

## Nutritional studies with Scaleless chickens

### I. THE SULPHUR-CONTAINING AMINO ACIDS\*

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#### 1. INTRODUCTION

Information concerning genetically determined differences in the requirements of chickens for specific nutrients is scant. Especially rare are examples of differences based on single genes. In practice recommended nutrient allowances for domestic fowl are based on optimum requirements determined experimentally, plus an added margin of safety to allow for breed and strain variations, variations in nutrient content of feedstuffs and some loss of nutrients during storage (Taylor, 1949). It is this safety factor that has tended to prevent the routine uncovering of small breed, strain or family differences. Females from highly inbred strains, however, will not uncommonly deviate widely enough from the norm in their requirements to produce embryos with characteristic deficiency symptoms. In such cases an improvement in hatchability and a reduction in anomalies will often result from a general enrichment of the diet. Such instances may be examples of the poor 'developmental homeostasis' (Lerner, 1954) of inbreds.

Occasionally breed differences in dietary requirements are uncovered in the course of deficiency assays. For example, Single Comb White Leghorns have been found to require less manganese, thiamine and vitamin D than Rhode Island Reds (Hutt, 1949). In resistance to nutritional encephalomalacia the White Leghorn breed differs significantly from heavy breeds of fowl (Howes & Hutt, 1952). Using breed crosses Howes & Hutt (1956) uncovered family differences in efficiency of thiamine utilization. They failed to find significant differences between thirteen strains of White Leghorns in this respect and found that only the Leghorns differed significantly from the other breeds studied, Rhode Island Reds, New Hampshires, Barred and White Plymouth Rocks. Davis *et al.* (1938) reported family differences among Single Comb White Leghorns in requirements for riboflavin. Lamoreux & Hutt (1948), developed strains differing in resistance to a dietary deficiency of riboflavin. MacDonald (1957, 1958) suggested that Australorps lack a gene present in Single Comb White Leghorns and active in the conversion of methionine to cystine.

Examples of strain differences in requirements for specific nutrients have been reported in mammals as well. For example, Kalter (1959) has reported differences

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between highly inbred strains of mice in susceptibility to an artificially induced riboflavin deficiency. In this case, however, acute deficiency symptoms were obtained in the offspring of depleted dams receiving galactoflavin, a riboflavin antagonist, and were more comprehensive than those found when dietary depletion alone was used. Part of the response noted could reflect side effects arising from the use of an antimetabolite.

Additional information on genetic differences in nutritional requirements may develop from some of the recent studies (Falconer, 1960; Abplanalp, 1962, for example) concerned with selection for growth rate on different planes of nutrition or alternatively on diets deficient in one or more individual nutrients, if success in altering growth rate in such cases proves to be at least partly due to altered nutritional requirements.

This report deals with the marked decrease in the requirement for the sulphur-containing amino-acids, methionine and cystine, in chickens homozygous for a single autosomal recessive gene, *scsc*. These birds lack scales and most feathers. The few feather follicles present are late in their differentiation and give rise to scattered down feathers and subsequently to an abnormal juvenile-type plumage (Abbott & Asmundson, 1957). It is the first of a series of studies of the comparative nutritional requirements of Scaleless birds. The almost complete absence of feathers suggested that Scaleless birds might have reduced requirements for nutrients known to be present in substantial amounts in feathers. For example the importance of the sulphur-containing amino-acids in hair and feathers is well known (Block & Bolling, 1951). It was felt that such a reduced need might not have been observed previously, since the majority of other avian plumage mutants which give rise to some degree of nakedness, are characterized by the presence of feather follicles, feather growth and either irregularity in feather form or a subsequent feather loss. Hutt & Sturkie (1938) reported that supplementation of the diet of Naked chicks with either cystine or cystine hydrochloride did not prevent the feather loss characteristic of this mutation. Naked neck (Greenwood, 1927) and recessive Aptyrylosis (Sturkie, 1942, 1950) together with Scaleless represent the only reported cases where follicle formation and subsequent feather growth is deficient. Naked neck and recessive Aptyrylosis, which are less extreme forms of nakedness than Scaleless, have not been examined from the point of view of possible changes in nutritional requirements.

