A comparison of biological methods with rats for determining the nutritive value of proteins

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The old-established growth method of Osborne, Mendel & Ferry (1919) for determining the protein efficiency ratio (PER) of protein sources has recently been suggested both in Canada (Chapman, Castillo & Campbell, 1959) and the USA (Derse, 1960) as a routine method for assessing the nutritive value of proteins. Njaa (1963) in a detailed study of the nitrogen balance sheet method for measurement of biological value (BV) (Mitchell, 1923–4a) considers that it and the growth method (PER) are preferable to other biological methods of protein assessment and that the former is more satisfactory than the latter in short-term experiments as it gives both more detailed information and more consistent results. Bigwood (1964) has also recommended BV as the most satisfactory estimate of protein quality. We have already reported (Henry & Toothill, 1962) a good correlation between the N balance sheet and the body-water (Miller & Bender, 1955) methods in assessing the nutritive value of a series of thirty-five protein sources. Sufficient quantities of twenty-four of them were available for the determination of their PER and the results are now reported for values obtained at an 8% level of protein intake.

Several methods of protein assessment are compared and the definitions of the terms used are:

$$\text{Biological value (BV)} = \frac{\text{Retained N} \times 100}{\text{Absorbed N}} \text{ (Mitchell, 1923–4a)};$$

$$\text{True digestibility (TD)} = \frac{\text{Absorbed N} \times 100}{\text{N intake}} \text{ (Mitchell, 1923–4a)};$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Gain in body-weight}}{\text{Protein intake}} \text{ (Osborne *et al.* 1919)};$$

$$\text{Net protein utilization (NPU)} = \frac{\text{Body N content with test protein} - \text{body N content with N-free diet}}{\text{N intake}}$$
$$\text{(Miller \& Bender, 1955)};$$

$$\text{Net protein ratio (NPR)} = \frac{\text{Gain in body-weight with test protein} + \text{loss in body-weight with N-free diet}}{\text{Protein intake}}$$
$$\text{(Bender \& Doell, 1957)}.$$

It is now usual to determine PER at a single (8–10%) level of protein intake. In the original method, however, Osborne *et al.* (1919) determined the PER of a protein at

several levels and accepted the peak value. Henry & Kon (1957) reported that the BVs of egg proteins and of casein did not change when the level of protein intake was increased from 4 to 8% but decreased progressively when the dietary protein level was increased to 12 and 16%. Results of a similar test with a poor-quality protein, wheat gluten, are now reported together with values for PER and for net protein utilization (NPU), calculated from body-water measurements (Miller & Bender, 1955), for these three protein sources determined at 4–20% levels of intake.

We have found (Henry, Cormack & Kosterlitz, 1961 and unpublished) that for cortisone-treated rats the BV of proteins is reduced but that their nutritive value as determined from N deposition in the liver is unchanged. It was, therefore, of interest to see in what way cortisone would affect the NPU, determined from body-water measurements, of those proteins already studied.

Working with several species of animal, a number of workers have reported higher N retentions when measured in balance experiments than by carcass analysis (cf. Butterworth, 1962). In this laboratory (Henry & Toothill, 1962) we have found that the N content of the carcasses of rats given experimental diets is slightly underestimated when it is calculated from equations relating body water to body N and derived from values for rats given our stock diet. For these reasons an experiment was arranged in which N retention from a 'N-free' diet and from good- and poor-quality proteins was determined in balance experiments and by direct and indirect (body water) determination of N in rat carcasses. The results are now reported.

EXPERIMENTAL

Animals. Rats of our hooded Norwegian strain were used. They were weaned at 21 days on to our normal stock diet (McKinlay, 1951).

