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The Nutritional Requirement of the Protein-Depleted Chicken

IV. Amino Acid Needs and Utilization during Repletion[1])

By J. D. SUMMERS and HANS FISHER

With 5 tables

(Received June 5, 1962)

The growth rate of protein-depleted chickens during repletion has been shown to vary with the type of depletion regimen and the level of protein in the repletion diet (SUMMERS and FISHER 1962). In the same study we observed that depleted birds required more dietary protein to achieve maximum weight gain than did control animals of the same starting weight.

The possibility that the optimum essential amino acid requirement pattern for repletion of malnourished subjects may differ from that of normal individuals has been suggested by SCRIMSHAW et al., (1958). FISHER et al., (1959) reported that chicks depleted for one week on a N (nitrogen)-free diet required a higher protein intake to achieve maximum growth, but that the requirement for lysine, as a percent of dietary protein, remained essentially the same as that of control animals of the same age but not the same body weight.

The present study was initiated to investigate the essential amino acid pattern required for repletion of malnourished chickens of similar body weight but of different age.

Experimental

In order to obtain a good response to amino acid supplementation, a protein level of 17% was chosen for the repletion diets. This level is sufficiently above that required for maintenance to ensure good growth, yet far enough below the amount required for maximum growth so that the response from amino acid supplementation would be on the steep portion of the dose-response curve. In the present study comparisons were made between controls and birds depleted 1) on an essentially N-free diet[2]), 2) on a 15% gelatin diet or

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[1]) Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Poultry Science, New Brunswick. Supported by a grant-in-aid from the National Science Foundation G-11399.

[2]) This diet supplied only trace amount etc. p. 47.

3) by total starvation. The depletion procedures and the maintenance of the animals have been previously outlined (SUMMERS and FISHER 1960). Growth and N utilization responses were determined for graded levels of the amino acids tryptophan, arginine, lysine and methionine.

Two lots of 8–10 male birds from a Vantress cross were used per treatment throughout these experiments. The composition of the experimental rations is given in Table 1.

At the start of each experiment 8–10 chicks, selected on a body weight basis from both the control and depleted groups were killed for carcass N- and moisture-determination. These values were required for calculating N retention and amino acid efficiency. At the end of each experiment, the birds were starved for 12 hours and then killed for carcass analysis. Moisture was determined by difference between wet and dry weight after drying in a forced draft oven at 85 °C until constant weight was attained. Carcasses were ground and thoroughly mixed; triplicate N determinations were then carried out on either individual or pooled samples by a semi-micro KJELDAHL technique.

Table 1. Composition of basal rations

Ingredient	Tryptophan expt.	Arginine expt.	Methionine & Lysine[1]) expts.
	%	%	%
Hydrolyzed casein[2])	20.24	—	—
Alfalfa meal	3.00	—	—
L-arginine HCl	1.20	—	—
DL-methionine	0.20	0.20	—
Glycine	1.20	1.20	—
Dextrin	30.00	—	—
Crude casein	—	19.77	—
Peanut meal + isolated soybean protein[3])	—	—	29.46
Mineral mix[4])	4.94	4.94	4.94
Corn oil	3.00	3.00	3.00
Fiber	3.00	3.00	—
Vitamins[4])	0.25	0.25	0.25
Choline Cl (70%)	0.30	0.30	0.30
Glucose monohydrate	—	to 100	to 100
Starch	to 100	5.00	—

[1]) For the lysine study, 0.34% DL-methionine was added to the basal diet, and for the methionine study, 0.43% L-lysine HCl was added to the basal ration.

[2]) Hy Case SF, Sheffield Chemical Corp., Norwich, N. Y.

[3]) 70% by weight, of the mixture was peanut meal and 30% isolated soybean protein. ADM Assay Protein C-1, Archer-Daniels-Midland Co., Cincinnati, Ohio.

[4]) For composition see SUMMERS and FISHER (1961).

Tryptophan. In these experiments tryptophan was given at graded levels from 0.08 to 0.30% of the diet. An acid-hydrolyzed, salt-free casein (Hy Case SF, Sheffield Chemical Co., Norwich, N. Y.) was used as the sole source of protein. A mixture of starch and dextrin, rather than glucose, was the source of carbohydrate; these carbohydrates helped prevent the diet from sticking to the beaks of the birds by making it less hygroscopic. The diets were adequately supplemented with glycine, arginine, and methionine, so that tryptophan would be the only limiting amino acid (FISHER et al., 1955). At the end of the experiment with birds that had been depleted on the N-free diet, the animals from each lot of 10 were randomly assigned into two groups of 5. Analyses were then carried out on these pooled samples (moisture was determined on individuals); thus, two N determinations were made per replicate, or four determinations per treatment group. For the birds that had been depleted by starvation or by giving the gelatin diet, the animals were also randomly assigned into 2 groups per lot, but N analyses were carried out on only one of the two replicates per treatment.

