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The N:H_2O ratio in the Sprague–Dawley rat and its variation with diet under the conditions of determination of net protein utilization*

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1. Analytical values are presented for body nitrogen, both on a fresh and dry carcass basis, body water and N to body water ratios for rats, of various ages, of the Sprague–Dawley Strain.

2. For 33- and 40-day-old animals, there were significant differences in body composition, including N:H_2O ratio, between animals given a protein-free diet and those given protein at the 10% dietary level. For all age groups the N and water percentages were higher in the carcasses of animals given the non-protein diet.

3. For all age groups there were significant negative correlations between the percentage of N in the dry carcass and the net dietary protein value (NDpV) of the diet. For 33-, 37- and 40-day-old animals there were significant negative correlations between the N content of the fresh carcass and the NDpV of the diet. For 33-day-old animals only, the correlation between N:H_2O ratio and NDpV was also highly significant.

4. Body N values calculated from N:H_2O ratio and from N:body-weight ratio were compared. At all ages, an equally good prediction was obtained from the N:body-weight ratio as from the N:H_2O ratio provided that the correct factors were used for animals given the non-protein and test diets.

The use of carcass analytical methods for the determination of net protein utilization (NPU) using young growing rats is now a well-established procedure (Miller & Bender, 1955; National Research Council, 1963). Short-cut methods involving body-water determinations followed by the use of a linear regression line relating age and the nitrogen to body-water ratio have been shown to give a reasonably accurate estimation of N retained for a specific colony of rats (Bender & Miller, 1953; Miller & Bender, 1955). Henry & Toothill (1962) have confirmed this good correlation but claim that more rats are needed to obtain the same order of accuracy as is obtained when using the balance sheet method for net protein value (NPV) of Mitchell (1923–4) and Mitchell & Carman (1926). Good correlations between body N and body water methods have also been recently demonstrated by Rafalski & Nogal (1966). It has also been shown (Donoso & Yanez, 1962; Rafalski & Nogal, 1966) that carcass N can be calculated with sufficient accuracy for the calculation of NPU from the body-weight using a N to body-weight ratio.

The use of N to body-water ratios for the calculation of NPU requires the constancy of the ratio at any specific age, i.e. that the ratio is independent of diet. The work of Miller & Bender (1955) has demonstrated that the ratio is constant for rats of their

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black and white hooded colony, and Rafalski & Nogal (1966) imply that this is so for rats of their Wistar Strain. However, Stucki & Harper (1962) have questioned the assumption 'that carcass N:H_2O ratios are constant at any given age regardless of the previous dietary treatment' and Henry (1965) showed that the N:H_2O ratio slightly but consistently underestimated the N content of rats on certain experimental diets, while Scrimshaw (1962) has pointed out that the ratio of gain in protein to gain in water may not always hold true for all types of dietary protein.

Whenever the use of short-cut procedures has been advocated it has been suggested that ratios be established for each particular colony of rats in each specific establishment. Over a period of more than a year analytical results had accumulated in this laboratory involving carcass N and water determinations upon the rats used here for NPU assays. Upon examination of these values it was soon obvious that the N:H_2O ratio at any specific age was not constant for this particular strain of rats. Consequently all the accumulated analytical values were examined and the results are now reported.

EXPERIMENTAL

Animals and technique

Analytical results were available for nearly 800 male rats of the Sprague–Dawley strain which had been used in this laboratory for the evaluation of protein quality by the NPU procedure. The information included age, body-weight, % H_2O (fresh basis) and % N (dry basis) of the animals. Only values for male rats were available since the routine procedure for protein quality evaluation in this laboratory had been the protein efficiency ratio (PER) method according to Campbell (1963) and thus regular monthly deliveries by air from London, England, of weanling male rats had been inaugurated.

Table 1. *Number and age of rats and length of experimental period*

Age (days)	No of rats	Age at beginning of experiment (days)	Period on test diet (days)
30	70	23	7
33	502	23	10
37	74	23	14
40	120	30*	10

* Seven days adjustment period (see below for explanation).

The NPU determinations had been made for various periods ranging between 7 and 14 days. Thus, since the experiments had been started with 23-day-old rats, the ages of the rats when the carcasses were analysed were 30, 33 and 37 days respectively. However, there were some additional experimental results from animals which had been given the stock diet for 1 week after arrival followed by the experimental diet for 10 days; the age of these animals was thus 40 days when the carcasses were analysed. These details, together with the number of animals concerned are shown in Table 1.