Experimental diets. The basal 'N-free' diet had the percentage composition: rice starch 64, sugar 12, potato starch 10, margarine fat 10, salts (de Loureiro, 1931) 4. The protein source to be tested replaced an equal weight of rice starch. To each kg of diet were added: thiamine 3·3 mg, riboflavine 5·5 mg, pyridoxine hydrochloride 3·3 mg, calcium pantothenate 11·0 mg, folic acid 0·6 mg, biotin 0·6 mg, *p*-aminobenzoic acid 11·0 mg, nicotinic acid 11·0 mg, inositol 110·0 mg, choline hydrochloride 1900 mg, menaphthone (2-methyl-1, 4-naphthaquinone) 2·2 mg, cyanocobalamin 20 μg. The diets were offered *ad lib*. In addition, 360 i.u. vitamin A, 26 i.u. vitamin D and 1·4 mg α-tocopheryl acetate were given separately by mouth, in arachis oil, to each rat at the beginning of an experiment and subsequently once a week in those experiments lasting for more than 10 days.

Sources of protein tested. The following protein sources tested by Henry & Toothill (1962) were used: vitamin-low casein (Genatosan Ltd), dried, defatted egg, soya-bean meal (sample 5), linseed meal, yeast and wheat gluten (Henry *et al*. 1961) and Agricultural Research Council samples of fish meal (FM 6), meat meal (MM 20), soya-bean meal (SB 1, 3, 5), cottonseed meal (CM 13, 19, 49), groundnut meal (GN 11, 12, 18), sunflower-seed meal (SF 1, 2, 3) and dried skim milk (SM 3, 7, 8, 13) (cf. Boyne, Carpenter & Woodham, 1961).

Determination of PER. Six litters of male rats, aged 23–26 days, were used in litter-mate comparisons in all éxperiments. Each rat was housed in a separate cage. The animals were given the experimental diets for 3 days to accustom them to the individual cages and to the change from the stock diet; subsequently records of weight increases and of diet intakes were kept for 4 weeks.

Determination of BV *and* TD *of proteins by the balance-sheet method (Mitchell, 1923–4a; Mitchell & Carman, 1926).* Groups of twelve female rats aged 25–26 days were used in all tests. Details of the cages and experimental procedure are given elsewhere (Henry, Kon & Watson, 1937; Henry, Kon, Lea & White, 1947–8; Henry & Kon, 1956).

Determination of NPU *by estimation of body water in rats.* NPU (carcass) (Miller & Bender, 1955) and NPR (Bender & Doell, 1957) were determined as described by Henry & Toothill (1962).

PER *determinations for comparison with* BV *and* NPU. The PER of the proteins listed on p. 126 was determined at an 8% level of protein intake in four experiments; vitamin-low casein was included as reference protein in each experiment.

Effect of level of protein intake. The PER and NPU (carcass) of whole-egg proteins, casein and wheat gluten were determined at 4, 8, 12, 16 and 20% levels of protein intake. BV and TD determinations were done at 4, 8, 12 and 16% protein levels.

Effect of cortisone. The BV, TD and NPU (carcass) of casein, soya-bean meal, cotton-seed meal (CM 13) and wheat gluten were determined at an 8% level of protein intake on normal rats and on litter-mates given daily a subcutaneous injection of 2·5 mg cortisone acetate (Cortellan; Glaxo Research Ltd). The design of experiments to study the effect of cortisone on the BV and TD of proteins has been described by Henry *et al.* (1961). For NPU (carcass) determinations groups of rats were given both the 'N-free' and the other diets with or without cortisone injections. No attempt was made to determine PER as cortisone administration usually causes weight losses.

Comparison of N retention as determined by the balance-sheet method and by carcass analysis. Eight litters of four 30-day-old female rats were used. One rat from each litter was killed at the beginning of the experiment and the carcass analysed for water and N as described by Henry & Toothill (1962); these values were used to estimate the initial body N content of litter-mates given the experimental diets. The remaining three rats in each litter were given the N-free diet, or diets in which casein, or wheat gluten supplemented with 1% DL-methionine, supplied 8% protein. These proteins were selected to be primarily deficient in methionine and lysine respectively. The rats were given the diets for 10 days, urine and faeces being collected, and were then killed with coal gas and the carcasses analysed for water and N as above. Two groups of four rats were allotted to each diet as in the determination of NPU by estimation of body-water, so that duplicate values were obtained for NPU (carcass).

