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

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SUMMARY

The study was carried out to determine the most adequate method for carcass nitrogen analysis in NPU assays and to asses 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 Portein Utilization (NPU) is commonly used, even though it is a cumbersome method. More often than not, differents 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-

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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 B_{13} 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 continous 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 celluflour were respectively used as sources of fat and

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.

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