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PROTEIN ASSAY

Assessing Protein Quality with the Individual Protein-Depleted Chick

ANNABEL BEATY, FRANCIS J. CONLAN, SANDRA McMINN, and S. M. WEISBERG

Research Laboratories Division of National Dairy Products Corp., Oakdale, Long Island, N. Y.

Techniques and equipment are described for carrying out protein assays on individual protein-depleted chicks. Assays have been carried out at dietary levels of 6.5, 9, and 12% of protein using a mixture of casein, gelatin, and DL-methionine as a standard of reference. Dairy products have been assayed as the sole source of protein and in combination with other proteins commonly used in experimental chick rations. Data are presented to show the differences in response of protein-depleted chicks and rats to three sources of protein.

In studying poultry nutrition and in evaluating poultry feeds and ingredients, the accepted procedure is to carry several chicks in one pen. Individual weights can be secured, but food consumption must be taken on the pen as a whole. The chick's requirement for unusually high temperatures during the first few days of life makes the battery brooder a necessity and has probably been the reason for the practice of group feeding throughout the entire test period. In determining amino acid requirements and evaluating protein quality in chicks, the group-feeding practice is also followed. The method of Grau and Almquist (*4, 5*) is widely used. Chicks are carried for 2 weeks on a good growing ration and are then distributed according to weight into groups for the 8-day assay period. The test protein is fed at a dietary level of 20% and the results are expressed in terms of per cent gain per day.

The rat has long been used to evaluate the nutritive value of protein and it seemed that some of the techniques might be applied to the chick. The weight regeneration method of Cannon (*3, 10*) appeared to be adaptable as work could be done on an older animal, and in the case of the chick, the period when extra heat is required would be past. The chick could be conveniently housed alone and feed and protein consumption data of individual chicks could be obtained.

Procedure

Day-old female New Hampshire chicks are reared for 10 days in battery brooders on a good growing mash. They are then placed in individual rat cages of the type shown in Figure 1. Light for the lower tiers is supplied by 15- or 25-watt light bulbs. The cages are held slightly forward over the pans to catch any food thrown out by the chicks. The pans are filled with sawdust and a strip of paper is placed over the sawdust under the food cups to facilitate recovery of spilled food. The food cup is secured in the rear of the cage with

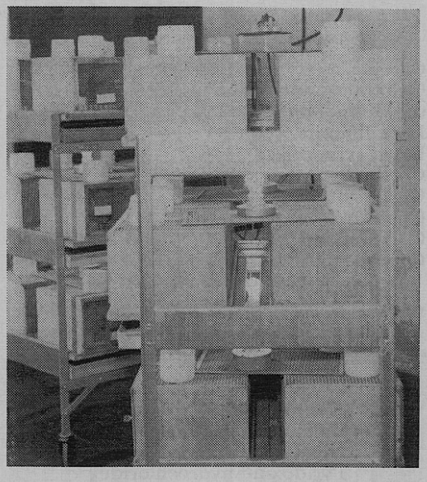

Figure 1. Cage stands for individual housing

a small spring suspended between the top and bottom of the cage, which prevents the cup from being upset and keeps the chick from roosting on it. The cup, which is a cold cream jar, can readily be removed and replaced behind the spring. The water cup, another cold cream jar, is placed in the front of the cage. The cup on top of the cage is used to store spilled food during periods when food consumption is being recorded.

During the chicks' first 10 days in individual cages, they are fed a complete diet as described by Briggs (*2*). They are then shifted to a protein-free diet or the Briggs diet without casein, gelatin, and DL-methionine. The composition of these two diets is shown in Table I, under preliminary rations. The chicks are weighed at the time they are placed on the protein-free diet and when they have lost approximately 25% of their original body weight they are fed the assay diets. This weight loss usually occurs in from 6 to 16 days. A few chicks are more resistant to weight loss and these have been placed on test on the 16th day even though they have not reached the desired weight loss. A weighed portion of diet is placed in a container bearing the chick's number and each day or as often as necessary the chick is fed from this container. At the end of the 2-week assay period, unused food and spilled food are weighed back. Each diet is analyzed for crude protein (N × 6.25) and this figure is used to calculate protein consumption. Weight gained during the 2-week assay period is divided by the weight of protein consumed to give the conventional protein efficiency figure of gain per gram of protein consumed.

In the course of this work, the same chick has been used for as many as three assays. If the chick had gained little or no weight during the first assay period, she was immediately subjected to a second assay period. If she had made a good recovery she was fed the protein-free ration for a few days until satisfactory weight loss occurred.