Differences in requirements for the sulphur-containing amino-acids (methionine and cystine, both normally satisfied by methionine supplied in the diet (Almquist, 1952) and here considered together) might be expected to be most evident during periods of exceptionally rapid growth of down or feathers; i.e. between the 9th and 18th day of incubation, between hatching and 7 or 8 weeks of age (in early feathering strains) and during the annual moult. The most suitable period to investigate in the present instance appeared to be between the 2nd and 5th week of age, for several reasons. (1) This is a period of extremely rapid growth with demands for rapid protein synthesis in all body parts as well as a period of continuous feather production in normal birds, the chick down being replaced by a succession of juvenile feather coats (Chu, 1938-9). (2) It is generally accepted that excesses of individual

amino-acids are not stored for considerable periods of time (Almquist, 1952). Accordingly it seems unlikely that differences in supplies of methionine and cystine available to Scaleless and normal day-old chicks in yolk form, based on a possible differential usage during embryonic development, would influence trials begun at 2 weeks of age. (3) Studies of growth rate of Scaleless birds fed natural diets (*ad lib.*) have indicated that their weight gain is equivalent to that of normally feathered siblings during this period (Asmundson & Abbott, 1958, and unpublished). Later, Scaleless birds tend to fall behind normal birds in weight. That this is due largely to their higher requirements for body maintenance is indicated by the markedly higher heat output of Scaleless than of normal birds (Abbott & Asmundson, 1958, and unpublished). In this regard Scaleless birds resemble homozygous Frizzle fowl (Benedict *et al.*, 1932).

## 2. MATERIALS AND METHODS

A total of 146 birds were used in these trials. Of these, half were Scaleless and half normal birds of similar breeding. They were raised in commercial battery brooders in a heated and ventilated room. The birds were divided into two separate trials, run 2 weeks apart. The composition of experimental groups is outlined in Table 1.

Table 1. *Design of experiment testing the effect of a diet deficient in methionine on normal (N) and Scaleless (Sc) chickens*

Trial No.	Full diet					Deficient diet				
	Pheno-type	Sex	No. of birds*			Pheno-type	Sex	No. of birds		
			(1)	(2)	(3)			(1)	(2)	(3)
1	N	♂♂	10	10	9	N	♂♂	10	10	10
	N	♀♀	10	10	10	N	♀♀	10	10	10
	Sc	♂♂	10	11	11	Sc	♂♂	10	8	7
	Sc	♀♀	10	9	9	Sc	♀♀	10	12	12
2	N	♂♂	7	7	7	N	♂♂	6	6	6
	N	♀♀	10	10	10	N	♀♀	10	10	10
	Sc	♂♂	7	8	7	Sc	♂♂	6	6	6
	Sc	♀♀	10	9	9	Sc	♀♀	10	10	10

\* (1) Assigned to group at hatching time;

(2) corrected for errors in sexing;

(3) remaining in group at sacrifice. One bird died on the 11th day (Sc ♂ Full diet) and two died during the Respiration trials (N ♂, Full diet and Sc ♂ Deficient diet, respectively).

Four errors in sexing at day-old changed the numbers in Scaleless groups. Sexers have been found to make a consistently larger proportion of errors in sexing Scaleless than normal chicks. The majority of the birds used came from line 24 and a few from line 13. These lines originated several years ago from crosses of New Hampshires with four other breeds of fowl (Abbott, 1958). Accordingly these lines show considerable variation in size and growth rate. Scaleless chicks of line 24 averaged

3.2 g. more in weight than normal siblings, while the larger chicks of line 13 did not differ significantly by phenotype in weight. Selection of birds for the experimental groups was made so that each group contained an equivalent set of birds, as follows: In each trial, sets of Scaleless sibs falling at day-old within the weight range 36–40 g. were assigned at random to the four Scaleless groups, normal sibs of the Scaleless chicks falling in the same weight range, were then assigned at random to the con-

Table 2. *Composition of diets*

Ingredient	Full diet (%)	Deficient in methionine and cystine (%)
Purified soybean protein*	22.00	22.00
Soybean oil, crude	3.50	3.50
Dicalcium phosphate	2.00	2.00
Calcium carbonate	2.50	2.50
Mineral mix †	2.30	2.30
Vitamin mix ‡	2.00	2.00
Choline chloride (0.25%)	0.80	0.80
Biotin	0.20 $\gamma$ /kg.	0.20 $\gamma$ /kg.
Vitamin E (20,000 I.U./lb.)	0.10	0.10
Vitamin B <sub>12</sub>	10.00 $\gamma$ /kg.	10.00 $\gamma$ /kg.
Vitamin A (20,000 I.U./gm.)	0.10	0.10
Vitamin D (1,500 I.C./gm.)	0.10	0.10
Glycine	0.30	0.30
Cellufour §	5.00	5.00
Cornstarch	58.90	59.30
DL-Methionine	0.40	—
	100.00	100.00

\* Drackett assay C-1 (82%).