Arginine. For these experiments, arginine levels from 1.0–1.5% of the diet were given, using crude casein, properly supplemented with glycine and methionine, as the protein source. For these experiments, as well as for the studies with lysine and methionine, glucose was the major dietary carbohydrate, since moisture uptake and consistency of the diet did not constitute problems.

At the completion of each experiment in this series, moisture was determined individually on all birds. N was determined individually on 5 birds, randomly chosen from each replicate of the groups depleted on the N-free diet, and on two pooled samples of 4 from one replicate for the animals previously depleted by starvation or by receiving the gelatin diet.

Lysine and methionine. Each of these amino acids was studied at 5 levels of supplementation. The protein source for these experiments was a mixture of peanut meal and isolated soybean protein, previously used in studying the protein needs of depleted birds (SUMMERS and FISHER 1962). Adequate methionine was added to all rations of the lysine series, and adequate lysine was similarly provided for the methionine studies.

At the end of each of these experiments moisture determinations were made on the carcasses of individual birds and N analyses were carried out on pooled samples of 4–5 birds per lot from each treatment. For the birds depleted on the N-free diet, carcass analyses were only carried out for those on the lowest level of amino acid supplementation and those at the level where maximum weight gains were obtained. For the animals previously depleted by starvation or by giving the gelatin diet, the birds from all levels of amino acid supplementation were analyzed.

Results

In discussing the results of these experiments we were primarily concerned in denoting *differences* in response rather than in pinpointing an absolute requirement. The interpretations to follow should be viewed in this light.

Tryptophan. The results in Table 2 show that the control animals made optimal gains at the 0.14% level of tryptophan; furthermore, the weight gains realized with the hydrolyzed casein diet equalled those obtained with any of the other protein sources studied (see Tables 3, 4, and 5). For the animals depleted on the N-free diet, the tryptophan requirement was somewhat higher than that of the controls yet, the maximum weight gains even at the highest level of tryptophan supplementation were about 100 grams below those of the control animals. The efficiency of tryptophan utilization as measured by carcass N gain per gram amino acid consumed was also superior for the control as compared to the N-free-depleted animals; the same was true for the N retention values.

The birds depleted on the gelatin diet behaved similarly to those depleted on the N-free depletion regimen; their maximum weight gains were also much inferior to those of the controls.

In sharp contrast with either of these two depletion groups, those depleted by starvation gained weight as well as the controls but required at least twice as much tryptophan to do so. Thus, the highest level of tryptophan supplied (0.30%) gave the best gains; we do not know whether still higher levels would have been even better.

Arginine. In this study (Table 3), the control animals showed a progressive increase in body weight with increasing amounts of arginine. The starved and N-free depleted animals also exhibited such increased response, but at the highest level of supplementation the gains were greater than those of the controls. This pattern was similar to that observed when low and high protein diets were fed to control and depleted birds (SUMMERS and FISHER 1962).

The birds depleted on the gelatin diet behaved differently from either the controls or the other two depletion groups. For these animals weight gain

Table 2. Body weight, feed efficiency and carcass composition of depleted and control birds given increasing levels of tryptophan