RESULTS

Table 1 gives PER values for twenty-three protein sources previously assayed by the balance-sheet and body-water methods (Henry & Toothill, 1962). No result was obtained for a poor-quality meat meal (MM 20) as three of the six rats lost

weight during the experiment. The table includes values for PER, NPU (balance), i.e. BV × TD ÷ 100, NPU (carcass) and NPR. All values are referred to casein = 100 to facilitate the comparison between methods, as the units in which results are expressed vary with the method of assessment. It can be seen that, with minor exceptions, the four methods place these proteins in the same order. There was a high correlation between PER and NPU (balance), NPU (carcass) and NPR with $r = 0.95$, 0.87 and 0.88 respectively. The agreement between PER and the other methods was excellent for good-quality proteins but progressively worsened as the quality of poorer protein sources deteriorated. In general, good agreement was observed when rats grew at a rate greater than 10–12 g/week.

Table 1. *Mean protein efficiency ratio (PER), with its standard error, of twenty-three proteins or protein sources (p. 126), PER, net protein utilization (NPU (balance) and NPU (carcass)) and net protein ratio (NPR) for these proteins when casein = 100*

		Casein = 100			
Protein source	PER	PER	NPU (balance)	NPU (carcass)	NPR
Casein	3·57 ± 0·029	100	100	100	100
FM 6	3·90 ± 0·058	109	107	100	103
SM 13	3·58 ± 0·037	100	100	105	109
SM 7	3·53 ± 0·081	99	97	103	108
SM 3	3·49 ± 0·079	98	96	109	110
Soya-bean meal + 2 g methionine/16 g N	3·47 ± 0·068	97	98	106	103
SM 8	3·00 ± 0·110	84	93	100	96
SB 5	2·95 ± 0·153	83	82	83	91
Soya-bean meal	2·83 ± 0·064	79	84	85	86
SB 1	2·82 ± 0·109	79	85	84	91
CM 49	2·71 ± 0·150	76	81	94	94
Dried brewer's yeast	2·24 ± 0·126	63	82	78	79
SF 2	2·24 ± 0·092	63	81	85	82
SF 3	2·20 ± 0·052	62	80	91	88
CM 19	2·13 ± 0·059	60	73	87	89
Linseed cake	2·11 ± 0·221	59	76	95	91
GN 12	1·70 ± 0·120	48	69	66	69
SB 3	1·66 ± 0·109	47	62	67	71
GN 18	1·55 ± 0·182	43	65	60	61
SF 1	1·37 ± 0·119	38	73	81	80
GN 11	1·15 ± 0·099	32	62	55	50
CM 13	0·90 ± 0·086	25	51	71	74
Wheat gluten	0·69 ± 0·064	19	65	64	71

Fig. 1 shows the effect of level of protein intake on BV, TD, NPU (carcass), NPR and PER for whole-egg proteins, casein and wheat gluten. TD was clearly independent of the level of protein intake for the three proteins. The curves for BV and NPU (carcass) were roughly parallel. Egg and casein values showed no change when the protein level was increased from 4 to 8%, but further increases in protein level caused a progressive decrease in these measurements, particularly in BV, that was more marked for egg than for casein. Values for wheat gluten decreased progressively as the dietary protein level increased, the greatest difference being between 4 and 8%. The two growth methods gave different responses. NPR decreased as dietary protein level

