

HEALTH PROTECTION BRANCH
OTTAWA
DETERMINATION OF PROTEIN RATING

I. APPLICATION

This method shall be used to determine the protein rating of foods under Sections B.01.062, B.01.063, B.01.064, B.01.065, B.14.073, B.14.085, B.14.086, B.14.087, B.14.088, B.14.089, B.14.090, B.21.027, B.22.027, B.22.029 and B.22.032 of the Food and Drug Regulations.

II. PRINCIPLE

A bioassay for the evaluation of the nutritional quality of the proteins, this method involves measurement of the protein efficiency ratio (grams gain per gram protein consumed) under standardized conditions. Male rats 20-23 days of age are fed ad libitum an otherwise adequate reference diet containing 10% protein supplied by a standard sample of casein. Foods to be assayed are added to the diet as the sole source of protein at the expense of casein and corn starch to maintain a protein level of 10%. Protein efficiency ratios (PER's) are calculated after 4 weeks and are adjusted to a constant value of 2.5 for casein. The protein rating is the product of the adjusted PER of the test food multiplied by grams protein in a reasonably daily intake of the test food. Foods containing much lipid (meats, nuts, whole milk power, etc) may require a lipid extraction. See Appendix A.

III. PROCEDURE

The test shall be carried out in accordance with the following instructions:

1. Animals

- (1) use weanling male rats of single strain 20-23 days of age, 10 for each diet.

2. Diets

- (1) use a basal diet of the following percentage composition on an air-dry basis:

Corn starch	80%
Corn oil or Cottonseed oil	10%
Non-Nutritive cellulose	5% (Note 1)
Salts, U.S.P. XIV	4% (Note 2)(1)
Vitamin mixture	1% (Note 3)
- (2) incorporate the protein food under test into the diet at the expense of corn starch to give 10% protein ($N \times 6.25$);
- (3) the protein content of the final diet should be within the range 9.7 to 10.3% as determined by analyses (Note 4);
- (4) supply the water and diet ad libitum.

3. Assay Period

- (1) use a 4 week period.

4. Cages

- (1) use individual cages which have screen bottoms and are provided with feeders which will reduce food spillage to a minimum.

5. Randomization

- (1) use a randomized block design in which blocks represent variations in

initial weight;

- (2) randomize the rats in each block for diet and cage;
- (3) a procedure for randomization is outlined in Appendix B.

6. PER Determination

- (1) in addition to the test group, maintain a reference standard group of rats on a diet consisting of the basal diet with casein at the level of 10% protein (N x 6.25) as determined by analyses;
- (2) for uniformity, this casein should be a product of uniform high quality and good stability;
- (3) the Test Diet High Nitrogen Casein approved by the Animal Nutrition Research Council and prepared by the Sheffield Chemical Company, Norwich, N.Y., has been found to be satisfactory;
- (4) all test animals must be maintained at a room temperature of $72 \pm 1^{\circ}\text{F}$ and a relative humidity of $45 \pm 5\%$;
- (5) for each animal, maintain a weekly record of body weight and food consumption.

IV. CALCULATIONS

- (1) At the end of 4 weeks calculate the PER for each food and for the Reference Standard casein using the following equation:

$$\text{PER} = \frac{\text{weight gain in grams}}{\text{weight of protein consumed in grams}}$$

- (2) Assuming that the casein has an average PER of 2.5 when determined under these conditions, adjust the PER of the test food as follows:

$$\text{Adjusted PER} = \text{PER (Test Food)} \times \frac{2.5}{\text{Determined PER of Standard Reference Casein}}$$

- (3) Calculate the protein rating (PR) of the test food as follows:

PR = Adjusted P.E.R. x grams protein in a reasonable
daily intake of the food (Note 5).

V. NOTES

- (1) Cellulose preparation used should be essentially free from nitrogen.
(2) Salts U.S.P. XIV consist of the following mixture:

Calcium carbonate (U.S.P.)	68.6 g	
Calcium citrate (U.S.P. Reagent)	308.3 g	
Calcium biphosphate (U.S.P. Reagent)	112.8 g	
Magnesium carbonate (U.S.P.)	35.2 g	
Magnesium sulphate, anhydrous (U.S.P. Reagent)	38.3 g	
Potassium chloride (U.S.P.)	124.7 g	
Dibasic potassium phosphate (U.S.P. Reagent)	218.8 g	
Sodium chloride (U.S.P.)	77.1 g	
Cupric sulphate (U.S.P.)	0.48 g	
Ferric ammonium citrate (U.S.P.)	94.33 g	
Manganese sulphate (U.S.P. Reagent)	1.24 g	12.2 g
Ammonium alum (U.S.P. Reagent)	0.57 g	
Potassium iodide (U.S.P.)	0.25 g	
Sodium fluoride (U.S.P. Reagent)	3.13 g	
		<hr/>
To make	100 g	
		<hr/>
To make		1000.0 g

