

Effect of sample preparation, and of fat and crude fiber in the diet, on the determination of net protein utilization (NPU)*

J. EDGAR BRAHAM¹ AND RICARDO BRESSANI²
Institute of Nutrition of Central America and Panama (INCAP)
Guatemala, C. A.

SUMMARY

The study was carried out to determine the most adequate method for carcass nitrogen analysis in NPU assays and to assess the effect of increasing levels of dietary fat and crude fiber on NPU values. The results showed that more reproducible results with a lower variation were found when nitrogen was determined on dry samples of the carcass than on the whole fresh or defatted carcass. It was also established that increasing crude fiber or fat up to 10% of the diet did not have any significant effect on NPU values.

INTRODUCTION

Among the biological methods for the evaluation of protein quality, Net Protein Utilization (NPU) is commonly used, even though it is a cumbersome method. More often than not, different laboratories arrive at different figures for a given protein, even within the same laboratory, in spite of standardized conditions, variation tends to be high. Although, in essence the method simply measures the amount of nitrogen deposited as tissue from the nitrogen consumed, the determi-

* This investigation was supported by a grant from the W. K. Kellogg Foundation.

1 Assistant Chief of the Division of Agricultural and Food Science, Institute of Nutrition of Central America and Panama.

2 Chief, of the same Division.

INCAP Publication I-355

Received: 26-3-1968

nation in itself is far from simple, since it requires the determination of nitrogen in the whole animal. Miller and Bender (1) obviated this by using a regression equation to predict body nitrogen by determining only the water content of the whole animal. Their results in rats were shown to be true for chickens also (2). This indirect method of determining total nitrogen, however, implies the determination of the regression equation for a given laboratory, since the equation worked out by one laboratory cannot be used by another. This determination can be made in the whole fresh animal, on an aliquot of the dry animal or on an aliquot of the dry fat-free animal.

The purpose of the work reported here was to evaluate whether or not the determination of nitrogen in these three different ways of preparing the samples affects the final result, and whether or not the percentages of certain dietary constituents, such as fat and crude fiber, have any significant effect on the determination of NPU. Previous studies showed that the length of the test as well as the protein level in the diet had a definite effect on the NPU value of proteins (3).

MATERIALS AND METHODS

Weanling rats of the Wistar strain from the INCAP colony were fed the diets shown in Table 1. For experiment 1, three different sources of protein were used: soybean meal, cottonseed meal and casein; all of which provided 10 percent of protein to the diet. Three groups of 10 rats each, consisting of five males and five females, all 25 days of age, were fed each of the diets containing the protein source. Additional groups, corresponding to each protein, were fed a nitrogen-free diet. Its composition was similar to the diets described in Table 1, except that the protein source replaced cornstarch. The average initial weight was 50 g for experiment 1 and 44 g for experiment 2. The animals were placed in individual all-wire screen cages with raised screen bottoms. Feed and water were supplied *ad libitum* for a period of 10 days, after which all the animals were killed with chloroform. After weighing, one group for each protein and for the nitrogen-free diet was either frozen or each rat placed directly into a Kjeldahl flask and digested using selenious acid as catalyst.

TABLE No. 1
COMPOSITION OF DIETS (%)

Ingredient	Experiment 1				Experiment 2	
	1	2	3	4	1	2
Soybean meal	20.0	—	—	—	20.0	20.0
Cottonseed meal	—	20.0	—	—	—	—
Casein	—	—	11.4	—	—	—
Minerals*	4.0	4.0	4.0	4.0	4.0	4.0
Cod liver oil	1.0	1.0	1.0	1.0	1.0	1.0
Cottonseed oil	5.0	5.0	5.0	5.0	5.0	—
Cornstarch	70.0	70.0	78.6	90.0	70.0	75.0
Vitamins**, ml	5	5	5	5	5	5

* Hegsted, D. M., R. C. Mills, C. A. Elvehjem & E. B. Hart.—Choline in the nutrition of chicks. *J. Biol. Chem.*, 138: 459-466, 1941.

** Manna, L. & S. M. Hauge.—A possible relationship of vitamin B₁₂ to orotic acid. *J. Biol. Chem.*, 202: 91-96. 1953.

After digestion was complete, the contents of the flask were diluted to 2000 ml with distilled water, and appropriate aliquots were taken for distillation, according to the directions of Hamilton and Simpson (4). The two remaining groups for each protein were weighed in metal trays and dried in a convection oven at 75°C until constant weight was reached. One group was ground in a micro Wiley mill with a 10 mesh sieve and homogenized in a mortar. Two-gram aliquots were digested as previously described and taken up to 250 ml. A 25 ml aliquot was taken for distillation. The remaining group was ground in the Wiley mill and then extracted for 48 hours with ethyl ether in a continuous extracting apparatus; then dried, weighed again and passed through a 60-mesh sieve. Aliquots of approximately 0.2 were taken for nitrogen determination.

