

# The Metabolism of Methionine by Single Comb White Leghorn and Black Australorp Chicks

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In 1941, Klose and Almquist reported that methionine was an essential dietary ingredient for growth promotion in the chick. Since then, many studies have been conducted to determine the sulfur amino acid requirements of the chick. The methionine requirement has been shown to be affected by the cystine, choline (Grau and Almquist, '43, Almquist and Grau, '45), protein (Rosenberg and Baldini, '57) and energy content of the chick's diet (Baldini and Rosenberg, '55). With substantially no cystine in the diet the results of feeding studies (Grau and Almquist, '43) have demonstrated that the chick utilizes methionine for cystine synthesis at a rate rapid enough to meet physiological needs for growth. Since the amount of cystine in the diet affects the chick's need for methionine, this requirement cannot be considered without consideration of cystine content.

Recently, McDonald ('57, '58) reported a breed difference in the metabolism of methionine. He found that Single Comb White Leghorn chicks, but not Australorp, gave a growth response to methionine supplementation of the basal diet. However, the Australorp chicks responded to as little as 0.078% of supplemental dietary cystine. Biochemical studies of the liver cysteine content of Single Comb White Leghorn and Australorp chicks, indicated that the Australorp chicks could not synthesize cystine at a rate that would meet physiological need for growth.

The current studies were initiated to determine the effect of cystine supplementation on the growth of Black Australorp and Single Comb White Leghorn chicks fed diets having a low cystine content, and also to study the utilization of methionine S<sup>35</sup> by the Black Australorp and Single Comb White Leghorn chick.

## EXPERIMENTAL

All chicks used in these experiments were obtained from outbred, closed flocks of the Breeding Section of the Poultry Research Branch at the Agricultural Research Center. Chicks were housed in electrically heated chick batteries having raised, wire floors. Feed and water were supplied ad libitum. Basal diets used are presented in table 1. Supplementation of the basal diet with methionine and cystine was made at the expense of cornstarch. The cystine and methionine content of the diets was determined microbiologically.

The method of Horn and Blum ('56) was used for the cystine microbiological assays except for the following two modifications: feed samples were hydrolyzed in an autoclave at 15 pounds pressure for two hours, and the sterilization time for the media was reduced to 2.5 minutes. Methionine was assayed by the method of Williams ('55) using a 24-hour reflux hydrolysis. *L. mesenteroides* P-60 (ATCC no. 8042) was used as the assay organism for both methionine and cystine.

### *The effect of cystine supplementation of basal diets on growth rate*

*Experiments 1 and 2.* Day-old straight-run chicks were placed randomly on the various dietary treatments outlined in table 2 for a 4-week experimental period.

*Experiment 3.* During the first week, the chicks were fed basal diet 3, supplemented with 0.3% of DL-methionine and 0.2% of L-cystine. At one week of age the chicks were placed randomly on the various treatments given in table 2, using duplicate groups of both breeds per treatment. The experiment was terminated when the chicks were 4 weeks old. Statistical analy-

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TABLE 1  
Composition of basal diets

Ingredient	Diet		
	1	2	3
	%	%	%
Alfalfa meal, 17% protein	—	5	5
Isolated soybean protein <sup>1</sup>	10	12	17
Casein, 88% protein	10	10	5
Gelatin	5	5	5
Fish solubles	3	3	3
Ground cellulose	3	3	1
Corn oil <sup>2</sup>	6	10	10
Mineral mix <sup>3</sup>	4.76	4.76	4.76
B-vitamin mix <sup>4</sup> (trituated in sucrose)	0.5	0.5	0.5
Vitamin A concentrate (10,000 I.U./gm)	0.05	0.07	0.07
Vitamin D <sub>3</sub> concentrate (15,000 I.C.U./gm)	0.003	0.003	0.003
Vitamin E concentrate (44 I.U./gm)	0.01	0.01	0.02
Choline chloride supplement (25% choline chloride)	0.5	0.6	0.8
Chlortetracycline	—	0.002	0.002
Antibiotic supplements <sup>5</sup>	—	—	0.02
DL-methionine	0.3	—	—
Cornstarch	56.87	46.59	47.84
Total	100	100	100
Protein % calculated	22.1	25.3	25.3

<sup>1</sup> Assay Protein C-1, Archer-Daniels-Midland Company, Cincinnati, Ohio.