- (3) The Vitamin Mixture consists of the following:

Vitamin A	500 I.U.
Vitamin D	100 I.U.
Vitamin E	10 I.U.
Vitamin K (Menadione)	0.5 mg
Thiamine	0.5 mg
Riboflavin	1.0 mg
Pyridoxine	0.4 mg
Pantothenic acid	4.0 mg
Niacin	4.0 mg
Choline	200 mg
Inositol	25 mg
Para-aminobenzoic acid	10 mg
Biotin	0.02 mg
Folic Acid	0.2 mg
Vitamin B ₁₂	2.0 µg

Add sufficient cellulose to make 1 gram

- (4) The total nitrogen procedures as outlined in Official Methods of Analysis, Association of Official Analytical Chemists are considered acceptable.

- (5) For example; if a food containing 10% protein ($N \times 6.25$) has an adjusted P.E.R. of 2.0 and if a reasonable daily intake of this food is 75 grams, the protein rating would be calculated as follows:

$$\begin{aligned}\text{Protein rating} &= 2.0 \times 0.1 \times 75 \\ &= 15\end{aligned}$$

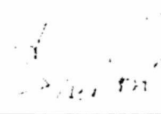
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VI. REFERENCE

- (1) Pharmacopeia of the United States of America XIV ed, 1950, p. 789.

The method described above, being comprised of six pages, Appendix A being comprised of two pages, A1-A2 inclusive, Appendix B being comprised of two pages, B1-B2 inclusive, and identified as FO-1, DETERMINATION OF PROTEIN RATING and dated October 15, 1981 is hereby designated the "Official Method" for the purposes of Sections B.01.062, B.01.063, B.01.064, B.01.065, B.14.073, B.14.085, B.14.086, B.14.087, B.14.088, B.14.089, B.14.090, B.21.027, B.22.027, B.22.029 and B.22.032 of the Food and Drug Regulations.



Acting Assistant Deputy Minister

Appendix A**I. APPLICATION**

This procedure shall be used for the defatting of samples prior to the determination of protein rating.

II. APPARATUS

- (1) Soxhlet apparatus, 3000 mL size. Johns Scientific Co., Catalogue No. 27614-208 or equivalent.

III. REAGENTS

- (1) Diethyl ether, reagent grade.

IV. PROCEDURE

- (1) freeze dry appropriate amount of sample or place sample in a shallow pan in an air oven at 50°C for four hr;
- (2) place approximately (ca) 500 g of sample in the soxhlet apparatus (Note 1) and continuously extract using diethyl ether for ca 1 hr (Note 2);
- (3) air dry fat-extracted sample in a shallow pan in a fume hood;
- (4) grind defatted sample to suitable particle size for mixing in diets for rat feeding studies.

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V. NOTES

- (1) If appropriate size thimbles are not available sample may be wrapped in cheese cloth.
- (2) Ether is extremely inflammable. Use great caution and defat sample in a fume hood.
- (3) A final concentration of 10-15% lipid in the test diet is acceptable.

Appendix B

The Randomized Block Design

In biological assays, variations due to individual animal differences are frequently encountered. While it is not always possible to completely eliminate all errors due to these differences, the use of animals of the same age, sex and strain, and the use of the Randomized Block Design minimize the errors due to the effects of individual differences.

The randomized block technique groups the animals into homogeneous groups referred to as blocks on the basis of some characteristic of the animal. Each block contains one of each diet being tested or compared. The number of blocks required in the Protein Rating procedure is 10.

I. PROCEDURE

- (1) Rank the weanling male rats in order of weight from the lightest to the heaviest. Any unusually light or heavy rats should be replaced.
- (2) Make a systematic selection from the ranked weights starting with the lightest (or heaviest). Each selection group is to be equal in number to the number of diets including control.
- (3) By a random procedure, assign one treatment or diet to each rat in the group. A new randomization should be made for each group. Under no circumstance should the assignment of treatment (diet) to ranking of animals be the same from group to group.
- (4) Assign at random the n animals in a group to groups of n cages in a bank of cages. Each group is referred to as a block.

- A determination involving 5 different diets would require 60 rats ($10(5 + 1)$). Graphically, the layout for a PER. Randomized Block Determination for this number of diets would be as follows:

Where

Cx refers to cage number X

Other randomization would give other caging arrangements. Further details on Randomized Block Design and the proper procedure for analysis of the data may be found in most statistical text books.