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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by Ju and Nasset (1959). Values from similar analyses on the adult chicken could not be found, even though this species was extensively used in depletion studies by German researchers at the turn of the century (Seitz 1906).

Depleted adult men, rats, and dogs have been employed in studies designed to evaluate the nutritive value of proteins and amino acid mixtures (Hoffman et al., 1948; Common 1948; Allison 1955).

The purpose of the present experiments was to obtain information on the carcass composition changes of the adult male chicken during depletion by starvation in preparation for an assessment of the amino acid needs and utilization for optimum repletion. Some preliminary work on energy and amino acid levels adequate for depleted roosters was also carried out.

Experimental

White Leghorn roosters, either 1 or $2^1/_2$ years old, were used for these studies. All birds had been maintained on a conventional stock diet until the start of these experiments. As controls, five 1-year-old and five $2^1/_2$-years-old normal, non-depleted animals were killed at the start of the experiment for determination of body composition. An additional 5 birds from each age group were starved for a 2-week period, with continuous access to tap water.

During the 2-week starvation period total excreta were collected every other day and nitrogen (N) was determined by a semi-micro Kjeldahl method. By the end of 2 weeks of starvation 1 bird had died and 2 others were on the point of death. These birds were bled prior to death for hematocrit and plasma protein determinations and then discarded. Excreta collections were stopped at that time but the remaining birds were starved for an additional 2 weeks, at which time 2 of the remaining animals were on the verge of death. All birds were now killed for carcass composition analysis.

Hematocrit and plasma protein values had been obtained for the control animals prior to killing and for the depleted animals after 2 weeks of starvation. Plasma protein was determined on an auto-analyzer, using a biuret method described by the manufacturer (The Technicon Co., Chauncey, N. Y.).

For the control and depleted animals, all feathers were removed, from 2 of the birds of each age group, in order to make separate N and fat analyses. Carcass and feathers were dried to a constant weight in a forced-draft oven at 85 °C, and moisture determined for the carcasses as difference between wet and dry weight. Both carcasses and feathers were then ground as finely as possible and sampled for N and fat determinations. Fat was determined by the procedure previously outlined (Summers and Fisher 1961). All N determinations were carried out in triplicate and the fat determinations in duplicate.

Preliminary experiments to determine energy and amino acid levels adequate for depleted roosters were carried out with birds depleted during 2 weeks of starvation. During the depletion period these birds had lost, on the average, 25% of their body weight. The following points were investigated:

1. The influence of energy intake on the N excretion of birds fed an essentially[2]) N-free ration (25, 35 und 45 gm food/kg body wt./day; eight birds were used for each level of food intake studied).
2. The N excretion of birds given an amino acid mixture which supplied an amount of N (155 mg N/kg body wt./day) equivalent to our best estimate of the endogenous excretion of normal birds (Leveille and Fisher 1958) and the essential amino acids in the quantities necessary for maintenance of normal animals (the diet was given to 10 depleted animals at the level of 25 gm/kg body wt./day).

[2]) This diet supplied only trace amounts of N in the form of certain vitamins such as choline, niacin, etc.

3. The influence of varying energy intake on the utilization of N supplied by an amino acid mixture adequate for maintenance of normal, non-depleted adult males (the diet which was given to 10 starved birds provided 280 mg N/kg body wt./day, LEVEILLE et al., 1960).

For these three experiments, total excreta collections were carried out on a 48-hour basis.

Results and Discussion

The composition values for carcass and feathers for normal birds from the two age groups are shown in Tables 1 and 2. No important differences due to age of birds were noticeable. Among the individual animals there were some large differences in composition; in some instances, there were 2-fold variations in fat content and concomitant differences in N and moisture content. Despite these variations in composition and differences in body weight, the water:N ratios were, essentially constant. This constancy of the water:N ratios was previously observed with the growing rat and chicken (BENDER and MILLER 1953; SUMMERS and FISHER 1961), while MITCHELL et al., (1931) and McNALLY (1955) have reported the relative constancy of the water content of fat-free tissue in adult chickens. The ratios for the adult rooster were considerably lower than those reported for the growing animals of either species. As has been done sucessfully for the growing animal, the constancy of the water:N relationship could be advantageously utilized in determining body N from a body water analysis.

Feather N content contributed approximately 19% to 24% of total body N. Since feather replacement continues throughout the life span of the bird, it is not surprising therefore that the essential amino acid requirements for maintenance should be influenced by the amino acid composition of feather protein (LEVEILLE et al., 1960).