Each experimental group consisted of ten animals so arranged that as far as possible all the groups had the same mean weight and that there was approximately the same weight distribution within each group. Rats were housed individually in wire-mesh cages in a room maintained at $23 \pm 1°$ with a relative humidity of 50–55%. Water and food were given *ad lib.*, and the weights of the rats, food consumed and food spilled were recorded daily. At the end of the experimental period the rats were killed with chloroform and incisions were made into the skull, thoracic and body cavities; and the carcasses were dried in a hot-air oven at 105° for 48 h. The dried carcasses were then ground to a powder in an electric mill and a portion was analysed for total N by a standard macro-Kjeldahl technique.

A basal diet as described by Campbell (1963) for PER and net protein ratio (NPR) determinations was used, i.e. maize starch, 80; maize oil or cottonseed oil, 10; non-nutritive cellulose, 5; salts (USP XIV), 4; and vitamin mixture, 1. Most of the protein foods were incorporated into the basal diet at the expense of maize starch to give 10% (9·7–10·3) protein; however a few of the diets had a protein level of 6·5%. The non-protein diet was the unmodified basal diet. Diets were given in the dry powdered form from containers designed to minimize spillage. The NPU values were calculated in a manner similar to that described for NPR by Campbell (Appendix A, National Research Council, 1963), comparing pairs of animals from the test group and non-protein group respectively ranked according to their initial weights. The mean of ten values was taken as the NPU of the protein concerned. Owing to the difficulties of accurate matching of weights for control and test groups, the procedure has now been replaced by the recommended group procedure as described by Miller (Appendix C, National Research Council, 1963). Forty-eight protein sources were examined and their determined NPU values ranged from 22 to 86. Details of these diets are shown in Table 2.

Table 2. *Protein content and NPU of diets fed*

No. of diets	NPU values found	Protein level (%)
1	22	10
14	30–39	10
10	40–49	10
7	50–59	10
9	60–69	10
2	70–79	10
1	85	10
2	20–30	6·5
2	30–39	6·5

RESULTS AND DISCUSSION

Body composition and diet

The relationship between the body composition of the rats and both diet and age is shown in Table 3. The separation into diets is merely into two groups, those given a non-protein and those given a test diet at the 10% level. For all age-groups the N percentage in both the fresh and dry carcass and the percentage of total water was higher for the animals given the non-protein diet than for those fed at the 10% protein level; this was only to be expected and reflected the lower level of fat in the carcasses of the animals given the non-protein diet. However, the $N:H_2O$ ratio was also higher in all age-groups given the non-protein diet although it was statistically significant only for the 33-day-old animals ($P < 0.001$), i.e. the group with the largest number of animals. For the 40-day-old animals, the other group containing a large number of animals, the difference was barely significant ($P < 0.10$). The difference of means was also highly significant when the results for all the age-groups were combined ($P < 0.001$). This of course would be influenced by the large number of animals in the 33-day-old group. It must, however, be recognized that the diets in the 10% protein group varied considerably, with NPU values ranging from below 30 to above 80. Correlation coefficients were calculated between the diet fed expressed as net dietary protein value ($NPU_{(op)} \times$ % protein)/100 and body composition for all age groups as defined by Platt & Miller (1959).

Expression as NDpCal% (Platt, Miller & Payne, 1961) would have been preferable but the energy values of the diets had not been determined. Since, however, all the diets were derived from the basal diet by replacement of the maize starch with the protein being assayed, the energy values of all diets were similar. There were highly significant ($P < 0.001$) negative correlations between the protein value of the diet expressed as NDpv and the carcass N% on a dry-weight basis. Highly significant negative correlations were also present between N% fresh body-weight and NDpv at 33 and 40 days; at 37 days the correlation was still significant ($P < 0.05$) but to a lesser degree. The % H_2O was also negatively correlated with NDpv at 30 and 37 days ($P < 0.05$) and 40 days ($P < 0.001$) but was not significant at 33 days.

Assuming the constancy of body composition on a fat-free basis (Moulton, 1923; Pace & Rathbun, 1945) all the above correlations could be explained by an increase in body fat with increasing NDpv. However in contrast to findings with other colonies of rats there was also a highly significant negative correlation ($R = -0.38$, $P < 0.001$) between the $N:H_2O$ ratio and NDpv of the diet for the 33-day-old animals, i.e. the group containing the largest number of animals. The regression equation linking $N:H_2O$ ratio (Y) with NDpv (X) was $Y = -0.068X + 4.08$. The correlation was caused by a decrease in the N% with increasing NDpv while the % H_2O remained unchanged. Similar correlations pertained, though generally to a lower degree of significance when the results for the non-protein-fed animals were excluded. Then, in addition, $N:H_2O$ ratios were negatively correlated with NDpv at both 33 and 37 days of age ($P < 0.01$ and < 0.05 respectively).