In protein assays carried out on rats, some standard of reference in the form of a high quality protein is frequently used. This serves to evaluate the performance of the particular group of animals and supplies a value with which unknowns can be compared. For the chick, the mixture of proteins used in the Briggs diet seemed to be a satisfactory standard. The complete diet contained approximately 26% of the protein derived from 20% of casein, 8% of gelatin, and 0.3% of DL-methionine. Assays have been carried out at dietary levels of 6.5, 9, and 12% of protein. The ratio of casein to gelatin and DL-methionine as it appears in the complete diet was maintained at each assay level. Other sources of protein were added to the basal diet in amounts sufficient to give the required dietary level of protein.

Composition of assay diets containing the three levels of casein, gelatin, and DL-methionine mixture is shown in Table I, under typical protein assay rations. Diets containing 12% of the protein from lactalbumin, casein, and dried skim milk are also shown. All other assay diets were prepared in the same manner by varying the amount of test material and Cerelose to give the desired level of protein. The percentage of protein in each case was determined by analysis of the ration. Cornstarch was initially used in these rations in anticipation of having to work with wet products. However, rations made with cornstarch tend to stick to the chick's beak and deform it.

Results

The results of three assays carried out during the first repletion period are shown in Table II. The diets were calculated to contain 6.5, 9, and 12% of protein, and the figures shown under per cent in ration are the values obtained by analysis of the completed rations. The casein, gelatin, and DL-methionine mixture was fed at all three dietary levels. Weight gain and food and protein consumption increased as the dietary level of the protein mixture was increased from 6.5 to 12%, but differences in gain per gram of protein consumed were not statistically significant.

Lactalbumin, when fed alone, was less effective than the casein, gelatin, and DL-methionine mixture. Subsequent experiments showed that a mixture of lactalbumin, gelatin, and DL-methionine was as effective as the equivalent casein blend in promoting weight gain in the protein-depleted chick. Weight gain and food and protein consumption also increased as the level of lactalbumin was increased in the ration, and differences in gain per gram of protein consumed at the three levels were not statistically significant. Casein was superior to lactalbumin as the sole source of protein for the chick. Dried skim milk protein fell between lactalbumin and casein, a finding which seems reasonable considering that skim milk contains a mixture of these two proteins.

Table I. Ration Composition[a]

Ingredients	Preliminary Rations		Typical Protein Assay Rations					
	Complete	Protein-free	Casein, gelatin, & DL-methionine			Lactalbumin	Casein	Dried skim milk
Casein	200	—	50	71.4	95.2	—	139.4	—
Gelatin	80	—	20	28.5	38.6	—	—	—
DL-Methionine	3	—	0.8	1.1	1.4	—	—	—
Lactalbumin	—	—	—	—	—	154	—	—
Dried skim milk	—	—	—	—	—	—	—	345
Corn oil	40	40	40	40.0	40.0	40	40.0	40
Chick salts	60	60	60	60.0	60.0	60	60.0	60
Choline chloride	2	2	2	2.0	2.0	2	2.0	2
Cornstarch	—	—	827.2	—	—	—	—	—
Cerelose	615	898	—	797.0	762.8	744	758.6	553
Total	1000	1000	1000.0	1000.0	1000.0	1000	1000.0	1000
% protein (N × 6.25)			6.35	9.18	12.22	12.12	12.17	12.12

[a]

Vitamin Supplement	Mg./Kg. Ration
Thiamine hydrochloride	8
Riboflavin	8
Calcium pantothenate	20
Nicotinic acid	100
Pyridoxine hydrochloride	8
D-Biotin	0.3
Folic acid	3
Vitamin B_{12}	0.02
Vitamin A acetate	3
Vitamin D_3	0.02
α-Tocopherol acetate	10
2-Methyl-1,4-naphthoquinone	1

Chick Salts	G./Kg. Ration
$CaCO_3$	15
K_2HPO_4	9
Na_2HPO_4	7.3
$Ca_3(PO_4)_2$	14
NaCl	8.8
$MgSO_4.7H_2O$	5
Ferric citrate (16.7% Fe)	0.4
$MnSO_4.4H_2O$	0.42
KI	0.04
$ZnCO_3$	0.02
$CuSO_4.5H_2O$	0.02

Table II. Protein Assays Carried Out during First Repletion Period

Protein Source	Protein % in ration (N × 6.25)	No. Chicks	Chick Wt., G. Initial	Chick Wt., G. Final	Chick Wt., G. Gain	Food Intake, G. Food	Food Intake, G. Protein	Gain,[a] G./G. Protein
			Assay 1					
Casein, gelatin, DL-methionine	6.35	13	175.5	233.7	58.2	279.8	17.76	3.26±0.29
Lactalbumin	7.18	12	165.2	175.8	10.1	150.2	10.78	1.05±0.20
			Assay 2					
Casein, gelatin, DL-methionine	8.42	12	193.2	320.2	127.0	388.6	32.72	3.75±0.22
Lactalbumin	9.17	12	184.8	206.8	22.0	189.9	17.35	1.25±0.25
			Assay 3					
Casein, gelatin, DL-methionine	12.22	12	149.4	363.6	214.2	486.9	59.50	3.59±0.09
Lactalbumin	12.12	12	144.2	179.9	35.8	175.7	21.29	1.46±0.24
Casein	12.17	12	149.8	235.2	85.4	285.5	34.74	2.41±0.19
Dried skim milk	12.12	11	140.3	202.0	61.7	250.1	30.31	1.92±0.18

[a] Mean and standard error of mean.