increased for the three proteins; PER rose to a maximum at 8% for egg proteins and casein; for wheat gluten there was a slight progressive increase from the 8 to the 20% level, and no value was obtained at the 4% level as all animals lost weight.
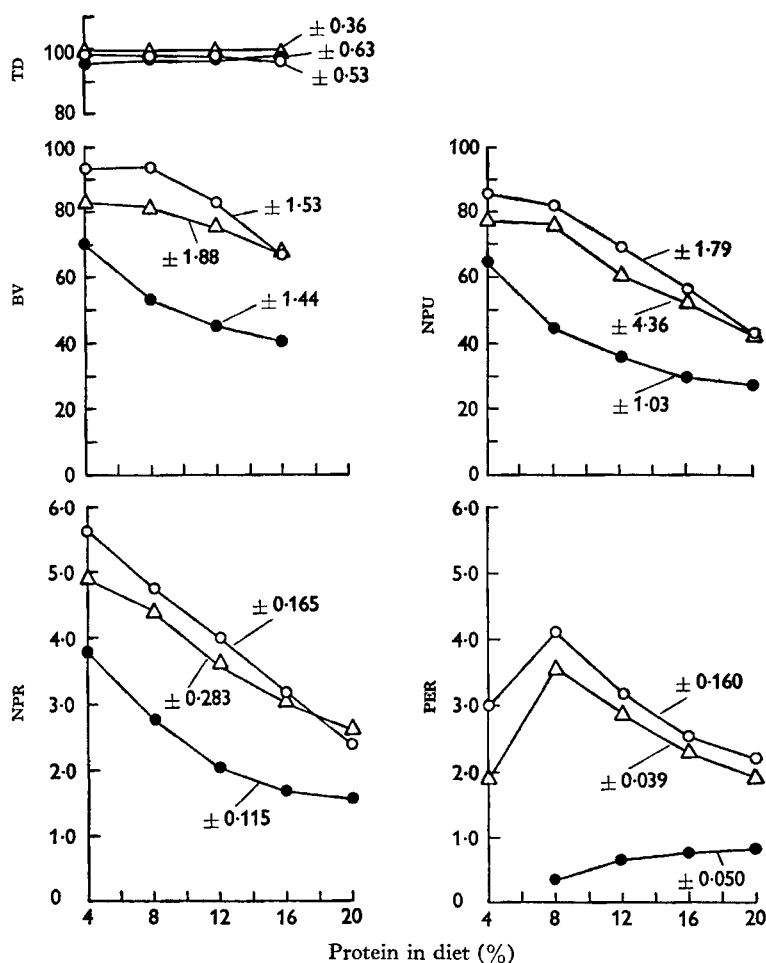

Fig. 1. Effect of level of protein intake on the biological value (BV), true digestibility (TD), net protein utilization by carcass analysis (NPU), net protein ratio (NPR) and protein efficiency ratio (PER) of whole-egg proteins (O—O), casein (△—△) and wheat gluten (●—●). SEs of the plotted points are given on each curve.

Table 2 shows the effect of cortisone on the NPU (carcass), NPR and NPU (balance) of casein, soya-bean meal, cottonseed meal (CM 13) and wheat gluten. Values for all three measurements with these proteins were lowered by cortisone. The decreases were more marked for NPU (carcass) and NPR than for NPU (balance), particularly with wheat gluten for which the 6% loss in NPU (balance) was not statistically significant.

Table 3 shows that, compared with carcass-N determination, the balance-sheet method overestimated both N lost by rats on a N-free diet and N retained from diets supplying 8% protein as casein or wheat gluten supplemented with methionine. On

all three diets N balances were reduced when carcass N was calculated from body-water measurements. There were marked individual variations between rats, and analysis of variance showed that, for casein and wheat gluten, N retentions calculated from balance and carcass-N determinations did not differ significantly but that results

Table 2. *Effect of cortisone on net protein utilization* (NPU *(balance) and* NPU *(carcass))* *and net protein ratio* (NPR) *of casein, soya-bean meal, cottonseed meal and wheat gluten*

	NPU (carcass)	NPR	NPU (balance)
Casein	74·3	4·38	89·8
Casein with cortisone	37·7	2·19	56·1
% decrease with cortisone	49	51	38
Soya-bean meal	57·7	3·34	67·5
Soya-bean meal with cortisone	33·2	2·17	47·4
% decrease with cortisone	42	35	30
Cottonseed meal	47·0	2·77	48·7
Cottonseed meal with cortisone	23·2	1·20	36·8
% decrease with cortisone	51	57	24
Wheat gluten	44·9	2·72	55·4
Wheat gluten with cortisone	29·3	1·85	52·2
% decrease with cortisone	35	32	6