† The grams per kilogram of analytical reagent grade chemicals in the mineral mix were as follows: 427.4 NaCl, 12.8 MnSO<sub>4</sub>.H<sub>2</sub>O, 27.8 FeSO<sub>4</sub>.7H<sub>2</sub>O, 3.4 CuSO<sub>4</sub>.5H<sub>2</sub>O, 3.0 ZnO, 0.9 Co(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>.4H<sub>2</sub>O, 0.4 KI, 171.3 MgSO<sub>4</sub>.7H<sub>2</sub>O, 128.2 KCl, 10.7 Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>.18H<sub>2</sub>O, 213.7 K<sub>2</sub>HPO<sub>4</sub>, 0.4 NaMoO<sub>4</sub>.2H<sub>2</sub>O.

‡ The composition of the vitamin mix expressed as grams per kilogram was as follows: 0.50 riboflavin, 0.50 thiamin HCl, 0.50 pyridoxine HCl, 1.49 calcium D-pantothenate, 4.96 niacin, 0.25 folic acid, 0.50 menadione (vitamin K), 991.30 cornstarch.

§ Purified wood cellulose.

trasting normal groups. This procedure resulted in each experimental group containing an equivalent number of birds of each family as well as in the exclusion of extreme deviates in size. An unequal sex ratio among birds hatched for the second trial resulted in a relative scarcity of Scaleless males meeting these weight standards; consequently, fewer birds were used in all male groups in this series.

All birds received a stock chick starter from 1–14 days of age. Beginning at 14 days, half of the normal and half of the Scaleless groups were fed a purified diet based on Drackett protein, containing 3.5% fat and adequate in all essential nutrients. The balance of the groups received the same diet-lacking methionine (Table 2). The

birds of trial 1 were slower in accepting the purified diet than those of trial 2. After two days, however, all had adjusted to the ration. This initial difference led to a small but fairly consistent difference in weight between replicate groups during the early part of the feeding trial.

The study was divided into four phases, an initial investigation of weight gain, food consumption and food efficiency during the first 14 days on the diets, a second check of these after 21 days, a period of collection of physiological data and a final collection of data at sacrifice. The experimental diets were fed throughout. Data obtained were as follows:

- (a) During the first 14 days on diet, at 24-hour intervals: individual bird weights, length of primary wing feathers in normal birds, food consumption and faecal output per group.
- (b) After 21 days on diet: individual bird weights, length of primary wing feathers in normal birds, food consumption and faecal output per group (28–35 days inclusive).
- (c) After 4–6 weeks on diet: respiratory quotient and heat output, packed cell volume, blood volume and differential white blood cell counts. Respiratory quotient and heat output were measured in a serial respirometer developed by Kleiber (1940), and modified to accommodate birds of 6 weeks age. Packed cell volume was obtained from microhematocrit readings. Blood volume was measured by the T-1824 method (discussed in Gregersen & Rawson, 1959) in the following manner: Evan's blue dye was injected into the left brachial vein and samples drawn from the right auricle after 10 and 20 minutes. After centrifugation and dilution, optical density readings ( $624\text{ m}\mu$ ) were made on a Beckman B spectrophotometer, plotted on semi-log paper and extrapolated to zero time. Calculations of blood volume and per cent whole blood were based on optical density of standard dye solutions and microhematocrit data as suggested by Gregersen (1944). Blood smears were stained with Wright's stain. With the exception of the differential white blood cell counts which were based on even, well-stained spreads from samples of five or six birds per experimental group, the above data were obtained in so far as possible on all 143 surviving birds. In two instances, heat output and respiratory quotient determinations were excluded because of faulty operation of the respirometer. In addition, five birds scheduled for the last run had to be omitted because they proved too large to fit in the respiration cages. Three blood volumes were omitted because of excessive turbidity of the samples.
- (d) At sacrifice (average age of birds, 60 days): individual weight of each bird and of its heart, thyroid and adrenal glands. The heart, thyroid and adrenals were prepared for subsequent histological study. The data concerning histological changes in these organs will be reported elsewhere.