As expected, and confirming the observations of Hegsted & Worcester (1947), there was a highly significant increase in weight with an increase in NDpv with correlation coefficients of between 0.88 and 0.93 for all age-groups, i.e. a relationship between protein quality and weight independent of food intake. At 33 and 40 days of age, i.e. 10-day experimental periods without or with 7-day adjustment periods, the regression equations relating NDpv and weight were $X = 0.106Y - 2.56$ and $X = 0.083Y - 3.58$ respectively, where X is NDpv and Y is body-weight in g. It should be emphasized that the maximum NDpv concerned was 8.5, i.e. a NPU of 85 at the 10% protein level.

Table 3. *The relationship of body composition of the rats with diet and age*

(Mean values with their standard errors)

Diet	Age (days)	No. of rats	Nitrogen % dry body-weight	*P*	Water (%)	*P*	Nitrogen (%)	*P*	N:H_2O ratio	*P*
Non-protein	30	18	10·73 ± 0·12	< 0·001	72·64 ± 0·94	< 0·05	2·71 ± 0·09	NS	3·66 ± 0·16	NS
10% protein	30	52	9·41 ± 0·09	—	74·42 ± 0·44	—	2·58 ± 0·03	—	3·59 ± 0·07	—
Combined	30	70	—	—	—	—	—	—	3·61 ± 0·06	—
Non-protein	33	102	10·20 ± 0·07	< 0·001	71·14 ± 0·24	NS	2·94 ± 0·03	< 0·001	4·16 ± 0·06	< 0·001
10% protein	33	400	8·88 ± 0·05	—	70·47 ± 0·13	—	2·60 ± 0·02	—	3·70 ± 0·02	—
Combined	33	502	—	—	—	—	—	—	3·79 ± 0·02	—
Non-protein	37	17	10·54 ± 0·11	< 0·001	72·14 ± 0·42	< 0·001	2·93 ± 0·05	< 0·10	4·08 ± 0·10	NS
10% protein	37	57	9·38 ± 0·09	—	69·79 ± 0·27	—	2·83 ± 0·03	—	4·06 ± 0·05	—
Combined	37	74	—	—	—	—	—	—	4·07 ± 0·05	—
Non-protein	40	33	9·94 ± 0·09	< 0·001	71·14 ± 0·48	< 0·001	2·86 ± 0·05	< 0·001	4·04 ± 0·07	< 0·10
10% protein	40	87	8·53 ± 0·09	—	68·55 ± 0·24	—	2·67 ± 0·02	—	3·90 ± 0·03	—
Combined	40	120	—	—	—	—	—	—	3·94 ± 0·04	—
Non-protein	All	170	10·24 ± 0·05	< 0·001	71·61 ± 0·21	< 0·001	2·90 ± 0·02	< 0·001	4·07 ± 0·04	< 0·001
10% protein	All	596	8·92 ± 0·03	—	70·13 ± 0·12	—	2·64 ± 0·01	—	3·78 ± 0·02	—
Combined	All	766	—	—	—	—	—	—	3·84 ± 0·02	—

NS, not significant.

Body composition and age

Regression equations relating the N:H_2O ratio with age have been reported by various workers. The various equations are summarized in Table 4. The regression equation obtained with this rat colony shows good agreement with those of the other workers and is especially close to that of Bender & Miller (1953).

The values used for the calculation of this regression equation were those for all the animals including those fed the non-protein diet. However, because of the difference in body composition (including N:H_2O ratio) produced by diet with this strain of rat one should not use a single age-related N:H_2O ratio for body N calculations.

Table 4. *Published values for regression equations relating age and N:H_2O ratio*

Investigators	Age range (days)	No. of rats	Strain	Regression equation
Dreyer (1957)	32–49	300	Wistar	$Y = 0.015X+3.39$ (male)
Bender & Miller (1953)	33–57	147	Black and white hooded	$Y = 0.024X+2.92$ (both sexes)
Miller & Bender (1955)	0–503	197	Black and white hooded	$Y = 4.83 - \text{antilog}_{10}(0.4304-0.0115X)$
Henry & Toothill (1962)	36–42	—	Hooded Norwegian	$Y = 0.034X+2.63$ (male) $Y = 0.034X+2.71$ (female)
This paper	30–40	766	Sprague–Dawley	$Y = 0.027X+2.86$ (male)

X = age in days, $Y = (100 \times \text{N}\,\%)/(\text{H}_2\text{O}\,\%)$.

