A Rapid Method for the Determination of Net Protein Utilization Values with Chicks*

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AMONG the methods used for the determination of the nutritive value of a protein, the determination of the Net Protein Utilization (NPU) which measures the amount of food nitrogen retained in the test animal, offers a useful and reliable method for the assessment of protein value. The method, however, suffers the disadvantage of needing a large number of nitrogen determinations; this shortcoming has been largely obviated by the method proposed by Bender and Miller (1953b) and Miller and Bender (1955), who demonstrated that the ratio of nitrogen to body water was a constant for a given age and, therefore, the nitrogen content of the body could be derived from the water content by means of a regression equation. Similar results were obtained by Dreyer (1957). This method reduces the nitrogen determinations to only that conducted on the food given to the animals.

The method of Bender has been used on rats as the experimental animal. The present study was undertaken to determine if chicks could be used, under practical laboratory conditions, for the determination of NPU values.

MATERIALS AND METHODS

For the determination of the total nitrogen content of chicks at different ages, one-day old New Hampshire chicks were fed a practical ration containing 20% protein. This diet contained per 100 grams a commercial concentrate 40g., mineral mixture (Salmina) 3g. and ground yellow corn 57g. Feed and water was supplied *ad libitum*. Groups of five chicks were sacrificed with chloroform each week, after overnight fasting, weighed to the nearest tenth of a gram and partially dissected to facilitate drying to constant weight at 100°C. In one experiment only male chicks were used, in another experiment straight run chicks were used and their sex was determined by direct observation of the sex organs after sacrificing the animals. Total nitrogen was determined on the whole dry carcass by the Kjeldahl procedure using selenious acid as the catalyst. After dilution, appropriate aliquots were taken for distillation which was carried out according to the directions of Hamilton and Simpson (1946).

For the actual determinations of NPU values, one day old New Hampshire chicks were fed the practical ration, containing 20% crude protein, for a period of 10 to 12 days after which the experimental animals were distributed by weight among the control diet and the experimental diets containing the protein under test. No group differed by more than four grams in total weight from any of the other groups. The control diet, containing 5% dry-egg protein, is shown in Table 1. The control diet should have been a protein free diet, but in order to prevent mortality in this group 5% dry-egg protein was added. The proteins under test were added at a level of about 10%, substituting for the egg

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TABLE 1.—*Composition of the control diet*

Ingredient	g.
Defatted dry egg	6.1
Corn starch	81.6
Cottonseed oil	5.0
Minerals[1]	3.0
Cod liver oil[2]	0.3
Alpha cell	4.0
Vitamin B solution[3]	5 ml

[1] Salmina, Riverside Co., Guatemala, C. A., contains 33% bone meal, 33% $CaCO_3$, 33% iodized salt and 1% minor elements.

[2] 1,800 I.U. vitamin A and 175 I.U. vitamin D per gram.

[3] Containing, per 5 ml.: 50 mg. inositol, 25 mg. menadione, 800 mg. choline, 5 mg. PABA, 50 mg. niacin, 10 mg. riboflavin, 10 mg. pyridoxine, 10 mg. thiamine, 30 mg. Ca pantothenate, 0.2 mg. biotin, 1 mg. folic acid, 15 mcg. vitamin B_{12}.

protein and part of the starch of the control diet. The added cellulose in the control diet was varied in the experimental diets according to the crude fiber content of the protein source. The experimental and control diets were fed for a 10 day period after which all animals were sacrificed with chloroform, weighed to the nearest tenth of a gram, partially dissected and dried to constant weight at 100°C. Total nitrogen was calculated from the water content of the carcass using the regression equation derived from the standard curve. NPU values were then calculated applying the regression equation derived from the standard curve, and from the formula derived by Bender and Miller (1953a),

$$NPU = \frac{B-(Bp-Ip \times NPUp)}{I}$$

where B equals the body nitrogen of the experimental group, Bp the body nitrogen of the control group, Ip the nitrogen intake of the control group, I the nitrogen intake of the experimental group and NPUp the NPU of the control protein, which was assumed to be 100.

