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EGG PROTEIN MIGRATION

Protein Distribution in Fresh and Stored Shell Eggs from Hens Fed Crude Cottonseed Oil

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Hens fed rations containing 2.5% crude cottonseed oil produce eggs that develop pink whites and large salmon-colored yolks after 6 months or more of cold storage. Protein migrates from the white to the yolk. The composition of the migrating protein was calculated from changes in protein distribution in whites and yolks of fresh and stored shell eggs as determined by paper electrophoresis. The migrating protein contained ovalbumin, conalbumin, and lysozyme, but no ovomucoid or ovoglobulin. Livetin migrated from yolk to white. The lipovitellenin band from 6-month-old eggs moved almost three times as far as that from fresh eggs during electrophoresis. Part of the lipovitellin was converted to a protein which behaved similarly to lipovitellenin under the conditions used for electrophoresis.

HENS FED RATIONS containing crude cottonseed oil produce eggs that develop viscous pink whites and large salmon-colored yolks after 6 months or more of cold storage (*11*). Migration of protein and water from the white to the yolk also occurs and is probably caused by a weakened vitellin membrane (*1*). Conalbumin migrates from white to yolk and iron from yolk to white (*10*). Evans, Bandemer, Davidson, and Schaible (*7*) reviewed the literature on pink white and salmon yolk discoloration of eggs.

Evans *et al.* (*6*) calculated the composition of the protein that migrated from the white to the yolk during storage of shell eggs from hens fed crude cottonseed oil, on the basis of the weight of protein that migrated, the methionine, cystine, and serine contents of the white and yolk proteins of fresh and 6-month-old eggs, and literature values (*9*) for the contents of these amino acids in the individual egg white proteins.

A more direct determination was desired of the amounts of different egg white proteins that migrate from the white to the yolk during storage of eggs produced by hens fed crude cottonseed oil. Evans and Bandemer developed procedures for the quantitative determination of the different proteins in egg white (*4*) and egg yolk (*5*). These methods have been used to determine the protein composition of whites and yolks in fresh and stored eggs; and these data were then used in calculations.

Experimental

Twelve pullets were housed in laying cages and fed a ration containing 2.5% of crude cottonseed oil. The basal ration to which the oil was added consisted of ground corn 34.5%, ground oats 20.0%, wheat bran 15.0%, flour middlings 10.0%, dehydrated alfalfa 3.0%, meat scraps 3.0%, dried milk 2.0%, fish meal 2.5%, soybean oil meal 2.5%, ground oyster shell flour 5.0%, steamed bone meal 1.5%, salt 0.6%, and fish oil (2000 units of vitamin A and 400 units vitamin D) 0.4%. Chicks hatched in April 1956 were placed in the laying cages as pullets at about the time they started to lay in September. Eggs were gathered twice daily, marked with pullet number and date, and placed in cold storage at 0° C. or used within three days as fresh eggs for protein separations.

Eggs from each of 11 pullets were studied fresh and after 6 months of cold storage. A sample consisted of two consecutive eggs from a pullet, and three samples (or six eggs) were used from each pullet for each period. Whites of two eggs carefully separated from the yolks were combined, weighed, mixed briefly in the Waring Blendor, and stored in the refrigerator until used. The two yolks were combined, weighed, mixed with an equal weight of 10% sodium chloride solution, and stored in the refrigerator until used. Eggs were saved for the fresh egg samples on Friday, Saturday, and Sunday of each week. Two eggs from each pullet were then used on Monday morning.

Protein distributions in the whites and yolks were determined by the procedures of Evans and Bandemer (*4*, *5*). Duplicate samples of egg whites from four pullets were used for electrophoresis on Monday afternoon and from another four on Tuesday afternoon. Yolk sam-

ples were similarly run in the electrophoresis apparatus over Wednesday and Thursday nights. Paper strips were dyed for 16 hours the night following the electrophoresis separation. The distance traveled by each protein fraction was determined by scanning the filter paper strips in the Analytrol recording scanner and integrator and measuring the distance of the peaks from the origin.

The nitrogen content of the whites and yolks was determined by the Kjeldahl-Wilforth-Gunning procedure (*2*), and the crude protein was determined by multiplying the determined nitrogen by 6.25. To determine the weight of egg white protein in each egg, the egg white weight was multiplied by per cent crude protein divided by 100. The weight of egg yolk protein was determined in a similar manner.

