# DETERMINATION OF NET PROTEIN UTILIZATION OF TWO LOW-COST, HIGH PROTEIN FOODS

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## SUMMARY

Net Protein Utilization of two low-cost, high protein foods, formulated for supplementary feeding, are determined. Thirty day-old albino rats are used for the determination of both NPU-operative and standard. Digestibility and Biological Value are determined simultaneously. Some practical aspects of the method are considered.

Both foods, in the form of biscuits, show good results from the study, especially the coconut biscuits. Growth of the animals fed these foods was impressive; those on coconut biscuits fared even better than those on skim milk. NPU-standard of the coconut biscuits was obtained to be 73, and NPU-operative, 61. The NPU-standard of soya biscuits was 68. These values are compared to that of skim milk, whose NPU-standard was obtained to be 84. The important aspects of the results obtained are discussed.

# INTRODUCTION

It has been realised that the mere chemical determination of foods do not reveal very much the nutritive values of the foods. Biological evaluations have gained recognition as extremely useful means of assessing protein quality of foods/diets. Methods such as Net Protein Utilization (NPU) have provided useful information. Workers who prefer this method claim that it is relatively easy and inexpensive to carry out. The determination of seven proteins can be tested simultaneously in 10 days, involving no nitrogen estimations other than on food (Miller & Bender, 1955).

In the classical method for the determination of Net Protein Value (N.P.V.), Biological Value (determined by nitrogen balance) is multiplied by Digestibility. Bender & Miller (1953), proposed to leave out the laborious nitrogen balance determination, and employ carcass analysis for the determination of N.P.V. In a further modification and simplification, it was proposed (Bender & Miller, 1953a) to eliminate carcass nitrogen analysis and rely on a predetermined ratio of nitrogen to water in the carcass. Thus, in the shortened method of NPU determination (Miller & Bender, 1955), only the water content of the carcass need be determined, and by the use of the said ratio, carcass nitrogen could be calculated. This method of NPU termination has become one of the

most widely used methods for evaluating the nutritive value of proteins.

In this presentation, results of a study of the NPU (determined by the method of Miller & Bender, 1955) of two low-cost, high protein foods are presented. Biological Value (BV) and Digestibility (D) were determined simultaneously. These foods, in the form of biscuits, have been developed by the Agricultural Products Utilization Division of MARDI for supplementary feeding purposes.

## TIME AND PLACE OF STUDY

The study was started in early May, 1976. The whole study took about 3 weeks, to include the preparation of the diets and determinations of body water and fecal nitrogen of the rats. Actual feeding of the animals was for 10 days only. Both chemical analysis and feeding of the animals were done at the Nutrition Research Institute, Unit Diponegoro, Jakarta.

## EXPERIMENTAL DIETS

The two foods studied, coconut biscuits and soya biscuits, were brought into Jakarta from Kuala Lumpur and stored in the refrigerator. The soya biscuits were found to be insufficient for

both NPU-standard and operative determinations. Hence only NPU-standard was done for these original soya biscuits. Subsequently, the formula as given by MARDI was followed and an attempt was made to bake these biscuits in the laboratory. Hereafter, the original soya biscuits from MARDI will be referred to as soya biscuits-MARDI, to distinguish it from those baked in the laboratory, which will be simply referred to as soya biscuits.

Besides these biscuits, a non-protein and skim milk powder diet were included as control and comparison, respectively. Hence there are a total of 7 types of experimental diets, namely: 1. non-protein, 2. skim milk, 3. coconut biscuits (NPU-operative), 4. coconut biscuit (NPU-standard), 5. soya biscuit (NPU-operative), 6. soya biscuit (NPU-standard), 7. soya biscuit-MARDI (NPU-standard). Each diet was determined in triplicate (except for soya biscuits-MARDI, which was done in duplicate).

For NPU-operative determinations, the biscuits were simply pounded lightly and presented to the rats in fine particles, with no additions whatsoever. After a chemical determination of the protein content of these biscuits, suitable dilutions were made to lower the protein content to 10% for NPU-standard determinations. Composition of the diets is shown in Table I.