Tryptophan level[1])	Body weight[2])	Feed Utilization	Carcass		Nitrogen gain/ gm tryptophan	Nitrogen retention
			Moisture	Nitrogen		
% of diet	gm	gm gain/gm feed	%	%	gm	%
			Controls			
0.08	144 ± 14[3])	0.16	72.5 ± ·3	10.73 ± ·31	10.0	27.5
0.11	219 ± 12	0.38	70.8 ± ·3	9.92 ± ·06	11.0	39.9
0.14	322 ± 8	0.52	71.3 ± ·2	10.14 ± ·14	11.6	53.2
0.17	330 ± 7	0.50	71.3 ± ·4	9.89 ± ·08	9.0	50.0
0.20	319 ± 7	0.47	71.2 ± ·3	10.17 ± ·04	7.7	49.8
			N-free			
0.08	126 ± 2	—	73.1 ± ·6	11.33 ± ·02	3.7	9.7
0.11	142 ± 4	0.17	71.9 ± ·4	11.11 ± ·16	7.1	26.0
0.14	189 ± 8	0.37	71.7 ± ·5	10.11 ± ·20	6.4	28.9
0.17	221 ± 11	0.37	70.4 ± ·6	10.12 ± ·10	6.8	35.7
0.20	225 ± 12	0.47	70.3 ± ·8	9.72 ± ·18	5.8	37.5
			Starved			
0.08	145 ± 4	0.05	71.1	12.09	7.2	17.3
0.11	182 ± 5	0.17	70.7	11.54	8.7	32.3
0.14	257 ± 7	0.32	70.6	10.74	8.6	37.8
0.17	304 ± 12	0.38	70.8	10.45	8.1	42.6
0.20	326 ± 14	0.41	68.4	10.27	7.5	48.5
0.30	353 ± 13	0.45	66.2	9.32	5.0	47.8
			Gelatin			
0.08	132 ± 9	0.04	73.7	11.23	2.6	6.7
0.11	154 ± 5	0.21	72.5	10.73	3.3	11.5
0.14	239 ± 19	0.34	70.6	9.66	6.5	32.7
0.17	212 ± 34	0.32	69.4	10.03	5.7	30.6
0.20	240 ± 17	0.35	70.2	9.78	5.1	32.5
0.30	254 ± 20	0.36	70.9	9.44	3.0	29.5

Average starting weight 118 gm for controls, 128 gm for N-free birds, 124 gm for starved, and 128 gm for gelatin birds.

[1]) Supplemented as L-tryptophan.

[2]) Final weights after 2 weeks on the experimental diets.

[3]) Average values with their standard errors for duplicate lots of 8–10 birds.

appeared to plateau at an arginine level of 1.2%. Furthermore, a maximum weight gain, even on the highest level of arginine supplied, was inferior to that obtained with the other groups.

The efficiency of arginine utilization and also N retention were similar for all but the gelatin-depleted birds.

Methionine. Table 5 gives the results of the methionine study. The controls and the birds depleted on the N-free diet showed a similar weight gain and gave an optimum response at the same level of methionine. The birds depleted by starvation gained slowly on these diets and showed little response to increased dietary methionine level. Unlike the similarity between all groups for N utili-

Table 3. Body weight, feed efficiency and carcass composition of depleted and control birds given increasing levels of arginine

Arginine level[1])	Body weight[2])	Feed utilization	Carcass Moisture	Carcass Nitrogen	Nitrogen gain/ gm arginine	Nitrogen retention
% of diet	gm	gm gain/gm feed	%	%	gm	%
			Controls			
1.0	260 ± 11[3])	0.41	68.1 ± ·3	8.74 ± ·15	1.3	45.0
1.1	270 ± 14	0.47	67.4 ± ·3	8.82 ± ·16	1.3	50.6
1.2	276 ± 13	0.42	67.8 ± ·3	8.59 ± ·18	1.1	45.2
1.3	305 ± 8	0.51	68.2 ± ·4	8.88 ± ·18	1.2	51.9
1.5	319 ± 9	0.52	68.7 ± ·3	9.22 ± ·14	1.1	57.0
			N-free			
1.0	232 ± 15	0.47	66.0 ± ·9	8.65 ± ·19	1.2	44.6
1.1	257 ± 11	0.54	66.1 ± ·7	8.63 ± ·17	1.1	41.0
1.2	308 ± 13	0.62	66.9 ± ·4	8.56 ± ·15	1.2	49.5
1.3	310 ± 11	0.64	67.3 ± ·3	8.79 ± ·15	1.1	51.5
1.5	362 ± 11	0.65	67.3 ± ·6	8.80 ± ·19	1.1	51.3
			Starved			
1.0	264 ± 14	0.27	64.7	8.76	1.3	41.1
1.1	297 ± 13	0.36	68.9	9.25	1.3	47.6
1.2	326 ± 12	0.39	66.4	9.11	1.2	46.7
1.3	378 ± 18	0.48	67.5	9.24	1.2	55.4
1.5	389 ± 14	0.53	67.6	9.55	1.1	55.1
			Gelatin			
1.0	230 ± 8	0.27	67.4	9.05	0.9	31.9
1.1	242 ± 9	0.34	68.1	9.05	1.0	30.0
1.2	266 ± 10	0.38	69.6	9.20	0.9	36.6
1.3	250 ± 8	0.32	67.7	9.29	0.8	38.6
1.5	284 ± 18	0.38	67.6	9.20	1.0	38.6

Average starting weight 118 gm for controls, 128 gm for N-free birds, 124 gm for starved and 128 gm for gelatin birds.

[1]) Supplemented as L-arginine HCl.