There were no consistent differences in feather length during the 3-week period of study. At later stages normal birds fed the deficient diet began to lose many of their feathers, which were brittle and easily broken. Accordingly, this report will be

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confined to comparisons of the two phenotypes fed the contrasting diets with respect to growth, food consumption and food efficiency, heat output and respiratory quotients, packed cell volume, blood volume, white blood cell counts and heart, thyroid and adrenal weights.

### 3. RESULTS

#### (1) *Growth, food consumption and food efficiency*

Growth, as measured by body-weight, of Scaleless birds fed either the full or the deficient diet was equivalent to or better than that of normal birds fed the full diet. At the same time normally feathered birds fed the deficient diet showed the expected rapid decline in weight gain. Figure 1 illustrates this for males and females in the

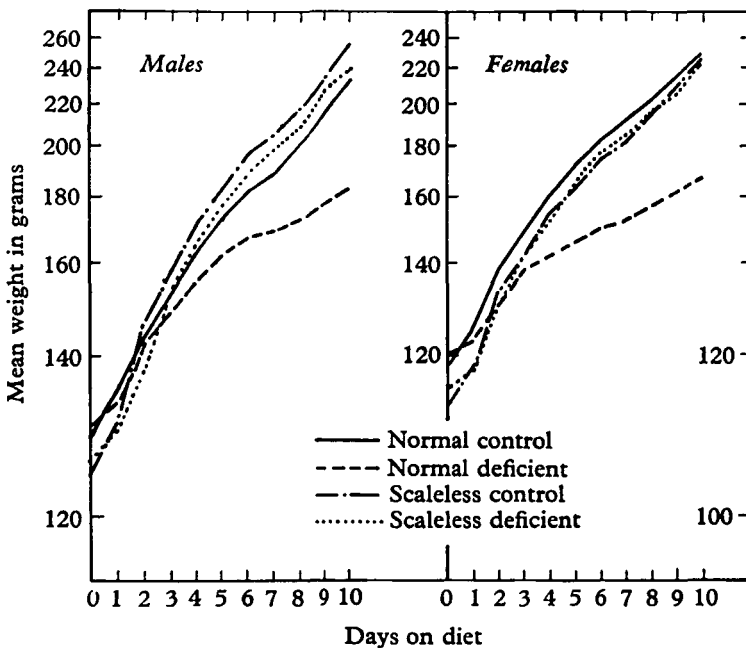


Fig. 1. Growth in normal and Scaleless birds on full diet (control series) and on a diet deficient in methionine (deficient series).

second trial. Similar results were obtained in both trials with both sexes. Birds in trial 2 gained at a higher rate, however, than those in the first trial, especially during the first 10 days. Their relative advantage was more marked in Scaleless than in normal birds. Aside from the initial difference in rate of accepting the experimental ration referred to above, there was no obvious difference between the two trials and after 3 weeks this difference had largely disappeared (Table 3). After 10 days on the experimental rations, Scaleless fed full and deficient diets were equivalent in weight; after 21 days Scaleless fed the deficient diet had dropped behind those fed the full

Table 3. Effect of a diet deficient in methionine and cystine on growth in normal and Scaleless chickens

		Mean body-weight in grams ± S.E.			
		Normal		Scaleless	
Sex	Trial	Full diet	Deficient diet	Full diet	Deficient diet
Pre-experiment (14 days)*					
♂♂	1	129.0 ± 5.1	129.9 ± 5.0	125.4 ± 5.9	132.3 ± 2.7
	2	133.7 ± 7.5	128.8 ± 9.8	123.8 ± 4.7	124.8 ± 4.5
♀♀	1	117.1 ± 3.8	118.8 ± 3.8	113.6 ± 5.2	117.6 ± 4.4
	2	118.8 ± 8.0	119.9 ± 6.0	111.8 ± 8.0	115.3 ± 4.5
10 days on diet (24 days)*					
♂♂	1	235.6 ± 12.0	180.6 ± 9.6	228.7 ± 13.1	240.1 ± 5.6
	2	246.2 ± 23.0	182.8 ± 19.1	254.4 ± 11.9	240.2 ± 8.3
♀♀	1	212.4 ± 7.2	154.1 ± 6.7	209.7 ± 9.5	202.3 ± 8.6
	2	227.4 ± 15.3	164.8 ± 9.4	225.7 ± 18.2	220.1 ± 8.5
21 days on diet (35 days)*					
♂♂	1	394.7 ± 21.1	216.1 ± 15.0	400.8 ± 43.0	396.4 ± 12.4
	2	395.8 ± 31.7	223.0 ± 10.4	429.8 ± 19.3	376.2 ± 7.0
♀♀	1	354.8 ± 13.7	186.2 ± 12.2	362.1 ± 33.8	325.7 ± 13.9
	2	373.6 ± 25.4	203.0 ± 16.6	353.1 ± 31.5	345.4 ± 13.3

\* Age of birds.

diet in gain, suggesting that the level of sulphur-containing amino-acids provided in the deficient diet had become slightly sub-optimal for Scaleless birds by 5 weeks of age.