TABLE I

COMPOSITION OF EXPERIMENTAL DIETS

| | Protein-free | Skim milk 10% | Coconut biscuit (op) | Coconut biscuit (std) | Soya biscuit (op) | Soya biscuit (std) | Soya biscuit MARDI (std) |
|---|---|---|---|---|---|---|---|
| Fat (coconut oil) g | 105 | 117 | - | - | - | - | - |
| Starch g | 756 | 631.5 | - | 689 | - | 613 | 308 |
| Glucose or sugar, powdered g | 156 | 60 | - | - | - | - | - |
| Saltmixture g | 21 | 24 | - | 30 | - | 30 | 20 |
| Vitamin mixture | + | + | - | + | - | + | + |
| Cellu flour g | 12 | 24 | - | 15 | - | 15 | 10 |
| Skim milk powder g | - | 343.5 | - | - | - | - | - |
| Coconut biscuit g | - | - | 1500 | 766 | - | - | - |
| Soya biscuit g | - | - | - | - | 1500 | 842 | - |
| Soya biscuit (MARDI) g | - | - | - | - | - | - | 662 |
| Total g | 1050 | 1200 | 1500 | 1500 | 1500 | 1500 | 1000 |

## EXPERIMENTAL ANIMALS

Albino rats from an inbred of the Lembaga Makan Rakjat strain from the Nutrition Research Institute, Unit Diponegoro were used. They were weaned on the 25th day and put on a stock diet for 5 days prior to the commencement of the actual feeding study. Hence at the start of the experiment, they were all 30 days old.

## METHOD

### NPU, BV AND D DETERMINATIONS

Each experimental cage housed 4 rats fed on a particular diet. It was ensured that the group weight of the rats totalled the same, correct within 2 grams. This initial group weight of the 4 rats on each diet was recorded. Subsequently, the group weight of the rats was taken daily, and weight change recorded.

Food and water were given ad libitum. The total amount of food prepared was weighed and kept in properly marked jars. The food was presented to the rats in pots designed to minimise spillage. Each day, these pots were filled up with fresh food. The water pots were cleaned and fresh water given.

Each experimental cage had a tray at the bottom, containing about 1 cm of saw-dust and 2 pieces of paper on top of the latter to absorb the urine and spilt water. Feces and spilt food

were collected on the papers. Every 3 days, these trays were changed for clean trays, papers and saw-dust. The feces and spilt food were weighed.

On the 10th day, all the rats, after weighing, were killed using ethyl ether. Incisions were made into the skull, thoracic and body cavities. The carcasses were placed on papers (about 10 pieces of papers, folded into small boxes, were used to absorb the fat), dried in the oven at 70°C, until constant weight (4 days). Water loss was determined.

The remaining food in the jars was weighed. All spilt food for the 3 collections were added to this. The remaining food in the pots on the day of the killing was also noted. The original amount of food in the jars, less these weights gave the amount of food taken by the rats for the 10-day period.

All feces collected for the 3 collections were pooled, weighed and ground. Fecal nitrogen was determined.

CHEMICAL ANALYSES OF DIETS AND FECES

Protein, fat, moisture and ash were determined for those diets whose NDpCal % were to be calculated. Protein was determined for all the diets. Procedures followed were standard methods, with modifications as made by the Unit Diponegoro.

Fecal nitrogen was determined by Kjeldahl method.

## CALCULATIONS

From carcass water content, body nitrogen content was calculated using the formula of Miller & Bender (1955). Net Protein Utilization values were then calculated by applying the eqqation of Bender & Miller (1953).

Digestibility was calculated using the formula as given by Miller & Bender (1955). Biological Value is then obtained from the relationship :

$$BV = \frac{\text{NPU-standard}}{D} \times 100$$

Net Dietary Protein Calorie % (NDpCal %) values of the biscuits were calculated thus :

$$\text{NDpCal \%} = \frac{\text{NPU-operative x protein content of diet x 4}}{\text{total calories per 100 g diet}}$$

## RESULTS

Percentage daily weight change of the animals during the experiment is given in Table II, and a graph plotted, as shown in figure 1. All animals, except those on non-protein diet, showed sharp increases in body weight. By the end of the feeding, all had gained over 40% of the original weight. The graphs for the coconut biscuits, both operative and standard, were the steepest and run close to each other. Those for the soya biscuits