[2]) Final weights after 2 weeks on the experimental diets.

[3]) Average values with their standard errors for duplicate lots of 8–10 birds.

zation and retention in the lysine study, in the methionine experiments these measurements were markedly inferior for the starved group.

For all groups on the arginine diets (and the lysine and methionine diets to follow) the carcass N content was significantly lower than on the tryptophan diets (Table 2).

Lysine. With the exception of the group depleted by starvation, the maximum weight gain response to dietary lysine was attained at a similar level of supplementation (Table 4). The starved birds, however, did not grow as well as the other groups on these diets and there was little response to increased dietary lysine level. Nitrogen retention and lysine utilization were similar for all groups.

Table 4. Body weight, feed efficiency and carcass composition of depleted and control birds given increasing levels of lysine

Lysine level[1])	Body weight[2])	Feed utilization	Carcass		Nitrogen gain/ gm lysine	Nitrogen retention
			Moisture	Nitrogen		
% of diet	gm	gm gain/gm feed	%	%	gm	%
			Controls			
0.70	311 ± 12[3])	0.47	66.7 ± ·4	8.14 ± ·10	1.6	40.8
0.78	330 ± 8	0.48				
0.87	336 ± 9	0.50	68.3 ± ·2	8.44 ± ·06	1.4	42.9
0.96	341 ± 10	0.46				
1.04	314 ± 10	0.43				
			N-free			
0.70	255 ± 8	0.41	65.9 ± ·3	8.45 ± ·13	1.6	46.2
0.78	305 ± 12	0.45				
0.87	379 ± 17	0.53	66.6 ± ·8	8.67 ± ·15	1.6	54.8
0.96	367 ± 15	0.63				
1.04	324 ± 9	0.62				
			Starved			
0.70	308 ± 18	0.35	66.5	8.36	1.5	39.3
0.78	294 ± 13	0.32	64.7	8.37	1.5	40.9
0.87	311 ± 13	0.42	67.4	8.37	1.2	42.8
0.96	288 ± 17	0.30	67.9	8.65	1.1	36.8
1.04	297 ± 14	0.34	66.6	8.65	1.5	38.9
			Gelatin			
0.70	261 ± 10	0.36	66.8	8.69	1.7	42.2
0.78	317 ± 9	0.44	67.3	8.26	1.6	44.2
0.87	371 ± 12	0.48	66.2	8.39	1.5	50.2
0.96	371 ± 12	0.46	68.0	8.75	1.4	48.8

Average starting weight 118 gm for controls, 128 gm for N-free birds, 124 gm for starved and 128 gm for gelatin birds.

[1]) Supplemented as L-lysine HCl.

[2]) Final weights after 2 weeks on the experimental diets.

[3]) Average values with their standard errors for duplicate lots of 8–10 birds.

Discussion

It is apparent from these studies that during repletion of birds of the same body weight, but depleted by various methods, the requirement for essential amino acids differs from that of controls and also among the depletion groups. In this study the depleted birds generally demonstrated a greater need for tryptophan than the controls, which in the case of the starved group was about double that of the controls. The much reduced gain of the birds depleted on the N-free diet suggested a greater need for other amino acids as well.

To test this hypothesis, two groups of birds were again depleted on a N-free diet and then given the hydrolyzed casein diet as before, supplemented with either (a) 0.3% L-tryptophan, or with (b) 0.1% L-phenylalanine, 0.2% DL-threonine and 0.4% L-leucine in addition to 0.3% L-tryptophan. The body weights of the latter group were 20% greater after 2-weeks of repletion than for the former group, indicating that this diet was deficient in one or more of

Table 5. Body weight, feed efficiency and carcass composition of depleted and control birds given increasing levels of methionine

Methionine level[1])	Body weight[2])	Feed utilization	Carcass		Nitrogen gain/gm methionine	Nitrogen retention
			Moisture	Nitrogen		
% of diet	gm	gm gain/gm feed	%	%	gm	%
			Controls			
0.15	244 ± 14[3])	0.27	66.5 ± ·7	8.19 ± ·15	2.1	31.1
0.25	294 ± 7	0.35				
0.33	335 ± 8	0.46	68.2 ± ·2	8.24 ± ·13	2.2	41.4
0.42	327 ± 10	0.45				
0.49	339 ± 14	0.45				
			N-free			
0.15	231 ± 12	0.35	66.7 ± ·7	8.67 ± ·21	2.6	38.7
0.25	337 ± 14	0.48				
0.33	370 ± 16	0.52	66.5 ± ·4	8.48 ± ·08	2.8	57.6
0.42	370 ± 7	0.54				
0.49	344 ± 15	0.53				
			Starved			
0.15	284 ± 15	0.30	67.8	7.83	2.7	31.0
0.25	279 ± 12	0.25	66.1	8.42	2.4	39.0
0.33	262 ± 14	0.27	66.5	8.15	1.6	29.0
0.42	274 ± 10	0.25	67.3	8.55	1.7	35.5
0.49	297 ± 14	0.34	66.6	8.65	1.5	38.9

Average starting weight 118 gm for controls, 128 gm for N-free birds, 124 gm for starved birds.