In Table 4 contributions to variance in 21-day body-weight and estimates of effects have been calculated according to Yates (1937). Phenotype, sex and diet effects were all highly significant as was the interaction between diet and phenotype.

Table 4. Contributions to variance in body-weight: normal and Scaleless birds fed full and methionine-deficient diets for 21 days

Source of variation	d.f.	Mean squares	Estimates of effects (g.)
Replicates	1	348.06	—
Phenotype (N vs. Sc)	1	25,680.06*	- 40.1
Sex (♂ vs. ♀)	1	6,765.06*	20.6
Diet (Full vs. Def.)	1	39,501.56*	49.7
Phenotype—Sex	1	689.06	- 6.6
Phenotype—diet	1	21,682.56*	36.8
Sex—diet	1	45.56	1.7
Phenotype—sex—diet	1	0.56	0.2
Error	7	170.21	—

\*P = < 0.01.

Although weight gains in Scaleless birds fed both diets were equivalent to or better than those of normal birds fed the full diet, Scaleless birds consumed more food. For example, during the first 10 days of the trial, Scaleless males fed full and deficient diets ate 11% and 17% more than normal males fed the full diet and gained 5.5% and 2% more weight, respectively. Normal males receiving the deficient diet ate 18% less food and gained 52% less weight than their controls. These relationships are expressed as food efficiencies, the average body-weight gain in grams per gram of food consumed, in Table 5. During the first 10 days of the experiment, Scaleless birds fed the full diet were slightly less efficient than normals. Between 11 and 21 days, food efficiency improved in normal birds but remained fairly constant in Scaleless birds fed both diets. Scaleless birds receiving the deficient diet were significantly lower in their food efficiency than those fed the adequate diet.

Table 5. *Mean weight gain, food consumption (FC) and food efficiency (FE) in normal and Scaleless birds fed full and methionine-deficient diets*

Pheno- type	Diet	Sex	1-10 days on diet			11-21 days on diet		
			Gain*	FC (g.)	FE Gain/FC	Gain*	FC (g.)	FE Gain/FC
N	Full	♂♂	10.9	18.7	0.58	14.1	22.5	0.63
N	Full	♀♀	10.2	17.5	0.58	13.1	20.0	0.66
N	Deficient	♂♂	5.2	15.3	0.34	3.4	11.1	0.31
N	Deficient	♀♀	4.0	13.5	0.30	3.2	9.7	0.33
Sc	Full	♂♂	11.5	20.6	0.56	15.8	27.3	0.58
Sc	Full	♀♀	10.5	19.9	0.53	12.7	24.9	0.51
Sc	Deficient	♂♂	11.1	21.8	0.51	13.4	26.1	0.51
Sc	Deficient	♀♀	9.4	20.1	0.47	11.3	24.1	0.47

\*g. per bird per day.

## (2) Faecal output

Faeces were partially dried before weighing. Expressed in terms of weight gains, no consistent differences were found between Scaleless and normal birds fed the full diet. However, for normal birds receiving the deficient diet, faecal output proportional to weight gain was consistently higher than for other groups. The data for faecal output (Table 6) show the same trends as those for gain and for food consumption. The inability of the normally feathered birds to use the methionine-deficient diet efficiently is shown by the high faecal output per gain ratio and the low gain per food consumption ratio. The differences of these ratios for normal birds on the deficient diet from those for the other six groups may also result in part from the higher water consumption of the former. Although water consumption was not measured, the more fluid condition at collection of the faeces from the normal birds fed the deficient diet, suggested that water consumption was highest by birds for which the diet was relatively most deficient.



