extent of N retention was less in week 3 than in week 1 (Table 3-A). In contrast, at maintenance levels of protein or less, urinary N excretion decreased with time more significantly than fecal N or N intake. Thus, the N balance improved with time and the improvement was greatest at zero protein concentration. Consequently, the BVs tended to decrease both in and below the maintenance range of N intake during the 3-week feeding period. These changes may be regarded as adaptations of the animals; but if the test period is long, the factor of aging of animals should also be taken into consideration.

As described above, the BV was not a fixed value but varied with the experimental conditions. Moreover, the changes in the BVs of wheat gluten and lactalbumin with the dietary protein levels were different (Fig. 4), and so the relative nutritive values of wheat gluten and lactalbumin varied at different protein levels. This means that differences in the qualities of the various proteins cannot be represented by a single correction factor determined at a given level, such as 9% protein (8). One of the most important problems in estimating protein requirements is how to represent protein qualities. For practical purposes, HEGSTED (16) proposed measuring protein quality by the slope-ratio assay and suggested defining the efficiency of utilization of a protein as the slope of the dose-response line around the requirement level relative to that of a standard protein such as lactalbumin. The nutritive value of wheat gluten relative to that of lactalbumin based on the slope-
ratio assay in this study was 22.5% in week 3 (Fig. 3-B), and this value is very similar to that of 21.8% for growing rats reported by Hegsted and Chang (17). The efficiency of utilization of lactalbumin, estimated as the slope of the regression line between N intake and N balance between zero and 3.90% protein, was 83% (Fig. 3-B). This figure is the same as that given by Block and Mitchell in their review (18) for growing rats.

In human subjects, Calloway and Margen (1) reported that the NPU of egg protein was about 65 rather than the expected value of 100. A similar value was obtained by Young et al. (2) and a still lower value was observed by Inoue and his co-workers (19). These values show the great variability in utilization of dietary protein and the importance of estimating the nutritive value relative to that of a standard protein. However, the problem of how to evaluate the efficiency of utilization of a standard protein remains to be solved.

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