[1]) The basal diet contained 0.20% cystine; supplementation was made with DL-methionine.

[2]) Final weights after 2 weeks on the experimental diets.

[3]) Average values with their standard errors for duplicate lots of 8–10 birds.

the 3 added amino acids[1]) for the N-free-depleted birds. We have previously shown that this diet was not improved by amino acid addition for normal, non-depleted birds (FISHER et al., 1955). These results therefore, provide further evidence of an altered pattern of amino acid requirement for variously depleted animals of similar body weight.

While a ready explanation for the differences in requirement observed between controls and depleted and also among the three depletion groups is not obvious from the work described here, we would suggest that these differences may be due to variations in the type and composition of the body proteins that are synthesized during repletion or during normal growth. We have previously described and emphasized (SUMMERS and FISHER 1960) the considerable differences that exist in body composition after depletion by the various means which were also employed in the present study. It seems particularly noteworthy that the birds depleted on the gelatin diet had a much reduced arginine requirement during repletion. Since gelatin is very high in arginine, one might speculate that an excess of this amino acid accumulated during the depletion period which could then be utilized during repletion.

The birds depleted by starvation had a much lower methionine and lysine requirement than either controls or the other depleted groups. Again, it appears not unlikely that specificity of protein loss during depletion, and, in this instance also, the much smaller loss of N during starvation (SUMMERS and FISHER 1960) could account for these differences. Protein digestibility differences offer another possible explanation for the inferior growth observed on the plant-protein diets used in the lysine and methionine series. In this connection it may be noted that the starved birds did particularly well on the hydrolyzed casein diet in which the N is, for the most part, provided in the form of free amino acids.

The significant difference in carcass N between the birds in the tryptophan series and those on the other amino acid diets was probably due to the different carbohydrate (starch and dextrin versus glucose) used in the former experiments (HARPER et al., 1953; SPIVEY et al., 1958).

Finally, it is of interest to note that the utilization of the limiting amino acid (carcass N gain per gm amino acid consumed) was similar at deficient and sufficient levels. This bears out our previously noted contention that the utilization of a limiting amino acid is not impaired when it is present at sub-optimal levels (FISHER and SHAPIRO 1961).

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Summary

The requirements of normal and of variously depleted birds of equal body weight were compared for tryptophan, arginine, methionine and lysine. Marked differences in require-

[1]) The amounts added corresponded to the difference in content for these amino acids between intact casein and casein after acid hydrolysis and subsequent salt removal.

ment were noted between control and depleted birds as well as among the differently depleted groups. Birds depleted by starvation had a higher tryptophan requirement and a lower requirement for methionine and lysine than either controls or birds depleted on a N-free or a gelatin-containing diet. Those depleted on a N-free diet gained less well on a hydrolyzed casein diet than controls or previously starved birds. This difference in weight gain could be overcome by supplementing the hydrolyzed casein with small amounts of leucine, phenylalanine, and threonine. The birds depleted on a gelatin diet had a significantly lower arginine requirement than any of the other groups. We conclude that in birds of similar body weight the state of depletion influences not only the quantitative needs for protein and amino acids but, perhaps more importantly, the amino acid pattern necessary for optimal repletion.

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The Nutritional Requirements of the Protein-Depleted Chicken

V. Effect of Depletion by Starvation on Body Composition and Subsequent Energy Needs of the Adult Rooster[1])

By J. D. Summers and Hans Fisher

With 7 tables

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The importance of body composition changes in growing chickens, depleted by different methods, on the subsequent need for and utilization of protein and essential amino acids during repletion, has been demonstrated in previous reports of this series (Summers and Fisher 1962a, b).

In contrast to the paucity of information on body composition of depleted animals during the growing stage, a considerable body of such data is available for mature animals, particularly for the rat. Detailed carcass-composition analyses on adult rats after exposure to different depletion regimens have been carried out by Addis et al., (1936), Widdowson and McCance (1956), and

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