Table 6. Faecal output (FO) in normal and Scaleless birds fed full and methionine-deficient diets for 10 days

Phenotype	Diet	Sex	FO* (g.)	FO/FC†	FO/gain
Normal	Full	♂♂	8.2	0.44	0.76
Normal	Full	♀♀	8.2	0.47	0.80
Normal	Deficient	♂♂	5.2	0.34	1.00
Normal	Deficient	♀♀	5.6	0.41	1.40
Scaleless	Full	♂♂	8.9	0.43	0.77
Scaleless	Full	♀♀	7.9	0.40	0.75
Scaleless	Deficient	♂♂	8.7	0.40	0.78
Scaleless	Deficient	♀♀	7.4	0.37	0.79

\* Average partially dried faecal weight/bird/day.

† Food consumption, grams/bird/day.

(3) Heat output and respiratory quotient

Heat output and respiratory quotient may be influenced by a number of factors: including bird size and body surface area, activity, interval since last food consumed and environmental temperature. The respiration trials were conducted in a basement room where temperature, light source and general conditions (noise and activity) were relatively constant regardless of the time of day. Most of the trials were run during the day; a few were run in the early evening. Repeat measurements on the same birds during these two periods were substantially the same. All birds were fasted for 24 hours immediately before study.

Table 7. Heat output and respiratory quotient of normal and Scaleless birds fed full and methionine-deficient diets for 6 weeks (Mean ± S.E.)

Phenotype	Sex	Full diet		Deficient diet	
		kcal/day/kg.	Fasting R.Q.	kcal/day/kg.	Fasting R.Q.
Normal	♂♂	107.9 ± 3.2	0.73 ± 0.01	144.9 ± 4.4	0.75 ± 0.01
Normal	♀♀	110.4 ± 2.9	0.74 ± 0.01	146.4 ± 5.1	0.76 ± 0.01
Scaleless	♂♂	165.1 ± 6.5	0.73 ± 0.01	184.2 ± 2.6	0.73 ± 0.00
Scaleless	♀♀	198.0 ± 5.3	0.73 ± 0.01	205.5 ± 3.7	0.74 ± 0.00

Scaleless birds showed a marked increase over normals in heat output, of the order of 52% in males to 80% in females (Table 7). These differences were similar to previous values obtained for normal and Scaleless birds fed natural diets (Abbott & Asmundson, 1958). The correction for surface area differences (Kleiber, 1947) does not materially alter this picture. Among normal groups those receiving the deficient diet had both a significantly higher heat output and respiratory quotient than those fed the full diet. Among Scaleless groups, heat output was slightly but not significantly higher in those fed the deficient diet; the respiratory quotient was not affected by the diet fed.

(4) *Packed cell volume and blood volume*

Packed cell volume (hematocrit) was significantly higher in Scaleless than normal birds regardless of diet fed (Table 8). Normal and Scaleless birds receiving the full diet did not differ significantly from each other in plasma volume or in whole blood volume. Normal birds and to some extent, Scaleless birds fed the deficient diet showed an increase in plasma and whole blood volume. One small Scaleless male that gained weight at a very slow rate was largely responsible for the high reading and consequent large error term of the Sc ♂♂ deficient group. If this bird is omitted, the blood volume is of the same order as for Scaleless females receiving this diet.

Table 8. *Determinations of packed cell, plasma and whole blood volumes of normal (N) and Scaleless (Sc) birds fed full and methionine-deficient diets for 6 weeks*

Pheno- type	Sex	Full diet			Deficient diet		
		Hematocrit (%) ± S.E.	Plasma volume (%) ± S.E.	Whole blood volume (%) ± S.E.	Hematocrit (%) ± S.E.	Plasma volume (%) ± S.E.	Whole blood volume (%) ± S.E.
N	♂♂	25.7 ± 0.9	6.8 ± 0.4	9.3 ± 0.5	26.4 ± 1.1	9.3 ± 0.9	12.6 ± 1.2
N	♀♀	25.2 ± 1.0	6.5 ± 1.2	8.9 ± 1.7	22.9 ± 0.8	9.5 ± 0.8	12.4 ± 1.0
Sc	♂♂	32.5 ± 0.8	5.8 ± 0.9	8.7 ± 1.2	31.1 ± 2.1	10.2 ± 2.0*	18.8 ± 6.2*
Sc	♀♀	32.8 ± 0.8	8.7 ± 1.1	12.8 ± 1.5	31.9 ± 1.3	7.1 ± 1.0	10.5 ± 1.2

\*High reading and S.E. due mainly to one runt bird.