<sup>2</sup> Stabilized by adding Tenox R, 500 mg/kg of corn oil, Eastman Chemical Products, Inc., Kingsport, Tennessee.

<sup>3</sup> Mineral mix supplied the following per cent of minerals in the diet: tri-calcium phosphate, 3.0; potassium phosphate dibasic, 0.9; sodium chloride, 0.5; magnesium carbonate, 0.262; ferric citrate, 0.05; zinc carbonate, 0.01; potassium iodide, 0.004; cupric acetate, 0.005; manganese chloride, 0.037; sodium selenate, 0.0004.

<sup>4</sup> Vitamin mix supplied the following vitamins in mg/kg of diet: thiamine-HCl, 30; riboflavin, 15; calcium pantothenate, 100; vitamin B<sub>12</sub>, 0.008; pyridoxine-HCl, 10; folic acid, 4; inositol, 50; menadione, 2.5; niacin, 100; biotin, 0.1.

<sup>5</sup> Contained zinc bacitracin, 25 gm of antibiotic/pound. Commercial Solvents Corp., New York.

TABLE 2  
Fourth-week body weights of Single Comb White Leghorn and Black Australorp chicks as affected by methionine and cystine supplementation of low-cystine basal diet

Basal diet no.	Supplements added		Experiment 1 <sup>1</sup>		Experiment 2 <sup>2</sup>		Experiment 3 <sup>3</sup>			
	DL-methionine	L-cystine	SC White Leghorn	Black Australorp	SC White Leghorn	Black Australorp	SC White Leghorn		Black Australorp	
	%	%	gm	gm	gm	gm	1	2	1	2
1	—	—	281	292						
	—	0.3	275	272						
2	—	—			249	340				
	0.30	—			300	360				
	0.30	0.20			321	338				
	0.40	—			306	357				
3	0.40	0.20			304	324				
	—	—					226	177	258	279
	0.15	0.15					290	257	336	336
	0.30	—					295	274	344	339
	0.30	0.20					296	258	346	348
	0.50	—					303	253	326	349

<sup>1</sup> Experiment 1, day-old chicks, 15 per treatment; basal diet 0.54% of L-methionine 0.10% of L-cystine by microbiological assay.

<sup>2</sup> Experiment 2, day-old chicks, 12 per treatment; basal diet 0.45% of L-methionine 0.11% of L-cystine by microbiological assay.

<sup>3</sup> Experiment 3, week-old chicks, 10 per treatment (each treatment replicated); basal diet, 0.40% of L-methionine, 0.11% of L-cystine by microbiological assay.

ses (analysis of variance and Duncan's new multiple range test) were made on growth data according to the methods outlined by Li ('57).

#### Utilization of L-methionine S<sup>35</sup>

Using sulfur<sup>35</sup>-labeled L-methionine, tracer studies were conducted of the amount of methionine utilized for cystine synthesis by each of the two breeds. Commercially obtained L-methionine S<sup>35</sup> was freed of radioactive impurities by paper chromatography before use.