Table 1. Carcass composition of normal adult roosters

Bird No.	Body Weight	Moisture	Nitrogen	Fat	Water:N
	gm	%	% dry wt	% dry wt	
		$2^1/_2$ years old animals			
1	2808	60.5	10.08	21.69	15.2
2	2755	62.9	10.21	19.66	16.6
3	2581	61.5	11.64	13.05	13.7
4	2653	64.0	11.16	15.17	15.9
5	2643	57.0	8.05	27.99	16.6
Average	2688	61.3	10.23	19.51	15.6
		1-year-old animals			
6	2411	61.0	10.52	20.53	14.9
7	2591	58.2	9.20	25.00	15.1
8	3115	59.6	9.53	26.01	15.5
9	2266	63.5	11.46	14.36	15.2
10	2503	63.1	11.11	14.45	15.3
Average	2577	61.1	10.36	20.06	15.2

Table 2. Feather weight and composition of normal adult roosters

Bird No.[1])	Feathers						Carcass Nitrogen
	Dry weight		Nitrogen	Fat	Nitrogen	Fat	
	gm	% dry carcass	% dry wt	% dry wt	As % of total body nitrogen	As % of total body fat	As % of fat- and feather-free bird
			$2^1/_2$ years old animals				
2	163.1	15.96	15.17	1.95	23.71	1.58	12.04
3	161.4	16.25	15.08	2.11	21.06	2.63	12.94
Average	162.3	16.11	15.13	2.03	22.39	2.11	12.49
			1 year old animals				
6	126.9	13.48	15.06	1.07	19.30	1.12	12.74
10	160.5	17.34	15.30	2.08	23.92	2.50	12.34
Average	143.7	15.41	15.18	1.89	21.61	1.81	12.54

[1]) These numbers correspond to those listed in Table 1.

Tables 3 and 4 give the carcass and feather composition values for the birds depleted by starvation. Again, as for the controls, there was little difference in composition between the two age groups.

The percentage weight loss during starvation was greatest for the 2 birds (1 and 9) that ended the depletion period with virtually no body fat. Both these birds were at the point of death when the depletion period was stopped. The very low carcass fat content of birds 1 and 9 after the starvation period would suggest that carcass fat is the main factor in determining survival time. This observation agrees with that of BAUR and FILER (1959) who showed that

Table 3. Carcass composition of adult roosters depleted by 4 weeks of starvation

Bird No.	Body weight			Carcass			Water:N
	Initial	Final	Loss	Moisture	Nitrogen	Fat	
	gm	gm	% of initial	%	% dry wt	% dry wt	
			$2^1/_2$ years old animals				
1	2390	1220	48.95	61.9	13.69	1.95	11.9
3	2660	1750	34.21	58.4	10.74	15.99	13.2
5	2880	1970	31.60	59.8	11.89	10.77	12.5
Average	2643	1640	38.25	60.0	12.08	9.57	12.5
			1 year old animals				
6	2810	1760	37.36	59.5	11.10	12.81	13.2
7	2390	1490	37.66	57.7	10.94	15.85	12.5
8	3300	2320	29.70	60.5	10.70	12.86	14.3
9	2750	1500	45.45	62.3	12.85	2.53	12.9
Average	2813	1768	37.54	60.0	11.40	11.01	13.2

starved pigs, with a high initial carcass fat content survived at least twice as long as those with a lower carcass fat content. The BAUR and FILER values for percentage carcass fat of pigs at time of death are very similar to those of birds 1 and 9 in our experiment (Table 3).

The water:N ratios for the starved birds, though fairly constant, are numerically lower than those of the control animals. From a comparison of the feather measurements (dry weight, % N) for normal and depleted birds (Tables 2 and 4), feathers appear to be an extremely stable portion of the body

Table 4. Feather weight and composition of adult roosters depleted by 4 weeks of starvation

Bird No.[1]	Feathers						Carcass Nitrogen
	Dry weight		Nitrogen	Fat	Nitrogen	Fat	
	gm	% dry arcass	%' dry wt	% dry wt	As % of total body nitrogen	As % of total body fat	As % of fat- and feather-free bird
			$2^1/_2$ year old animals				
1	154.5	33.26	15.43	1.25	37.42	21.30	13.14
3	153.6	21.11	15.36	1.45	32.91	1.89	11.96
Average	154.1	27.19	15.39	1.35	35.17	11.60	12.55
			1 year old animals				
6	147.9	20.74	15.33	1.58	28.69	2.56	11.58
8	170.0	18.55	15.54	1.27	26.93	1.82	11.40
Average	159.0	19.65	15.44	1.43	27.81	2.19	11.49