(5) *Differential white blood cell counts*

Heterophil and lymphocyte counts differed between normal and Scaleless disease-free birds (Table 9). Scaleless birds receiving the full diet were higher in heterophils and lower in lymphocytes than normal birds. Scaleless males were also higher than normal males in thrombocyte count. White blood cell counts obtained on birds fed the deficient diet indicated that Scaleless were closer than feathered birds to normal values. Normal birds fed the deficient diet were as high or higher than Scaleless birds in heterophil and equivalent in lymphocyte counts. No significant group differences were found in monocyte, basophil or eosinophil counts.

(6) *Heart, thyroid and adrenal weights*

The heart, thyroid and adrenal glands were enlarged both absolutely and especially relative to body-weight in Scaleless birds in the comparison to feathered birds receiving the full diet (Table 10). In this respect they resemble homozygous Frizzle fowl (Boas & Landauer, 1934; Landauer & Aberle, 1935). The deficient diet caused little change in either organ or body-weight in Scaleless birds but did affect a reduction in the three organs and in body-weight of normally feathered birds. The relative decline however was much greater in body than in organ weight, so that the ratios of organ to body-weight of the normal birds increased on the deficient diet to the point that they were nearly as large as the corresponding figures for Scaleless.

Table 9. Differential white cell counts\* in normal (N) and Scaleless (Sc) 8 week-old chickens fed full (F) and methionine-deficient (D) diets for 6 weeks

Phenotype	Sex	No. of slides		Monocytes		Basophils		Eosinophils	
		F	D	F	D	F	D	F	D
N	♂♂	21	15	1.5 ± 0.2	1.9 ± 0.3	1.5 ± 0.2	1.9 ± 0.4	0.9 ± 0.2	1.6 ± 0.5
	♀♀	13	15	1.7 ± 0.3	1.3 ± 0.2	2.0 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.7 ± 0.3
Sc	♂♂	12	10	3.1 ± 0.6	1.5 ± 0.3	2.0 ± 1.0	2.2 ± 0.3	1.3 ± 1.0	1.7 ± 0.4
	♀♀	6	18	1.7 ± 0.5	2.4 ± 0.4	2.6 ± 0.2	1.6 ± 0.2	1.1 ± 0.3	1.4 ± 0.2
Heterophils									
N	♂♂	21	15	12.2 ± 0.7	17.5 ± 2.1	38.2 ± 1.0	35.4 ± 4.6	44.0 ± 2.9	41.8 ± 3.6
	♀♀	13	15	11.4 ± 1.7	13.7 ± 1.8	35.5 ± 4.8	41.1 ± 4.9	46.6 ± 3.6	37.9 ± 4.1
Sc	♂♂	12	10	16.9 ± 2.4	14.6 ± 2.9	44.3 ± 3.8	37.8 ± 4.8	33.3 ± 3.2	43.2 ± 4.1
	♀♀	6	18	17.7 ± 1.8	15.7 ± 1.7	35.0 ± 7.2	35.7 ± 3.2	40.6 ± 3.7	41.6 ± 3.5
Lymphocytes									

\*Counts are expressed per 100 cells (based on 200 cell counts).

Increase in total body-weight is however not a particularly reliable reference point for individual organs characterized by different growth rates and, in the case of endocrine glands capable of rapid response to a variety of trigger mechanisms. Under conditions of dietary deficiency in normal birds the loss is most evident in bone and muscle and less so in endocrine organs. The fact that the change in diet

Table 10. *Heart, thyroid and adrenal weights of normal (N) and Scaleless (Sc) chickens fed full and methionine-deficient diets for 6 weeks*

Phenotype	Sex	Full diet		Deficient diet	
		(g.)	As % body-weight	(g.)	As % body-weight
Heart weight					
N	♂♂	4.497	0.587	3.067	0.704
N	♀♀	3.791	0.539	2.478	0.691
Sc	♂♂	6.034	0.789	6.309	0.823
Sc	♀♀	4.962	0.765	5.241	0.786
Thyroid weight					
		(mg.)	As % body-weight	(mg.)	As % body-weight
N	♂♂	22.4	0.0029	15.9	0.0037
N	♀♀	23.3	0.0033	12.4	0.0035
Sc	♂♂	31.4	0.0041	31.2	0.0041
Sc	♀♀	32.3	0.0051	31.6	0.0047
Adrenal weight					
		(mg.)	As % body-weight	(mg.)	As % body-weight
N	♂♂	45.4	0.0059	32.4	0.0074
N	♀♀	46.8	0.0067	25.4	0.0071
Sc	♂♂	60.3	0.0079	62.1	0.0081
Sc	♀♀	52.7	0.0081	47.8	0.0072

affected the three organs differently in the two phenotypes is of considerable interest. The increased heart size of normal birds suggests that methionine deficiency like low temperature, may act as a stress factor as well as differentially affect the growth of different body parts. The relatively larger adrenal weight of normal birds receiving the deficient diet may also suggest a disproportionate increase in protein catabolism (Sahyun, 1948) which would also agree with the data of Table 6, especially if increased faecal output reflects in part an increased urine production.