**Experiment 4.** Six Single Comb White Leghorn and 6 Black Australorp chicks were fed basal diet 1 from one day of age. Starting the second day, they were injected intramuscularly with carrier-free L-methionine S<sup>35</sup> (20 microcuries per chick per day) for 4 consecutive days and killed on the 5th day. The droppings were collected daily and the total S<sup>35</sup> content determined<sup>1</sup> (table 3). The carcasses were pooled by breed and homogenized in a Waring blender. To provide an optimum hydrolysis period for methionine liberation with least destruction of cystine, the homogenized carcasses were hydrolyzed with 6 N HCl for three hours at 110°C, filtered, residue rehydrolyzed for 7 hours and filtered; three-hour and 7-hour filtrates for each breed were combined and reduced in volume *in vacuo*. Aliquots of the hydrolysates were used for isolation of methionine S<sup>35</sup> and cystine S<sup>35</sup> by means of ion exchange

resins. One-milliliter aliquots of the hydrolysates were placed on 0.9 by 100-cm columns of Dowex 50 (hydrogen form) and eluted according to the procedure of Stein and Moore ('50). One-milliliter aliquots of the fractions collected from the ion exchange columns were evaporated to dryness and counted in a windowless gas-flow counter. Corrections were made for self-absorption and decay. Identity of the methionine and cystine peaks was confirmed by paper chromatography.<sup>2</sup>

Table 4 (experiment 4) shows the per cent of total carcass sulfur S<sup>35</sup> found in the cystine fraction. In order to determine the specific activity of the cystine S<sup>35</sup> found in the carcass, larger columns (5 by 50 cm) of Dowex 50 resin (hydrogen form) were used to isolate larger quantities of cystine S<sup>35</sup>. The cystine S<sup>35</sup> thus obtained was recrystallized three times and checked for purity by microbiological assays and paper chromatography. The specific activity of the cystine S<sup>35</sup> isolated from the carcass hydrolysate of the two breeds of chicks is given in table 4 (experiment 4).

<sup>1</sup> Droppings homogenized in Waring blender were made up to volume and aliquots wet-ashed with concentrated nitric acid, residue taken up in 10 ml of H<sub>2</sub>O. One-milliliter aliquots of the ashed solution were dried in stainless steel planchets and counted.

<sup>2</sup> Solvent systems for paper chromatography; methyl ethyl ketone, propionic acid, water (75, 25, 30) and *tert.* butanol, formic acid and water (75, 15, 15).

TABLE 3  
Retention and excretion of L-methionine S<sup>35</sup> administered intramuscularly to Single Comb White Leghorn and Black Australorp chicks  
Experiment 4

Breed	Days after initial dose				Total <sup>1</sup>
	1	2	3	4	
	%	%	%	%	%
S <sup>35</sup> content of droppings					
SCW Leghorn	15.19	12.76	9.34	7.26	44.55
Black Australorp	12.34	11.43	9.64	8.68	42.09
S <sup>35</sup> content of carcass <sup>1</sup>					
SCW Leghorn		56.73			
Black Australorp		56.90			
Recovery of S <sup>35</sup> administered <sup>1</sup>					
SCW Leghorn		101.28			
Black Australorp		98.99			

<sup>1</sup> Per cent of total dose administered.

TABLE 4  
Conversion of carrier-free L-methionine S<sup>35</sup> to cystine S<sup>35</sup> by Single Comb White Leghorn and Black Australorp chicks

Experiment no.	Hydrolysate	Resin column no.	% <sup>1</sup> recovered as cystine S <sup>35</sup>	Average %	Specific activity cpm/mg
<i>Carcass hydrolysate</i>					
4	SCW Leghorn	L 1B	33.90	35.71	93,811
4	SCW Leghorn	L 1E	37.52		
4	SCW Leghorn	L 1D			
4	Black Australorp	1A	31.72	30.75	81,683
4	Black Australorp	1B	29.75		
4	Black Australorp	1C			
5	SCW Leghorn	SB	32.39	32.73	
5	SCW Leghorn	SD	35.24		
5	SCW Leghorn	SF	30.57		
5	Black Australorp	SA	27.28	26.19	
5	Black Australorp	SC	26.63		
5	Black Australorp	SE	24.67		
<i>Liver hydrolysate</i>					
6	SCW Leghorn	LIA	27.30	29.08	
6	SCW Leghorn	LIC	30.86		
6	Black Australorp	AIA	21.53	21.85	
6	Black Australorp	AIB	22.18		

<sup>1</sup> Per cent of total radioactivity recovered as cystine S<sup>35</sup> from carcass- or liver-tissue hydrolysate.