The results of three assays carried out during the second or third repletion period are shown in Table III. Conditions to which the chicks were subjected preceding the second and third repletion periods have varied considerably and because of this no specified weight loss has been adhered to. Chicks which had been on a high quality protein during the previous assay period were maintained on the protein-free diet until they lost approximately 20% of their weight at the close of the previous assay period. Those which had gained little weight were kept on the protein-free diet until they had lost approximately the same amount of weight which they had gained during the previous assay period. As in the case of the first repletion period the weights of some chicks plateau before the desired weight loss occurs, and these chicks have been put on test after approximately 2 weeks on the protein-free ration. Chicks which had not gained during the previous assay period were immediately placed on the new test ration. A diet group in second and third repletion periods was composed of chicks from several diet groups of the preceding period. As the proteins assayed represent a wide range of nutritive values, this procedure tended to equalize the groups with respect to dietary history.

The nutritive values of the two proteins which have been assayed in successive repletion periods (casein, gelatin, and DL-methionine mixture and lactalbumin) increase slightly from the first through the third repletion period. This is probably due to a greater depletion of protein reserves as the chick is subjected to continued inadequate protein nutrition.

The crude feather meal protein fed in assay 4 was inefficiently used for weight regeneration, but when combined with lactalbumin the nutritive value of a 50 to 50% mixture was significantly superior to either lactalbumin or feather meal protein alone. The crude feather meal was a commercial, dried product which contained 91% of protein. The poultry feed fortifier protein was equal to the casein, gelatin, and DL-methionine mixture. This product contained an assortment of whey products, which supplied 60% of the protein, and fish meal, which supplied 40% of the protein.

When the amino acid content of the ration containing 9% of the protein from lactalbumin was compared with that of the ration containing 9% of the protein from the casein, gelatin, and DL-methionine mixture, the lactalbumin ration was found to be deficient in arginine, methionine, and glycine. The casein, gelatin, and DL-methionine ration used for this comparison contained

Table III. Protein Assays Carried Out during Second and Third Repletion Periods

Protein Source	Protein % in ration (N × 6.25)	No. Chicks	Chick Wt., G. Initial	Chick Wt., G. Final	Chick Wt., G. Gain	Food Intake, G. Food	Food Intake, G. Protein	Gain,[a] G./G. Protein
			Assay 4 (2nd repletion period)					
Casein, gelatin, DL-methionine	8.42	8	208.9	330.0	121.1	365.8	30.80	3.77±0.26
Lactalbumin	9.17	8	216.9	252.6	35.7	227.1	20.84	1.66±0.15
Crude feather meal	8.90	6	188.5	183.8	−4.7	104.1	9.27	0.38[b]
Lactalbumin + crude feather meal[c]	8.83	8	218.0	301.3	83.3	348.6	30.78	2.72±0.23
Poultry ration fortifier	8.92	8	203.1	327.7	124.6	401.8	35.84	3.50±0.21
			Assay 5 (3rd repletion period)					
Casein, gelatin, DL-methionine	8.50	9	198.7	333.9	135.2	383.0	32.55	4.24±0.36
Lactalbumin	9.02	9	195.9	233.6	37.7	218.7	19.73	1.92±0.28
Lactalbumin + 3 amino acids[d]	9.68	9	195.2	261.2	66.0	256.2	24.80	2.62±0.14
			Assay 6 (2nd repletion period)					
Casein, gelatin, DL-methionine	9.02	12	261.6	436.2	174.6	515.9	46.53	3.71±0.13
Lactalbumin	9.00	11	241.6	286.1	44.5	285.2	25.66	1.74±0.17
Casein	9.18	12	238.4	327.9	89.5	375.8	34.50	2.54±0.16
Lactalbumin, gelatin, DL-methionine	8.98	12	253.5	401.5	148.0	463.8	41.65	3.53±0.16

[a] Mean and standard error of mean.
[b] Four chicks lost weight.
[c] 4.5% of protein from lactalbumin and 4.5% from crude feather meal.
[d] 0.109 gram arginine, HCl, 0.09 gram DL-methionine, and 0.4 gram glycine per 100 grams diet.