## 4. DISCUSSION

The marked decrease in the requirement of Scaleless chickens for the sulphur-containing amino-acids indicates that a single gene can substantially change the needs for an essential amino-acid. In this case it is evident that the lower requirement for methionine is secondary to the absence of scales and feathers.

Weight gains of Scaleless birds fed the deficient diet were found to be equivalent to those receiving the full diet during the early part of the feeding trial. After 3 weeks Scaleless birds receiving the deficient diet had dropped behind Scaleless on the full diet, indicating that the lack of sulphur-containing amino-acids begins to limit growth in these mutants after 5 weeks of age. This may be about the age at which the need by normal birds for methionine and cystine for body growth exceeds the requirement for feather growth. In spite of this decrease in gain, after 6 weeks feeding of the experimental rations Scaleless receiving the deficient diet did not differ from those fed the full diet in other respects such as viability, heat output and weights of the heart and endocrine glands.

The continued growth of feathers on normal birds fed a diet deficient in methionine indicates that feather growth takes precedence over gain in weight. While early feather growth may be a nutritionally expensive process it appears to be an efficient part of the birds system for maintaining its body temperature since the difference between the two phenotypes increases with age. This is so whether the comparison is based on food consumption or food consumed per unit of gain. The use of Scaleless and normal birds would seem to offer an opportunity to measure accurately food requirements for feather growth and the effect of feathers on food requirements at different temperatures. Such a study would contribute to the understanding of gene-environment interaction.

This work provides information on the capacity of the body to vary in response to drastic changes in its environment. Adaptive responses to hypothermic stress in this case included enlargement of the heart, thyroid and adrenal glands. The increase in size of these organs as well as the increased heat output of Scaleless birds, increased packed cell volume and alterations in white blood cell counts measure the phenotypic flexibility of the bird, which allows it to adapt to basic changes in metabolism. Similar changes in the endocrine glands and in the heart and blood contents were reported by Landauer & David (1933) for homozygous Frizzle birds.

The use of mutants in nutritional studies offers opportunities to study certain problems not easily approached by other means. For Scaleless birds the methionine-deficient diet provided adequate amounts of the essential amino-acids whereas this diet severely limited the growth of normally feathered birds. As would be expected (Rose, 1938) feathered birds reduced their food intake on this diet while the food intake of Scaleless birds increased over the amount consumed of the full diet. Acceptance of food then was a function of the composition of the diet and the phenotype. The higher food intake of Scaleless birds apparently was related directly to their increased requirements for body temperature maintenance.

The study further suggests that the Scaleless gene could replace its normal allele in the appropriate nutritional environment. A diet somewhat less deficient in

methionine than the one considered here should allow Scaleless but not normal birds to grow and reproduce. Temperature control would increase the advantage of Scaleless over feathered birds under such circumstances. This possibility is at present being examined.

#### SUMMARY

Young birds homozygous for the recessive gene, *sc*, had a significantly lower requirement than their normally feathered siblings for the sulphur-containing amino-acids, methionine and cystine. When fed a synthetic diet lacking in methionine, feathered birds showed the expected abrupt decrease in weight gain while Scaleless birds grew at a rate equivalent to feathered birds receiving the full diet. Scaleless birds receiving the full diet exceeded feathered birds in weight gain during the first part of the feeding trial but they required more food per gram of gain. Between Scaleless groups those fed the deficient diet dropped behind those on full diet in gain after 3 weeks but even after 6 weeks these did not differ in other respects. On the other hand among normal birds those receiving the deficient diet were higher in heat output and had relatively larger thyroid and adrenal glands than those receiving the full diet.

Differences between the two phenotypes are accounted for by (1) the difference in nutritive requirements determined by feather growth, and (2) the resultant differences in heat loss which accounted for the increase in heat output, packed cell volume and heart, thyroid and adrenal gland size of Scaleless birds.

The use of mutants such as Scaleless in studies concerned with requirements for specific physiological or growth purposes is suggested.

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