In some experiments the nitrogen content of the dry carcass was determined by the Kjeldahl procedure in order to determine the accuracy of the values predicted by the N/H_2O ratios.

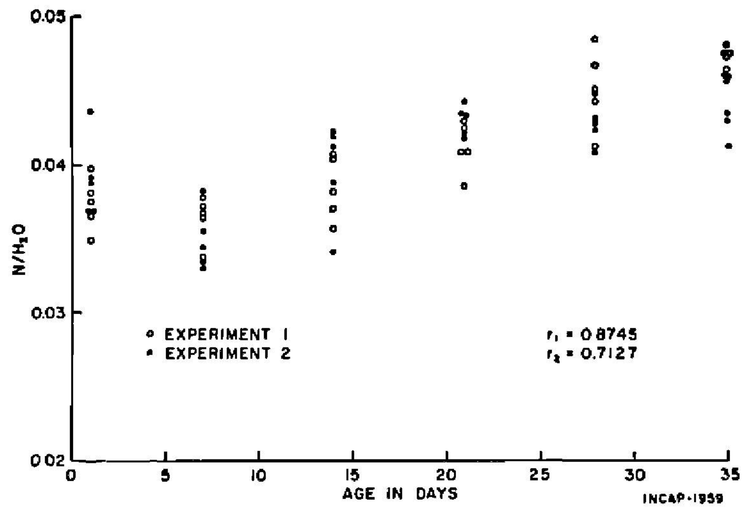

FIG. 1. Relationship of age to nitrogen/body water ratio in New Hampshire chicks.

RESULTS

Standard curves. Two experiments were run to determine the normal nitrogen content of chicks at different ages. In experiment 1, only male chicks were used, in experiment 2, both males and females were used. Since no sex differences were observed at the ages studied, the data for both experiments have been pooled as shown in Fig. 1, which is a scattergram of the N/H_2O ratios at different ages and in Fig. 2, where the regression line between the two variables studied is shown. The correlation coefficients were 0.8745 and

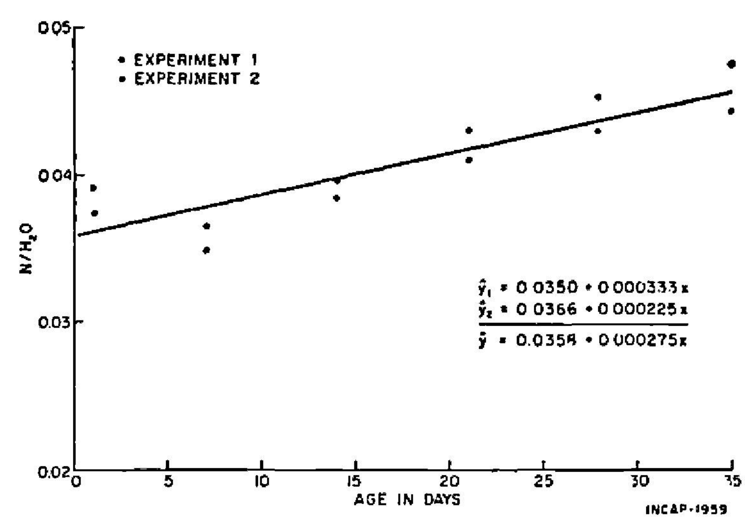

FIG. 2. Relation of body nitrogen/body water ratio to age in New Hampshire chicks.