Table IV. Results of Protein Assay Using Protein-Depleted Rats

Protein Source	Protein % in ration (N × 6.25)	Rat Weight, G. Initial	Rat Weight, G. Final	Rat Weight, G. Gain	Food Intake, G. Food	Food Intake, G. Protein	Gain,[a] G./G. Protein
Casein, gelatin, DL-methionine	9.61	199.7	233.3	33.7	136.3	13.10	2.57±0.06
Casein	9.53	195.6	234.8	39.2	138.7	13.22	2.96±0.03
Lactalbumin	9.88	199.4	249.7	50.2	140.8	13.91	3.61±0.14

[a] Mean and standard error of mean.

5.72% of the protein from casein, 2.56% of the protein from gelatin, and 0.1% from DL-methionine. The amino acid values were taken from published tables (*1*) and were not determined on the preparations fed. In assay 5 the deficit of these three amino acids was corrected by adding crystalline amino acids to the lactalbumin ration. The addition of these three amino acids significantly increased the nutritive value of lactalbumin.

Assay 6 was carried out at a dietary level of 9% of protein and during a second repletion period, but it adequately confirms the findings of assay 3 (12% of protein, first repletion period) with respect to the superiority of casein over lactalbumin as the sole source of protein for the chick. When fed in combination with gelatin and DL-methionine, casein and lactalbumin give approximately equal performance. Lactalbumin was substituted for casein on an equal protein basis.

To compare chick and rat responses, three of the proteins assayed on chicks were fed to protein-depleted rats according to the method of Cannon as modified by Wissler (*9*). Mature rats were fed a protein-free ration until they had lost approximately 27% of their original body weight. The depletion period was 32 days. During the 10-day assay period they were fed diets containing 9% protein. Each assay diet was fed to 9 rats. The results of this rat assay on the casein, gelatin, and DL-methionine mixture, casein and lactalbumin are given in Table IV. The composition of the casein, gelatin, and DL-methionine mixture was the same as that used in the chick ration containing 9% protein from this source. The mixture of casein, gelatin, and DL-methionine was significantly lower in nutritive value than the other two proteins for the rat. Lactalbumin was significantly superior to casein.

Discussion

In biological studies, it is most desirable to work directly with the species of animal to which the results will be applied. The data reported herein show that it is practical to apply to the chick, with a minimum of modification, certain techniques and equipment intended for rats. Considering the well known differences in amino acid requirements of chicks and rats and the differences in growth response to the same protein noted in these data, the chick should be the test animal of choice in evaluating proteins intended for poultry rations.

In early exploratory work, two 4-week growth tests were carried out on chicks. In these tests, chicks were held in battery brooders for 3 weeks on a ration containing one half the amounts of casein, gelatin, and DL-methionine present in the complete ration shown in Table I. They were then placed in individual cages and fed test rations containing approximately 13% of the protein from the casein, gelatin, and DL-methionine mixture, casein, and lactalbumin. Average gain per gram of protein consumed under these conditions were as follows: casein, gelatin, and DL-methionine mixture, 2.23 grams; casein, 1.34 grams; and lactalbumin, 0.71 gram. Whereas these three protein sources occupied the same relative positions with respect to nutritive values as they did in the weight regeneration method, the growth response of casein and lactalbumin was small and it seemed unlikely that satisfactory assays could be carried out at lower levels of protein in the ration.

Using the protein-depleted chick, it is possible to carry out satisfactory assays at dietary levels of 6.5, 9, or 12% of protein. Thus it is possible to assay a wide variety of poultry ration ingredients including those which contain a small amount of protein. The authors' experience has mostly been with rations containing 9% of protein as products in which interest centered could be fitted into this type of ration.

The superiority of lactalbumin over casein, as the sole source of protein for the rat, has been adequately demonstrated (*6*, *8*), but there are species differences in this respect as shown by Mueller (*7*). The results of the present study show that casein is superior to lactalbumin as the sole source of protein for the chick. However, no single source of protein is ever relied upon in any practical poultry ration.

The quality interrelationships of individual milk proteins to cereal and seed meal proteins as they occur in poultry rations is beyond the scope of this paper. This assay method could, however, be applied to such mixtures.

Acknowledgment

The authors wish to express their thanks to Ida Joppert, for preparation of diets, and to John Lomot, for the analysis of diets and ingredients.

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Corrections

Spectrophotometric Semimicrodetermination of Ergosterol in Yeast

On page 36 [J. AGR. FOOD CHEM. **5,** 360 (1957)] in Figure 1, curve 1 should represent 24(28)-dehydroergosterol and curve 2, ergosterol.

O. N. BREIVIK

Stability of Certain B Vitamins Exposed to Ethylene Oxide in the Presence of Choline Chloride

On page 957 [J. AGR. FOOD CHEM. **4,** 956 (1956)] in the second line of the first column, the word "corn" should have been "cornstarch."

H. A. BAKERMAN