[1]) These numbers correspond to those listed in Table 3.

and apparently subject to little depletion. The only exception is the feather fat content, which was significantly reduced during depletion ($P < 0.05$). In the depleted animals, feathers contributed approximately $^1/_3$ of the total carcass N. Thus, when composition analyses are expressed on a whole- carcass basis, the high N content of feathers tends to mask the very considerable N loss from the featherless carcass. The carcass N content, expressed on a feather- and fat-free basis (Tables 2 and 4) was relatively constant, regardless of the state of starvation of the animal. This is in agreement with similar observations for the rat (WIDDOWSON and McCANCE 1956) and for the pig (BAUR and FILER 1959).

Table 5 gives the hematocrit and plasma protein values for both normal and starved birds. For the normal animals the hematocrit values were relatively constant, showing much less variation among individuals than the plasma protein or other composition values. Among the depleted animals, the $2^1/_2$-year-old birds had significantly ($P < 0.05$) higher hematocrit values and lower (but not significantly so) plasma protein values than the 1-year-old birds.

Table 6 shows the N excretion values for the depleted animals during the first 2 weeks of starvation. For 6 of the 10 animals, the N excretion per kg body weight increased only slightly during the 2-week depletion period. Three of the 4 birds (birds 2, 4, and 10) that showed a steadily increasing rate of N excretion died either at the end or shortly after the 2-week starvation period, and the fourth (bird 9) was on the point of death at the end of 4 weeks of starvation. As previously mentioned these results are probably a reflection of an initially low carcass fat content. N excretion during a short starvation period might possibly give some indication of body composition.

Table 5. Hematocrit and plasma protein values for normal and starved adult roosters[1])

Bird No.	Normal		Starved	
	Hematocrit	Plasma protein	Hematocrit	Plasma protein
	%	gm/100 ml	%	gm/100 ml
		$2^1/_2$ year old animals		
1	47.4	3.86	50.0	4.2
2	43.1	4.33	57.5	2.9
3	46.7	3.86	52.9	3.8
4	48.4	3.89	57.1	3.7
5	41.4	3.86	48.4	4.0
Average	45.4	3.96	53.2	3.7
		1 year old animals		
6	44.1	3.82	48.6	4.4
7	44.9	4.34	44.4	4.5
8	41.8	4.32	40.5	4.3
9	46.7	4.58	45.0	4.5
10	45.3	3.38	34.3	3.0
Average	44.6	4.09	42.6	4.1

[1]) Hematocrit and plasma protein values for the depleted birds were determined after 2 weeks of starvation; bird numbers correspond to those listed in the previous tables.

The N loss of the 6 birds that exhibited a more uniform excretion pattern averaged 359 mg N/kg body wt./day. LEVEILLE and FISHER (1958) reported the average daily endogenous N excretion to be about 150 mg N/kg body wt./day. The difference between these two values represents an estimated 1.25 gm (approximate) of body protein catabolized daily/kg body wt. to furnish the basal energy needs of the starving animal.

Section A, Table 7 gives N excretion values of birds fed varying amounts of an essentially N-free diet following a 2-week starvation period. For both the first and second collection periods there were no differences among the three groups, and the N excretions for days 5–6 and 7–8 are essentially equal to the endogenous excretion reported for the adult rooster by LEVEILLE and FISHER (1958). It was therefore concluded that 25 gm of feed/kg body wt. (90 calories of metabolizable energy) supplied the energy needs of the depleted animal given an essentially N-free diet.

Section B of Table 7 shows that birds first depleted by 2 weeks of starvation, and then given a N-free diet for 4 days at the rate of 25 gm/kg body wt./day (90 calories of metabolizable energy), could be maintained in N balance with 155 mg N/kg body wt./day; the N was supplied by the amino acid pattern found suitable for maintaining normal birds at the endogenous excretion level (LEVEILLE et al., 1960).