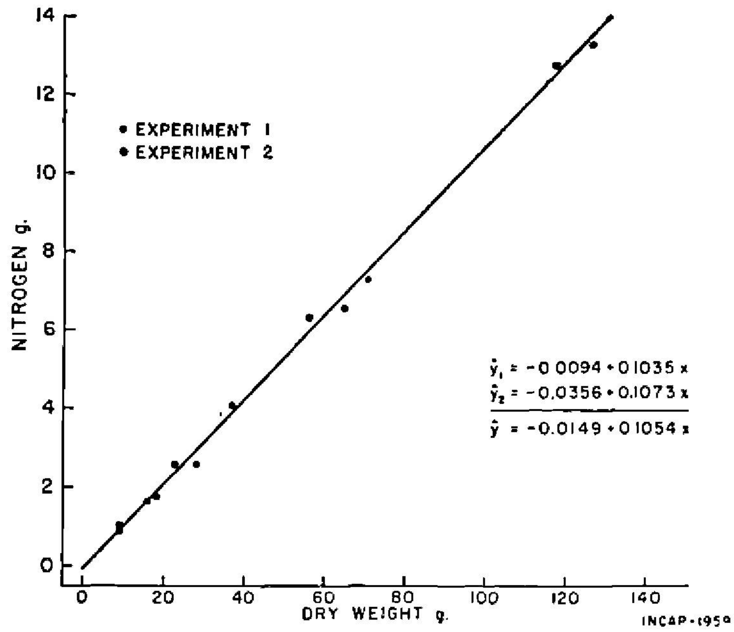

FIG. 3. Relation of total body nitrogen to dry weight in New Hampshire chicks.

0.7127 for experiments one and two, respectively, and the calculated regression equations for each experiment as well as the equation for the pooled data are shown in Fig. 2.

The correlation between dry weight and nitrogen was also studied and Fig. 3 shows the regression line between these two variables. The correlation coefficients were 0.9944 and 0.9970 for experiments one and two, respectively, and the calculated regression equations are shown in the figure.

Table 2 shows the predictions for experiment 2 data calculated by the equation derived from the data obtained in experiment 1, and Table 3 shows the predictions for experiment 1 data on experiment 2 equations. Nitrogen values predicted from the relation between dry weight and nitrogen (equation 1) and between N/H_2O and age (equation 2), as well as the differences between observed and predicted values are shown on both tables. It is evident from these data that one equation predicts as well as the other, the mean deviation over the predicting range being about the same magnitude, but of opposite signs. However, when actual NPU values were determined, only equation 2 was found to predict adequately the nitrogen content of the carcass as shown on Table 4 where the predicted values for both equations as well as the chemical determination of a group of chicks is shown. The protein source in the ration was cottonseed oil meal which supplied 10 percent of protein. It can be seen that equation 1 based on the dry weight-nitrogen correlation predicts values which are too high as compared to either equation 2 or to the actual chemical determinations. Evidently, the correlation between dry weight and nitrogen is true only when the test protein is fed at the same level used in the determination of the values for calculating the regression line.

Table 5 shows the net protein utilization of several proteins as determined with chicks. As can be seen, the values obtained agree with those reported by Miller and Bender (1955) using their method and by Block and Mitchell (1946–

TABLE 2.—*Predictions for experiment 2 data based on experiment 1 equations*

Age (days)	Nitrogen				
	Observed	Predicted from equation 1	Δ_1	Predicted from equation 2	Δ_2
1	1.0100	0.9718	−0.0382	0.9160	−0.0940
7	1.7281	1.8288	+0.1007	1.8560	+0.1279
14	2.5902	2.3763	−0.2139	2.7326	+0.1424
21	6.5130	6.7005	+0.1875	6.3605	−0.1525
28	6.3185	5.8456	−0.4729	6.5276	+0.2091
35	12.7166	12.2327	−0.4839	13.3336	+0.6170
Mean deviation			−0.0728		+0.1416