Predictions of body N from body-weight or body water

It has been shown (Donoso & Yanez, 1962; Rafalski & Nogal, 1966) that NPU can be calculated with almost equal accuracy from body N, body water or body-weight, using with the last two appropriate factors for conversion of the data into body N. Campbell (1963) and Henry (1965) have pointed out that if a body-weight factor is used to calculate NPU it becomes the same as NPR adjusted to a percentage scale, i.e. protein retention efficiency (PRE) as described by Bender & Doell (1957) since both are calculated from the difference in weight between rats given a non-protein diet and those given a test diet.

With the exception of the 30-day-old animals given a non-protein diet, the values obtained for total body N were very close whether determined directly or calculated from either body-weight or body water. Correlation coefficients were also calculated between the determined and the two calculated N values for the various age and diet groups using the factors shown in Table 3. These results are shown in Table 5. It can be seen that with the exception of the 30-day-old animals given the non-protein diet all the correlations were highly significant, with the coefficient being slightly higher for the prediction from body-weight than for the prediction from body water. The generally accepted view (Moulton, 1923; Pace & Rathbun, 1945) is that at 'chemical maturity' body composition on a fat-free basis is constant and thus body water should give a better prediction of total N than body-weight. The very slight difference observed in the correlation coefficients in no way challenges this accepted viewpoint; it is, however, suggested that under the conditions of the experiments here reported (10% protein diets, Sprague–Dawley rats, 30–40 days of age), the prediction of body N can be obtained with sufficient accuracy from the body-weight for the calculation of NPU, confirming the results of Donoso & Yanez (1962) and Rafalski & Nogal (1966). Thus although the value so calculated becomes theoretically identical with the PRE value of Bender & Doell (1957), there would appear to be as much justification in retaining the term NPU for values calculated in this manner as for values calculated from body-water determinations.

Table 5. *Correlation coefficient between determined body nitrogen and nitrogen calculated from body-weight or body water*

Diet	No. of rats	Age (days)	Correlation coefficient: Calculated from body-wt	Correlation coefficient: Calculated from body water
Non-protein	18	30	0·23	−0·24
10% protein	52		0·86	0·77
Combined	70		0·90	0·82
Non-protein	102	33	0·55	0·28
10% protein	400		0·95	0·93
Combined	502		0·96	0·95
Non-protein	17	37	0·83	0·66
10% protein	57		0·93	0·90
Combined	74		0·96	0·94
Non-protein	33	40	0·95	0·92
10% protein	87		0·96	0·94
Combined	120		0·97	0·96

Factors used for calculation are those shown in Table 3.

One must distinguish two factors concerned with body N predictions with this strain of rat. Firstly one can use either body-weight or body water to predict total body N with equal accuracy provided that the appropriate factors are used for the varying ages and whether the rats are given non-protein diet or test diet. However, in addition, with this particular colony, there are changes in N:H_2O ratio, i.e. changes in body composition on a fat-free basis, dependent on diet, indicating that the N:H_2O ratio used should not only be dependent on age but also on the expected NPU of the diet being assayed—a clearly impossible situation.

The greatest effect of diet upon body composition was with the 33-day-old rats. Values of NPU calculated from N:H_2O ratios could be in error by as much as 15–20 units if the combined ratio was used for non-protein-fed and test-fed animals rather than the use of two ratios, i.e. not recognizing the difference in body composition due to diet. No error would occur using N analysis results. These are, however, younger animals than those normally recommended for NPU determinations (National Research Council, 1963) which are 38 days old upon analysis (7 days adjustment, 10 days experimental from weaning). These would correspond to the 40-day-old animals described above in which the effect of diet upon body composition was less pronounced, although even here errors of 5–6 units could be caused by the use of a combined ratio for non-protein-fed and test-fed animals. It would thus seem that for rats of the Sprague–Dawley strain only NPU values based upon carcass N analysis are valid although predicted values derived using different ratios for non-protein-fed and test-fed animals would be closer than those predicted using a single ratio for any particular age.

The above results were obtained with diets whose protein concentration did not exceed 10%, but when diets are measured under operative conditions (Miller & Payne, 1961) and protein concentrations are higher body composition changes may be more marked. Work is in progress to determine body composition changes at higher levels of protein intake.

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