Table 6. Body weight changes and nitrogen excretion of adult roosters during a 2-week starvation period

Bird No.	Body weight Initial	Final	Loss	Nitrogen excretion mg/kg/day days 1–2	3–4	5–6	7–8	9–10	11–12	13–14
	gm	gm	% of initial							
				$2^1/_2$ years old animals						
1	2390	1650	31.0	356	408	412	427	388	499	483
2	2680	1610	39.9	341	508	845	915	1076	1160	—
3	2660	2050	22.8	249	238	291	320	318	340	346
4	2720	1580	41.9	387	521	835	870	969	1041	1186
5	2880	2250	21.9	286	211	274	283	282	316	325
Average	2646	1828	31.5	324	377	531	563	607	661	585
Average body weight (gm)[1]				2446	2344	2240	2134	2016	1902	1828
				1 year old animals						
6	2810	2050	27.1	178	344	387	424	381	417	403
7	2390	1760	26.4	251	365	418	370	325	335	338
8	3300	2570	22.1	238	285	341	299	271	289	259
9	2750	1880	31.6	353	511	665	672	712	747	692
10	2470	1470	40.5	447	589	971	964	1052	1141	1069
Average	2744	1946	29.5	293	418	556	542	548	586	552
Average body weight (gm)[1]				2534	2404	2320	2218	2124	2034	1946

[1]) Average of weights recorded at time of collection every other day.

Section C of Table 7 indicates that, when depleted birds (2 weeks of starvation) are given a level of dietary N sufficient for repletion to commence (280 mg N/kg body wt.), the dietary energy level has to be increased above the amount necessary to maintain normal, undepleted animals. BENDITT et al., (1948a, b),

Table 7. Effect of varying energy or N intake on nitrogen excretion of starved adult roosters

A

Diet intake[1]) mg/kg body wt./day	N excretion[2]) days 1–2	3–4	5–6	7–8
25	311 ± 37[3])	193 ± 16	146 ± 8	138 ± 10
35	329 ± 29	194 ± 11		
45	335 ± 40	213 ± 11		

[1]) The birds were starved for 2 weeks, then given the diet which contained essentially no N and supplied 90 calories per kg. body wt. of metabolizable energy. For its composition see LEVEILLE and FISHER (1958).

[2]) Mg N/kg body wt./day.

[3]) Average value for 8 birds ± standard error.

B

	N balance[1]) Days		
	1–2	3–4	5–6
	— 6 ± 3[2])	+ 6 ± 3	+ 5 ± 3

The birds were starved for 2 weeks, then given an essentially N-free diet for 4 days; this was followed by the test diet given at the rate of 25 gm/kg body wt./day and supplying the level of essential amino acids necessary for the maintenance of nitrogen equilibrium in normal adult roosters (LEVEILLE et al., 1960).

[1]) Average value for 10 birds ± standard error.

[2]) Mg N/kg body wt./day.

C

Supplemental diet intake[1])	N balance[2]) Days						
gm	1–2	3–4	5–6	7–8	9–10	11–12	13–14
0	— 174 ± 42[3])	— 90 ± 11	— 56 ± 12				
10						— 11 ± 17	
20				— 32 ± 12	+ 12 ± 16		+ 15 ± 6

[1]) The birds were starved for 2 weeks, then given a ration which supplied 280 mg N/kg body wt./day and 90 calories of metabolizable energy at the rate of 25 gm/kg body wt./day. The supplemental diet intake refers to additional N-free diet.

[2]) Mg N/kg body wt./day.

[3]) Average value for 10 birds ± standard error.

and ALLISON (1958) reported similar findings for the depleted rat and dog; the former workers interpreted the increased energy needs as being necessary to meet the extra cost of tissue synthesis when adequate protein is supplied. We interpret this greater need for energy for depleted *vs.* undepleted birds as a reflection only of changes in body composition. Since the energy needs of animals are probably more closely related to their lean body mass than to body weight, the N content of the carcass might serve as a better measure for a feeding standard, particularly under conditions of malnutrition. Indeed, calculations show that the absolute energy needs of the starved birds are little different from those of normal animals when expressed on a carcass N, rather than a body weight, basis[3]).

Summary

Carcass composition, plasma protein, and hematocrit determinations were carried out on normal adult male chickens, and on adult males depleted by starvation for 4 weeks. For the first 2 weeks of depletion, N excretion was also determined for the starved animals. The following observations were recorded:

[3]) From the data previously presented it can be shown that normal birds on 25 gm of diet intake consumed 3.43 calories/gm carcass N. This is approximately equivalent to an intake of 35 gm for depleted birds (3.56 calories/gm carcass N).

1. During 2 weeks of starvation the adult rooster lost approximately 25% of his body weight, and between 10 and 20% of his carcass N.
2. There was no loss in either weight or N content of feathers upon depletion; there was, however, a significant loss in feather fat.
3. The survival time of starved birds appeared to be related to their initial, prestarvation fat reserves.
4. The energy needs for repletion of depleted roosters, expressed on a body weight basis, were considerably higher than those necessary for maintaining normal animals. This appeared to be primarily a reflection of the greater proportion of 'lean body mass' (carcass N) in the starved birds.

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