Table 3. *Mean nitrogen retentions in* 10 *days, by groups of eight rats, from a N-free or* 8% *protein (casein or wheat-gluten supplemented with methionine) diet calculated from* (1) *analysis of food and excreta,* (2) *analysis of carcasses for N,* (3) *estimation of body N from body-water determinations. Also net protein utilization* (NPU) *values for casein and wheat gluten calculated from these values*

Measurement	Method of calculation	N-free diet	Casein diet	Wheat-gluten diet
N balance (mg N retained)	Analysis of food and excreta	− 306·1 ± 8·4	289·6 ± 68·0	181·5 ± 27·5
	Analysis of carcasses for N	− 254·5 ± 24·8	259·1 ± 90·8	123·8 ± 15·7
	Analysis of carcasses for water	− 391·3 ± 25·7	93·1 ± 86·7	0·0 ± 41·5
NPU	Analysis of food and excreta	—	81·8 ± 2·73	67·5 ± 2·72
	Analysis of carcasses for N	—	63·7 (58·7, 68·6)	49·1 (53·8, 44·4)
	Analysis of carcasses for water	—	60·6 (58·2, 62·9)	50·7 (49·0, 52·4)

Values are mean values with their SE except when they are the mean of two values only, which are given in parentheses.

calculated indirectly from body water were significantly lower than either. With the N-free diet, N losses calculated from balance values were intermediate between the two estimates from carcass analyses, and all differences were significant. The table includes NPU values calculated from the balance values and from carcass N determined directly or from the water content. The two values derived from carcass analysis agreed well but were lower than those derived from balance values.

DISCUSSION

It is clear from Table 1 that, under standard experimental conditions, PER, NPU (balance), NPU (carcass) and NPR ranked the twenty-three proteins for which a PER value was obtained (see p. 127) in essentially the same order. Differences between methods were observed, however, when experimental conditions were changed (level of protein intake, Fig. 1) or metabolic disturbances induced (cortisone treatment, Table 2). Chapman *et al.* (1959) studied six protein sources of different quality and observed a greater spread between good- and poor-quality proteins for PER than for NPU or NPR. They also found good reproducibility for the PER of casein. Our results confirm both of these findings. The PER value of casein given in Table 1 is the mean of four separate determinations of 3·63, 3·52, 3·58 and 3·54; a further value of 3·52 was obtained in the experiment to study the effect of level of protein intake. These figures for casein are rather higher than those, determined at similar levels of intake, reported in the literature. An explanation probably lies in the observations of Keane, Smutko, Krieger & Denton (1962, 1963) that higher PER values are obtained when diets are given moistened with water than when given dry. The latter appears to be the more general practice, but in this laboratory such diets are mixed to a thick paste with water to minimize scattering.

The results shown in Fig. 1 are in accord with the findings of other workers. Maximum values for PER have been reported at lower protein concentrations for good- than for poor-quality proteins (Osborne *et al.* 1919; Barnes, Maack, Knights & Burr, 1945; Yang, Clark & Vail, 1959; Morrison & Campbell, 1960*a*, *b*; Keane *et al.* 1962), although Jansen (1962) observed little change for casein and rolled oats as the dietary protein level was increased from 8 to 16 %. His results for wheat gluten supplemented with lysine were similar to those now reported for unsupplemented wheat gluten. TD was independent of the level of protein intake, as was also found by Forbes, Vaughan & Yohe (1958) and by Munaver & Harper (1959). Maximum BVs are found at rather lower protein levels for poor- than for good-quality proteins (Mitchell, 1923–4*b*; Forbes *et al.* 1958; Rippon, 1959; Yang *et al.* 1959). The sharp decrease in the BV of wheat gluten, in which the first limiting amino acid is lysine, between the 4 and 8 % protein levels is similar to that previously observed for a lysine-deficient sample of dried skim milk (Henry & Kon, 1957); the probable reasons for the finding were discussed by these authors. As would be expected, the curves for BV and NPU (carcass) were very similar; those for NPR resembled them rather than the curve for PER.

These observations that maximum BVs, but not PER, are usually found at an 8–10 % level of protein intake and are largely independent of protein quality may partly explain the greater spread in PER than in BV when determinations are made at a constant protein level (see Table 1).