TABLE II

AVERAGE PERCENTAGE DAILY WEIGHT CHANGE OF YOUNG WEANLING ALBINO RATS FED ON VARIOUS DIETS

| days / diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| non-protein | -1.2 | -4.0 | -4.1 | -5.2 | -7.0 | -8.4 | -9.4 | -11.0 | -12.9 | -14.3 |
| skim milk | 8.1 | 11.7 | 18.4 | 22.8 | 26.6 | 31.0 | 34.6 | 39.6 | 41.3 | 46.7 |
| coconut biscuit (op) | 7.1 | 15.3 | 19.7 | 27.6 | 33.5 | 38.3 | 47.3 | 54.0 | 57.8 | 65.4 |
| Coconut biscuit (std) | 12.4 | 17.2 | 23.0 | 28.7 | 34.6 | 39.6 | 45.8 | 51.8 | 55.0 | 58.7 |
| Soya biscuit(op) | 7.5 | 11.4 | 17.6 | 22.5 | 26.6 | 31.0 | 36.3 | 40.9 | 44.7 | 48.7 |
| Soya biscuit(std) | 11.3 | 13.0 | 17.6 | 19.3 | 25.1 | 28.1 | 31.8 | 35.6 | 40.8 | 41.5 |
| Soya biscuit (MARDI) (std) | 9.8 | 16.2 | 17.4 | 16.3 | 25.4 | 29.4 | 31.6 | 35.6 | 43.4 | 40.6 |

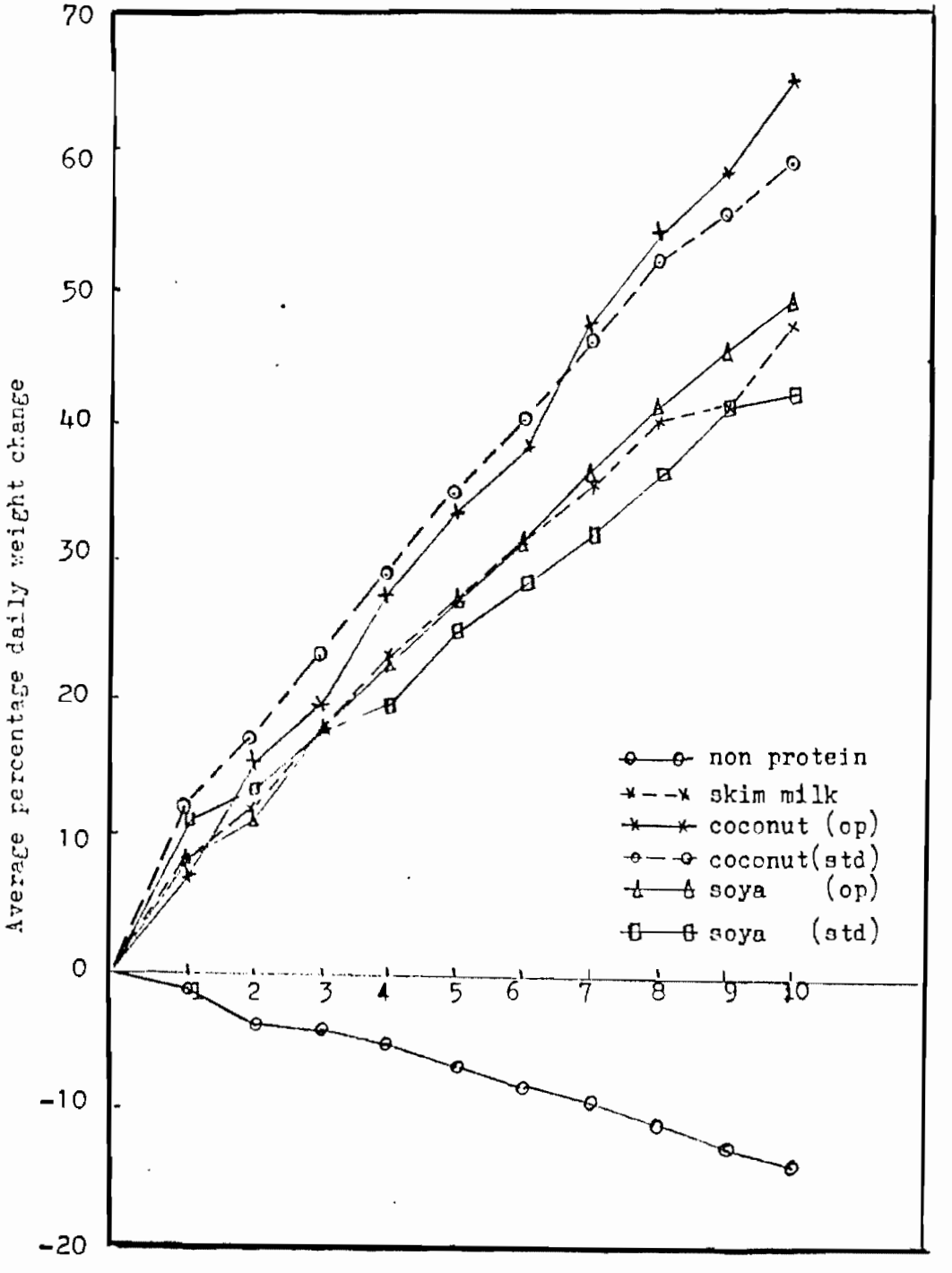


Fig. 1 . Average percentage daily weight change of young weanling albino rats fed on various diets.

