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## Minimum Dietary Protein required to Maintain Nitrogen Equilibrium in Adult Rats

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Five diets containing 11.6, 9.2, 7.0, 5.0 and 4.0 per cent protein were fed *ad-libitum* to rats for 14, 17, 14, 21 and 28 days respectively and nitrogen balance was measured. Results indicate that the proteins level required for the maintenance of body weight and nitrogen balance in equilibrium is 5.0% when fed *ad-libitum* to the adult rats under the present experimental conditions.

The minimum protein requirement is not a well established quantity either for the rats or for other species. Barnes *et al.*<sup>1</sup> reported that the proteins requirement of young and adult rats were nearly the same in terms of egg protein (11 mg N/100 Cm<sup>2</sup>/day). The requirement for egg protein for maintenance of nitrogen balance found by Forbes *et al.*<sup>4</sup> was 0.9 mg N/day/kg<sup>0.75</sup> body weight. The National Research Council<sup>8</sup> recommended 880 mg protein/day/Kg<sup>0.734</sup> body weight. Njaa<sup>9</sup> indicated that protein level required for the maintenance of body weight of young rats was 2, 3 and 5 per cent of the diet for egg albumin, casein and pea meal respectively. The present study was conducted to find the

level of dietary protein adequate to maintain nitrogen equilibrium in adult rats.

### MATERIALS AND METHODS

Twenty adult rats (6 months old) of Sprague-Dawley strain were used in this study. The animals had been raised on stock diet from weanling till adult age. The rats were distributed into five groups of four each and were kept in separate metabolic cages in a room maintained at 70-72°F.

Five experimental diets containing 11.6, 9.2, 7.0, 5.0 and 4.0 per cent protein were prepared (Table 1).

TABLE 1. Composition of Experimental Diets.

Ingredients	A	B	C	D	E
Casein	11.6	9.2	7.0	5.0	4.0
Maize starch	77.4	79.8	82.0	84.0	85.0
Corn oil	5.0	5.0	5.0	5.0	5.0
Vitamin mixture	2.0	2.0	2.0	2.0	2.0
Mineral mixture	4.0	4.0	4.0	4.0	4.0
Total	100.00	100.00	100.00	100.00	100.00

The diets were allotted to the group of rats at random and were fed *ad-libitum* for 14, 17, 14, 21 and 28 days respectively. A preliminary period of 2 to 3 weeks was allowed prior to the actual experiment to adjust the rats to new feeding regimen.

Nitrogen balance, body weight and feed consumption were measured twice a week. Urine, faeces and shed hair were collected in 1 N H<sub>2</sub>SO<sub>4</sub> from the individual animal and three or four days pooled collection from each rat was homogenized in a blender for 2-3 minutes. A 5 ml of homogenized sample in duplicate was analysed for nitrogen content by Kjeldahl method. The samples of diet were analysed for nitrogen (Kjeldahl method) and calorie (Ballistic bomb calorimeter) contents.

### RESULTS AND DISCUSSION

Average weight changes, food intake and nitrogen balance data are shown in Table 2. Food intake was calculated as Kcal/Kg<sup>3/4</sup>/day. The rats consumed almost the same amount of each diet, but gained weight and nitrogen in proportion to the protein content of the diet. On 5% protein diet the rats maintained both body weight and nitrogen balance, the

average intake per Kg<sup>3/4</sup>/day was 150 Kcal and 335 mg nitrogen.

When the level of protein was reduced to 4% the growth ceased and the animals remained in negative nitrogen balance, indicating that this level of protein in the diet could maintain only the body weight of the adult rats.

Dietary nitrogen consumed is ultimately either stored in the form of body protein or eliminated as urea. Obviously positive nitrogen balance values must be paralleled by a proportional increase in body weight apart from changes in fat content.

A factor 30 has been used in converting nitrogen weight into live weight (Costa<sup>3</sup>). It is apparent (Table 2) that increase in body weight is proportion to the amount of nitrogen retained in the body of rats with the first three levels of protein. When the protein level in the diet was reduced to 5.0 or 4.0% the changes in body weight differed from the calculated values. The rats gained more weight than the calculated values from the nitrogen balance presumably due to fat deposition thus representing a change in the composition of body.