The differences between N retentions calculated from balance and carcass-N determinations (Table 3) can be attributed, at least in part, to inevitable analytical errors. Although not significant, the differences in N retention from casein and wheat gluten are in the direction observed by other workers (cf. Butterworth, 1962). They lend some support to the view that the discrepancy is largely caused by errors in the

collection and analysis of excreta (Becker & Harnisch, 1958; Müller, Wilmes & Knappen, 1960; Butterworth, 1962; Chalupa & Fisher, 1963). Such errors will be of less significance with a N-free diet when smaller amounts of N are excreted.

Table 4. *Mean values for nitrogen content of carcasses of rats, given a stock, nitrogen-free or 8% protein (casein or wheat gluten) diet, found by analysis or calculated from body-water determinations*

| | Rats | | N in carcass | | Calculated N/found N (%) |
Diet	No. and sex	Age (days)	Found (%)	Calculated (%)	
Stock	8 ♀♀	30	2·65	2·63	99
N-free	8 ♀♀	40	3·06	2·86	93
8% casein	8 ♀♀	40	3·03	2·85	94
8% wheat gluten with methionine	8 ♀♀	40	3·00	2·85	95
Stock*	3 ♂♂, 3 ♀♀	38	2·71	2·72	100
N-free*	3 ♂♂, 3 ♀♀	38	2·81	2·67	95
8% casein*	3 ♂♂, 3 ♀♀	38	2·83	2·62	92

* Calculated from Henry & Toothill (1962).

Scrimshaw (1962) advises caution in the calculation of NPU (carcass) from body-water measurements and points out that 'evidence is accumulating that the inherent assumption, i.e. that the ratio of gain in protein to gain in water is constant, does not hold good for all types of dietary protein', and Stucki & Harper (1962) query the assumption 'that carcass $N:H_2O$ ratios are constant at any given age regardless of the previous dietary treatment'. Some support for these doubts is evident from the values involving carcass analyses now presented. Table 4 gives the N content of litter-mate rats given our stock diet or the three experimental diets, determined directly or calculated from body-water measurements, together with earlier results (Henry & Toothill, 1962). Although very few determinations were made, it is clear that the $N:H_2O$ ratio determined with rats given our stock diet predicts accurately the N content of such rats but that it slightly, but consistently, underestimates N in rats given experimental diets. Standal (1963), on the other hand, found that the N content of the carcasses of rats given five experimental diets (one with no protein and four with 10% protein) was from 1 to 20% higher when calculated from water than from N analyses; he determined the relationship between water and N in the bodies of seventy-seven rats given diets containing six types of protein of varying quality. We have already pointed out (Henry & Toothill, 1962) that a slight error is inherent in calculating N in the body from its water content, but concluded that the error would be constant for the N-free and other experimental diets so that the NPU (carcass) values quoted in that paper would not be affected. This view is supported by the excellent agreement for both casein and wheat gluten when NPU (carcass) is determined by direct or indirect carcass-N measurement (Table 3).

Two further discrepancies between NPU (balance) and NPU (carcass) are evident in the tests with cortisone. In the first place the extent of the reduction in NPU (balance) varied with the quality of the protein, whereas the reduction in NPU (carcass) was

similar for the four proteins tested. Secondly the reduction was more marked for NPU (carcass) than for NPU (balance). One cause of this finding is undoubtedly the error arising from the application of the N:H$_2$O conversion factor determined on normal rats to animals treated with cortisone: body water was reduced by such treatment but the change in body N may not have been proportional. The percentage loss in nutritive value of these proteins was very similar when measured as NPU (carcass) or NPR. This observation together with the close correlation for these quantities evident from Table 1 (cf. p. 128) support the views of Campbell (1961) and Njaa (1963) that NPU (carcass), calculated by the method of Miller & Bender (1955), is the same as NPR, since the dominating factor in both terms is the difference in weight between groups of rats given a N-free diet and the test protein. It may be concluded that, for a rapid routine assessment of protein quality, the simpler determination of NPR can replace that of NPU (carcass).