(operative and standard) were considerably lower and run close to the skim milk curve. The animals on the non-protein diet, on the other hand, showed daily decline of body weight. By the end of the ten days, they had lost 14% of the weight at start of the experiment.

The NPU, BV and D, and NDpCal % obtained for the biscuits are presented in Table III. These are compared with that for skim milk.

## DISCUSSION

### DIGESTIBILITY AND BIOLOGICAL VALUE

Digestibility is obtained by determining the proportion of the total nitrogen intake that is absorbed by the body. D values for skim milk, coconut biscuit and soya biscuit-MARDI are similar. The efficacy of absorption of these three foods may be said to be similar. The approximately 90% of D values obtained is close to absorption values of animal proteins. However, it does not mean that the quality of these three proteins are the same (further discussion below).

Soya biscuits and soya biscuits-MARDI are essentially the same, as explained in Experimental Diets. It is interesting to note, however, that the D values obtained for the former is slightly lower. The reason for this is not clear. It could be that the baking of the soya biscuits in the laboratory had altered

TABLE III

NPU, BV, D AND NDpCAL % OBTAINED FOR THE BISCUITS, COMPARED TO THAT FOR SKIM MILK

| | | NPU-std | NPU-op | NDpCal% | D | BV |
|---|---|---|---|---|---|---|
| skim milk | XP320 | 85 | - | - | 92 | 93 |
| | XP321 | 84 | | | 90 | 93 |
| | XP322 | 82 | | | 92 | 89 |
| | average | 84 | | | 91 | 92 |
| coconut biscuit | XP320 | 74 | 66 | 10.03 | 93 | 80 |
| | XP321 | 71 | 56 | 8.51 | 93 | 77 |
| | XP322 | 75 | 61 | 9.27 | 94 | 80 |
| | average | 73 | 61 | 9.27 | 93 | 79 |
| soya biscuit | XP320 | 60 | 55 | 7.74 | 85 | 70 |
| | XP321 | 58 | 55 | 7.74 | 83 | 70 |
| | XP322 | 56 | 49 | 6.90 | 85 | 66 |
| | average | 58 | 53 | 7.46 | 84 | 69 |
| soya biscuit MARDI | XP321 | 67 | - | - | 90 | 75 |
| | XP322 | 68 | | | 90 | 75 |
| | average | 68 | | | 90 | 75 |

the proteins somewhat. The availability of amino acids in the biscuits might have been lowered. It was noted during the baking that the biscuits were somewhat charred. This over-heating could be one of the reasons for the lowered availability of the amino acids (FAO/WHO, 1973).

Biological Value of a protein is a measure of the proportion of the nitrogen absorbed that is retained by the body. The coconut biscuits gave a good BV of 79. The soya biscuits from MARDI also gave a BV of the same order, although slightly lower. But the D values of skim milk and these two biscuits gave similar results. Hence, although absorption of skim milk protein and those of the biscuits are the same, the proportion that is retained is considerably higher in the skim milk protein. From this, it is clear that D and BV alone does not give a complete picture of the quality of the protein.

As discussed under Digestibility, there is again a difference in BV values for the soya biscuits. Similar explanations may be given for this. The lower BV for soya biscuits baked in the laboratory may be due to a lowered availability of amino acids, resulting in lowered nitrogen retention, and hence Biological Value.

NET PROTEIN UTILIZATION

NPU is the percentage of the food protein (nitrogen) consumed which is retained or utilized by the body. It gives an overall

picture of the digestibility and the efficiency of utilization of the absorbed amino acids. For routine purposes, the determination of NPU alone, without D or BV suffices to evaluate a protein. This obviates the analyses of fecal nitrogen, which can be quite tedious.