TABLE 3.—*Predictions for experiment 1 data on experiment 2 equations*

Age (days)	Nitrogen				
	Observed	Predicted from equation 1	Δ_1	Predicted from equation 2	Δ_2
1	0.9090	0.8990	−0.0100	0.8953	−0.0137
7	1.6628	1.6640	+0.0012	1.7450	+0.0822
14	2.6047	2.9860	+0.3813	2.6976	+0.0929
21	4.0548	4.0311	−0.0347	4.0623	+0.0075
28	7.2862	7.6108	+0.3246	6.9511	−0.3351
35	13.2691	13.5293	+0.2602	12.4159	−0.8532
Mean deviation			+0.1556		

TABLE 4.—*Nitrogen content of the carcass of chicks determined by Equation 1, Equation 2 and by direct chemical analysis**

Chick No.	Nitrogen content of carcass		
	Equation 1	Equation 2	Chemical analysis
1	5.3078	3.8623	3.0393
2	4.5595	3.2595	3.5105
3	3.0285	3.4819	2.9846
4	4.0535	3.1618	3 3889
5	4.4224	3.1181	3.8753
Total	23.3717	16.8836	16.7986
N.P.U.	78.0	57.5	56.8

* Protein source: Cottonseed meal furnishing 10% of protein in the ration.

47) by their balance sheet procedure; however, the results obtained for corn are much higher and those for milk lower than the values reported by either of the above mentioned workers. These differences could be ascribed to a species difference since both Mitchell and Bender used rats as the experimental animal.

DISCUSSION

The method, under the experimental conditions used here, seems to be practical and could be used to evaluate the supplementary value of protein feeds in poultry feed formulations. Cereal grains gave higher values than those obtained with rats; this may be due to a species difference in relation to the utilization of the type of carbohydrate of cereal grains or it could be due to the fact that the proteins of cereal grains contain factors which cause a greater retention of nitrogen in chicks, a possibility that warrants further investigation.

The use of chicks for the evaluation of net protein utilization values appear less suitable than the rat when very accurate

TABLE 5.—*Net protein utilization of selected proteins*

Protein source	NPU values	Standard error	No. of replicates[1]	Value of Miller and Bender[2]	Value of Block and Mitchell[3]
Soybean oil meal	66.8	0.9	5	56.0	72.0
Corn, whole	74.3	2.4	5	55.0	53.0
Rice	71.7	3.8	2	—	70.0
INCAP Vegetable Mixture 9	62.8	1.5	40	—	—
Skim milk	56.4	0.8	4	75.0	79.0
Cottonseed oil meal	63.0	2.2	4	58.8	53.0
Sorghum	71.1	1.9	6	—	—
Sesame oil meal	66.6	0.6	2	—	65.0

[1] Each replicate consists of five chicks per groups.
[2] Miller and Bender (1955).
[3] Block and Mitchell (1946–7).

determinations are sought. In the first place, determinations on individual chicks are obviously impractical and the utilization of groups of chicks rule out the possibility of determining individual variations within the group. The standard error of NPU values for the same protein tested in different groups of chicks is, however, relatively low as shown on Table 5. Another disadvantage is the relatively high requirement of protein for the chick as compared to the rat and since the NPU determination is usually carried out at a 10% protein level in the diet to make certain that none of the dietary protein could be used as a calorie source, this implies that the newly hatched chick cannot be used directly for NPU determinations unless a period of adequate protein feeding has been induced previous to feeding the 10% protein diet. The chick, then, is used at a different biological age than the rat since the latter is used at 21–22 days immediately after weaning.

These disadvantages are not, however, so overpowering to render the method with chicks unreliable, especially if one considers that NPU figures are at best, an approximate value subjected to wide variations due not only to the method used, but to the physiological state of the animal.

SUMMARY

The relation between dry weight and total nitrogen to body water ratio and age in New Hampshire chicks was determined. In both cases high correlations were found between the variables studied. Using the regression equations, body nitrogen could be predicted with accuracy when the protein content of the diet was 20%. When the protein level in the diet was 10%, only the equation for nitrogen to body water and age gave satisfactory results. Preliminary NPU determinations of several protein sources were found to be in good agreement with the values reported by other authors except for corn and milk.

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