*Experiment 5.* Three Single Comb White Leghorn and three Black Australorp two-day-old chicks were injected intramuscularly with carrier-free L-methionine S<sup>35</sup> (22.64 microcuries per chick per day) for 4 consecutive days and killed on the 5th day. These chicks had been fed basal diet 3 from one day of age. The entire chick carcass was homogenized separately in a Waring blender, hydrolyzed and fractionated on ion exchange resins by the same procedure described in experiment 4. The per cent of total radioactivity found in the cystine fraction for each chick is given in table 4 (experiment 5).

*Experiment 6.* Two Single Comb White Leghorn and two Black Australorp three-week-old chicks were injected intramuscularly with carrier-free L-methionine S<sup>35</sup> (28.28 microcuries per day per chick) for 4 consecutive days and killed on the 5th day, having been fed basal diet 2 from one day of age. Livers were removed, hydrolyzed and fractionated on ion exchange resins as described in experiment 4. The

per cent of the total radioactivity found in the cystine fraction for each liver hydrolysate is shown in table 4 (experiment 6).

#### RESULTS AND DISCUSSION

Growth studies outlined in the first three experiments (table 2) show no growth response with either breed of chick when cystine was added to diets containing adequate methionine. The data were analyzed statistically, with no significant difference found in the growth of the two breeds. In experiment 2, no growth response was obtained from methionine supplementation of the basal diet fed to Black Australorp chicks. It appears, therefore, that basal diet 2 contained an adequate amount of sulfur amino acids to meet the requirement of the Black Australorp chick but not of the Single Comb White Leghorn chick.

In experiment 3, the diet was modified to reduce the sulfur amino acid content in the basal diet. Here, a significant difference (5% level of significance), was

obtained when the growth of both breeds fed the basal diet was compared with the growth of chicks receiving supplementary methionine or methionine plus cystine. These results are contrary to those obtained by McDonald ('57), who found that the addition of methionine to his basal diet used for Australorp chicks resulted in a growth depression rather than a growth response. However, the results of the tracer studies with L-methionine  $S^{35}$  show that less methionine is converted to cystine by the Australorp chicks than by Single Comb White Leghorn chicks. The cystine  $S^{35}$  isolated from the Black Australorp carcass hydrolysate had a lower specific activity than the cystine  $S^{35}$  isolated from the Single Comb White Leghorn carcass hydrolysate. This indicates that a definite breed difference exists in utilization of methionine for cystine synthesis. Even though the rate of conversion of methionine to cystine was not as great for the Black Australorp chicks as for the Single Comb White Leghorn chicks, the growth studies indicate that the rate was adequate to meet physiological needs.

#### SUMMARY

The utilization of methionine for synthesis of cystine by Single Comb White Leghorn and Black Australorp chicks was studied, using a low-cystine content, semi-purified diet. A growth response was obtained from methionine supplementation of the basal diet. With either breed of chick no additional growth response was obtained from cystine supplementation of a diet containing adequate methionine.

Experiments using L-methionine  $S^{35}$  to study the utilization of methionine for cystine synthesis showed a breed difference in the amount of methionine converted to cystine. More of the radioactive methionine was converted to cystine  $S^{35}$  by Single

Comb White Leghorn chicks than by the Black Australorp chicks. The cystine  $S^{35}$  isolated from the carcass hydrolysate of Single Comb White Leghorn chicks had a higher specific activity than that isolated from the carcass hydrolysate of the Black Australorp chicks. Because of the good growth rate obtained with methionine supplementation of a low-cystine content basal diet, the conversion of methionine to cystine appears to be adequate to meet the physiological needs for growth in both breeds studied.

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