NPU values obtained in the study indicate promising protein quality of the biscuits, especially the coconut biscuits. The difference in values for BV and D for the two soya biscuits has been discussed. This is of course reflected in their NPU values. One tends to accept the NPU value obtained for soya biscuit-MARDI since NPU-standard for soya beans alone, as obtained by Unit Diponegoro is 55 (Lie et al, 1974). Hence the soya biscuits, with added egg and wheat flour should give a higher value than the 58 given by soya biscuits baked in the laboratory. This has shown how the primitive baking in the laboratory has destroyed or lowered the availability of some amino acids, hence lowering its protein quality.

The NPU determinations are done with ad libitum intakes of food. The total daily calorie intake is important, for if this is reduced below a certain level, an increasing amount of protein is burnt for energy purposes, and consequently, the efficiency of utilization of protein decreases. Thus Forbes and Yohe (1955)

reported no change in the biological value of a diet when the food intake of rats was reduced from 8 to 6 g per day; but a decrease from 99 to 69 when the food intake was further reduced to 4 g per day.

Under conditions of calorie restriction, the protein value of a diet will depend upon the energy available for protein anabolism rather than on the concentration and the nature of the protein it contained. Miller & Payne (1961) give some discussion on this and presented an equation for the prediction of the protein value of diets when fed under conditions of caloric restriction.

These are important considerations to be borne in mind in the evaluation of protein quality of a food. Protein malnutrition in man is frequently associated with low calorie intakes of foods which may have an adequate protein value if consumed in unrestricted amounts.

NET DIETARY PROTEIN CALORIE %

NDpCal % is a measure of the proportion of the calories of the diet that is present as utilizable protein. The proportion in the diet of utilizable protein should be 8.0% of the caloric intake at birth, i.e. the diet should have a NDpCal % of 8.0 (Platt & Miller, 1959). During the weaning period, the proportion declines so that after 1 to 2 years, the composition of an adequate diet is similar to that needed by the adult, i.e. about 5 % of

utilizable protein (Payne, 1969). Lie et al (1974 a) suggests that since no conclusive figure is yet available for the pre-school children, an NDpCal % of 6.5 may be arbitrarily used as a minimum.

NDpCal % of both the coconut and soya biscuits give respectable results, especially the former. In view of the above discussion that the NPU-operative obtained for the soya biscuits baked in the laboratory may have been falsely lowered, the NDpCal % might be deemed to be higher than the 7.46 obtained.

## REFERENCES


1. BENDER, A.E. AND MILLER, D.S. (1953) : A new brief method of estimating Net Protein Value : Biochemical Journal, 53, vii.

2. BENDER, A.E. AND MILLER, D.S. (1953a) : Constancy of the N/H20 ratio of the rat and its use in the determination of the Net Protein Value : ibid, 53, vii-viii.

3. FORBES, R.M. AND YOHE, M. (1955) : Effect of energy intake on the biological value of protein fed to fats : J. Nutr., 55, 499-506.

4. LIE, G.H., OEY, K.N., PRAWIRANEGARA, D.D., HERLINDA, J., SIHOMBING, G., JUS'AT, I. (1974) : Nutritive value of vaious legumes used in the Indonesian diet : Presented at the 1st Asean workshop on grain legumes, Bogor, Indonesia, Jan. 15-20, 1974.

5. LIE, G.H., OEY, K.N., PRAWIRANEGARA, D.D., HERLINDA, J., SIHOMBING, G., JUS'AT, I. (1974a) : Available sources of food in Indonesia (for the improvement of the nutritional status of children) : Presented at the 3rd National Pediatric Congress, Surabaya, Indonesia, July 1-6, 1974.

6. MILLER, D.S., AND BENDER, A.E. (1955) : The determination of the NPU of proteins by a shortened method : Br. J. Nutr., 9, 382-388.

7. MILLER, D.S. AND PAYNE, P.R. (1961) : Problems in the prediction of protein values of diets : calorie restriction : J. Nutr., 75, 225-230.

8. PAYNE, P.R. (1969) : Effect of quantity and quality of protein on the protein value of diets : Voeding - 30e Jaargang, no. 4, 182-191.

9. PLATT, B.S. AND MILLER, D.S. (1959) : The net dietary-protein value (N.D.-p.v.) of mixtures of foods - its definition, determination and application : Proc. Nutr. Soc., 18, vii-viii.

10. REPORT OF A JOINT FAO/WHO AD HOC EXPERT COMMITTEE (1973) : Energy and protein requirement : FAO nutritional meetings report series no. 52, WHO technical report series no. 522, Rome, 1973.