TABLE 2. Average Weight Changes, Food Intake, Nitrogen Balance of Adult Rats Fed Various Levels of Protein.

Diet Protein per cent	No. of rats	Days	Weight change		Intake		Nitrogen balance	
			Calculated*	Found	Kcal/Kg <sup>3/4</sup> /day	mgN/Kg <sup>3/4</sup> /day	mgN/Kg <sup>3/4</sup> /day	As per cent of N intake
11.6	4	14	+40.5	+40	152	749	+195	+26
9.2	4	17	+30.0	+30.0	148	579	+120	+21
7.0	4	14	+7.0	+10.5	145	446	+38	+9
5.0	4	21	+3.0	+8.0	150	335	+5	+2
4.0	4	28	-17.0	-2.0	147	252	-45	-17

\*Nitrogen retained  $\times$  30

The nitrogen balances and change in body weight in all the diets are plotted against the nitrogen intake (Fig. 1). The regression line for body weight ( $Y = .09 \times -24$ ) passes the line of equilibrium at 270 mg N/Kg<sup>3/4</sup>/day, approximate to the 4% protein diet. The second regression line for nitrogen balance ( $Y = .49 - 168$ ) crosses the line of balance at 340 mg N/Kg<sup>3/4</sup>/day or 5.0% protein level. These lines indicate that the protein level required for the maintenance of body weight and nitrogen balance in equilibrium is 5.0% when fed *ad-libitum* to the adult rats under the present experimental conditions.

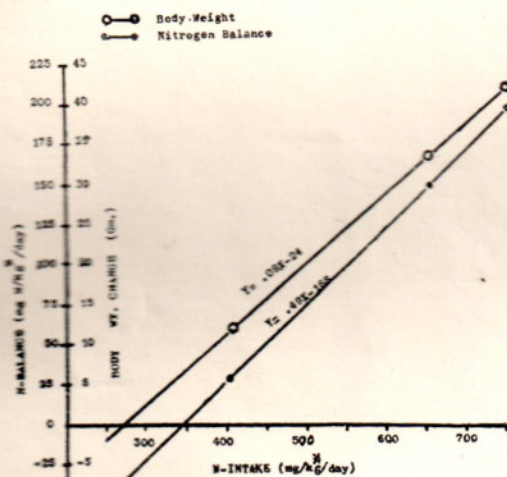


Fig. 1 Nitrogen Intake versus Nitrogen Balance and Weight changes

Weight maintenance depends upon the balance of the gains and losses of a number of body components, the more important of which are fat, protein and water. Regimens likely to produce no change in body weight, therefore, do not depend solely on caloric intake but also on the other dietary factors, particularly protein. However, since the caloric intake is known to influence the protein value of the diets, these factors must be considered together.

Net dietary protein values which are a measure of the utilizable protein in the diet (Platt and Miller<sup>10</sup>), were calculated as  $NDpcals\% = NPU \times P$  where P is the percentage of energy supplied by the protein and was calculated from

$$\frac{25 \times N\%}{\text{Metabolizable energy/gm}}$$

and were found 3.5 and 2.8 for the diets containing 5% and 4% protein respectively.

Miller and Payne<sup>5,6</sup> have shown that diets having NDpcals 4% met the protein requirements for maintenance of animals fed *ad-libitum* and caloric requirements for maintenance have been reported 140 Kcal/Kg<sup>3/4</sup>/day (Brody<sup>2</sup>), 160 Kcal/Kg<sup>3/4</sup>/day (Smuts<sup>11</sup>), 1935 and 150 Kcal/Kg<sup>3/4</sup>/day (Miller and Payne<sup>7</sup>).

In this experiment the animals maintained their body weights with 147 Kcal/Kg<sup>3/4</sup>/day (NDpcals. 2.8%) and 150 Kcal/Kg<sup>3/4</sup>/day (NDpcals. 3.5%) and this agrees well with the above workers. The 4% protein diet could not satisfy protein requirement so the animals were in negative nitrogen balance. The maintenance of weight on this diet could be explained on the basis of changes in the composition of the body.

The present work suggests that a caloric intake of 150 Kcal/Kg<sup>3/4</sup>/day of a diet of 3.5 NDpcals is completely adequate for maintaining weight and nitrogen equilibrium in adult rats.

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