For the second experiment, only one source of protein was used, soybean meal (Diet 1, Table 1), with different levels of fat and crude fiber (see Table 3). Refined cottonseed oil and cellulflour were respectively used as sources of fat and

crude fiber, replacing an equal weight of corn starch from the basal diet No. 1 (shown in Table 1). After 10 days, the animals, 6 per group (3 males and 3 females), were sacrificed with chloroform. Nitrogen was determined in two-gram aliquots of the dry animal. In all cases NPU was calculated by subtracting the average total nitrogen content of the corresponding nitrogen-free group from the total nitrogen content of each individual rat in the experimental groups and dividing by the corresponding nitrogen intake.

RESULTS AND DISCUSSION

Table 2 shows the results of the nitrogen determination on the three different samples. There was no significant difference between the three methods of preparing the sample for analysis. However, more reproducible results, and less variation were encountered when nitrogen was determined on the dry samples than when it was done either on the whole fresh or on the dry defatted animal. Moreover, this procedure was less time-consuming and certainly more economic than any of the other two. The fact that some nitrogenous constituents might be removed by the solvent could be responsible for the slightly lower values obtained when the sample was defatted.

Table 3 shows that when either crude fiber or fat were increased to a level of 10 percent of the diet, there were no significant differences in the results. The level of 5% of either

TABLE No. 2

EFFECT OF SAMPLE PREPARATION ON THE NITROGEN DETERMINATION AND NPU CALCULATION

Nitrogen determined in	Soybean meal		Cottonseed meal		Casein	
	NPU	S.D.	NPU	S.D.	NPU	S.D.
Whole fresh rat	61.0	6.4	43.6	7.3	70.7	9.5
Dry rat	57.3	6.8	44.4	5.1	68.2	4.8
Dry defatted rat	55.4	7.6	41.9	6.9	63.0	7.8

F 1.36, non-significant between treatments.

TABLE No. 3

EFFECT OF THE FAT AND CRUDE FIBER PERCENTAGE IN THE DIET
ON THE DETERMINATION OF NPU

Percentage of crude fiber in basal diet ¹	NPU	S. D.	Percentage of fat in basal diet ²	NPU	S. D.
0.8	56.2	7.2	1.7	54.0	7.3
2.0	55.0	6.9	2.0	59.8	5.4
4.0	60.8	5.3	4.2	55.4	5.8
6.0	61.5	11.5	6.0	59.1	9.1
8.0	62.5	7.8	8.0	55.0	8.9
10.0	59.1	4.9	10.0	55.4	4.0

F test for crude fiber: non-significant.

F test for fat: non-significant.

¹ The weight of cellulflour indicated replaced an equal weight of corn starch in basal diet (Table 1).

² The weight of refined cottonseed oil indicated replaced an equal weight of corn starch in basal diet (Table 1).

constituent usually added to standard or reference diets for the determination of NPU seems adequate and the additional amount afforded by the protein source would probably not affect the end value to a significant degree.

These dietary components can, however, affect other measures of protein quality such as protein efficiency ration (PER), through a lowering of the amount of feed consumed. The effect becomes more significant as the length of the experimental period increases as with PER, but will have no effect on NPU, which is carried out for a 10 day experimental period as shown in the present paper.

RESUMEN

Efecto de la preparación de la muestra y del contenido de lípidos y fibra cruda en la dieta sobre la determinación de la utilización proteica neta (UPN)

El presente estudio se realizó con el propósito de encontrar el método más adecuado para la determinación del nitrógeno del carcás en ensayos de UPN y determinar el efecto de varios niveles de grasa y de fibra cruda

sobre el UPN de las proteínas. Valores de UPN más reproducibles y con menos variación se encontraron cuando el nitrógeno del carcás se determinó en muestras secas que en muestras de carcás fresco o desgrasado y seco. Además, se demostró que cuando la cantidad de grasa o de fibra cruda se aumentan en la ración hasta un 10%, no tienen ningún efecto significativo sobre los valores de UPN.

BIBLIOGRAPHY

- (1) Miller, D. S. & A. E. Bender.—The determination of the net utilization of proteins by a shortened method. *Brit. J. Nutrition*, 9: 382-388, 1955.
- (2) Braham, J. E., R. Bressani & M. A. Guzmán.—A rapid method for the determination of net protein utilization values with chicks. *Poultry Sci.*, 42: 1423-1247, 1963.
- (3) Braham, J. E., L. G. Elías, S. de Zaghi & R. Bressani.—Effect of protein level and duration of test on carcass composition, net protein utilization (NPU) and on protein efficiency ratio (PER). *Nutritio et Dieta*, 9: 99-111, 1967.
- (4) Hamilton, L. F. & S. G. Simpson.—Talbot's quantitative chemical analysis. 9th ed. New York, Macmillan Co., 1946.