In general, and in spite of the greater amount of time and labour involved, balance methods are preferred to the simpler growth methods as they give more precise information (Njaa, 1963; (USA) National Research Council, 1963; Bigwood, 1964). Growth methods are, however, simpler than balance methods for the routine assessment of protein quality, and good correlation has been found between NPU (both balance and carcass) and PER (Block & Mitchell, 1946–7; Henry *et al.* 1947–8; Bender, 1956). PER has been criticized by Bender & Doell (1957) because no value is obtained if the quality of the protein is too poor to permit growth; they consider that the inclusion of a control group given a N-free diet in the determination of NPR overcomes this difficulty. However, workers have asked for further information on the significance and value of such a group (Campbell, Sabry & Cowan, 1963; (USA) National Research Council, 1963).

Campbell *et al.* (1963) conclude that the choice of method should primarily be determined on the basis of precision and reproducibility. Of the growth methods studied by them, they found that NPR values were no more stable than those for PER, whereas in this laboratory PER correlated with NPU (balance) rather better than did NPR (Table 1 and p. 128). Good reproducibility for the PER of casein has been observed in Canada (Chapman *et al.* 1959) and in this laboratory (p. 131), and Derse (1960) has reported good agreement between laboratories for three proteins of different quality when a standard procedure was adopted. On the other hand, discrepancies were observed between two laboratories when NPU (carcass) was determined from body-water measurements (cf. Henry & Toothill, 1962). There were similar discrepancies for NPR values calculated from the same data. It may be concluded that, at present, PER can be considered a more satisfactory growth method than NPR when used for the evaluation of proteins for growing children. The failure of the former to yield values for proteins of poor quality is not serious since, as Campbell (1961) has pointed out, a protein that will not support growth in the rat is unlikely to be of value for malnourished infants unless it can be shown to have supplementary properties.

SUMMARY

1. The protein efficiency ratio (PER) was determined on rats, at an 8% level of protein intake, for twenty-three protein sources for which values for net protein utilization (NPU), determined from both balance data and carcass analysis, and net protein ratio (NPR) have already been reported (Henry & Toothill, 1962).

2. PER values were highly correlated with those for NPU (balance), NPU (carcass) and NPR with $r = 0.95$, 0.87 and 0.88 respectively.

3. The biological value (BV), true digestibility (TD), PER, NPU (carcass) and NPR of whole-egg proteins, casein and wheat gluten were determined at 4–16% (BV and TD) or 4–20% (PER, NPU (carcass) and NPR) levels of protein intake.

4. Responses were similar for BV and NPU (carcass). Increasing the protein level from 4 to 8% had no effect for whole-egg proteins and casein but caused a sharp drop for wheat gluten; there was a continuous drop for all three proteins with further increases in protein concentration. TD was not affected by level of intake for the three proteins. PER increased between 4 and 8% and then fell continuously to the 20% level for egg proteins and casein; for wheat gluten there was a continuous slight rise from the 8 to the 20% level of intake; no value was obtained at the 4% level. NPR dropped steadily from the 4 to the 20% level for all three proteins.

5. NPU, determined both from balance data and by carcass analysis, of casein, soya-bean meal, cottonseed meal and wheat gluten was reduced by a daily injection of 2.5 mg cortisone acetate. The reduction in NPU (balance) decreased with protein quality from 38% for casein to 6% for wheat gluten; the reduction in NPU (carcass) was independent of protein quality and amounted to 35–50%.

6. N retention from a 'N-free' diet and from diets containing 8% protein as casein or wheat gluten was determined from balance data or from analysis of the carcasses for N, determined directly or calculated from body-water values. NPU values for casein and wheat gluten were calculated from the three sets of data.

7. Compared with analysis of the carcasses for N, the balance-sheet method over-estimated both the N lost with the 'N-free' diet and the N retained with casein and wheat gluten. The discrepancies were accentuated when N in the body was calculated from its water content. For both proteins, NPU (carcass) was independent of the method of determining body N; the values were lower than for NPU (balance).

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