The eggs used were produced between December 1, 1956, and January 22, 1957. The two eggs stored for 6 months before using were the next two eggs produced by a pullet after the two used fresh. Bandemer, Evans, and Davidson (*3*) observed that yolks, but not whites, of eggs from a particular pullet increased in weight for the first 6 or 7 months of production. It was believed that if eggs produced close together were used, differences in yolk size would be negligible. However, total protein weights of the stored eggs (produced later than the fresh ones) were greater than those of the fresh eggs. Corrections were therefore necessary. The corrected average yolk protein weight for the 6-month-old eggs was adjusted so that the total yolk plus white protein weight of the 6-month-old eggs was equal to that for the fresh eggs.

Results and Discussion

Average weights of the egg white and yolk proteins are given in Table I. Egg white protein decreased and egg yolk protein increased considerably in weight during 6 months of cold storage, demonstrating migration of protein from the white to the yolk. Earlier work (*6*) has shown a slight loss in yolk protein rather than a gain during storage. This discrepancy could be due to a difference in the protein content of the fresh and stored eggs or to differences in handling when separating white from yolk.

Ovalbumin is well separated from the other white and yolk proteins during electrophoresis (*4*) (Figure 1). Fresh eggs contained an average of 2.33 grams of ovalbumin, and 6-month-old eggs contained 2.30 grams using the uncorrected values for protein in egg white and yolk; its distribution was unchanged in normal eggs during storage (*8*), and the same was anticipated in eggs from hens fed cottonseed oil. If the 0.12 gram of excess protein in the stored eggs

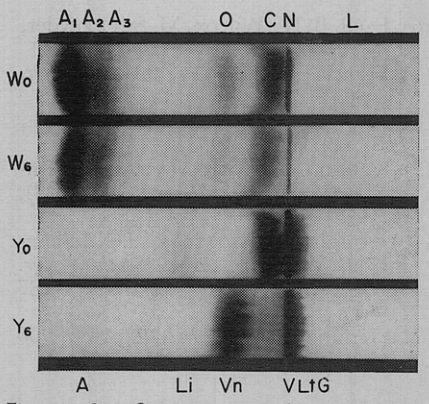

Figure 1. Separation of egg white proteins and separation of egg yolk proteins by filter paper electrophoresis

Spinco ridgepole paper electrophoresis cell filled with 0.05M barbital buffer of pH 8.6 and current of 5 ma. for 16 hours. Filter paper strips stained with bromophenol blue.

Wo. White from fresh eggs
W6. White from 6-month-old eggs
Yo. Yolk from fresh eggs
Y6. Yolk from 6-month-old eggs

Protein bands.

A_1. Ovalbumin A_1
A_2. Ovalbumin A_2
A_3. Ovalbumin A_3
O. Ovomucoid plus ovoglobulin
C. Conalbumin
L. Lysozyme
Li. Livetin
V. Lipovitellin
Vn. Lipovitellenin
Lf. Lipoprotein F
G. Protein G

came from a larger amount of white protein in the 6-month-old eggs than in the fresh ones, this extra protein would contain 0.08 gram of ovalbumin, and the 6-month-old eggs would contain 2.40 grams rather than the 2.30 grams of ovalbumin. The apparent gain in weight is explained on the basis that the yolks of the eggs when placed in storage were larger than those of the eggs used fresh.

The weight of yolk protein of the 6-month-old eggs was corrected so that the total protein weights of fresh and 6-month-old eggs were equal. There was an average migration of 0.18 gram of protein from the white to the yolk using the corrected yolk protein weights of Table I.

Differences in the distance traveled during paper electrophoresis by the egg white proteins from fresh and from stored shell eggs were not large (Table II), but the proteins of stored eggs appeared to travel slightly farther than those from fresh eggs. Similar results were obtained with normal eggs (*8*).

Speeds of migration during paper electrophoresis of the egg yolk proteins in fresh eggs (Table II) were similar to those observed earlier from normal eggs (*8*), but some differences in migration of yolk proteins in stored eggs were observed. The main change was in the speed of migration of lipovitellenin (Figure 1). In 6-month-old "cottonseed" eggs (eggs from pullets fed crude cottonseed oil) it migrated nearly three

Table I. Weight of Egg Proteins (N × 6.25) in Fresh and Stored Shell Eggs from Pullets Fed Crude Cottonseed Oil

	Av. Weight of Protein in One Egg, Grams	
	Fresh eggs	*6-mo.-old eggs*
White	3.54	3.36
Yolk	2.35	2.65
Whole egg	5.89	6.01
Yolk (corr.)[a]	2.35	2.53
Whole egg (corr.)[a]	5.89	5.89

[a] Calculation of corrected values in text.

Table II. Distances in Millimeters Traveled by Proteins during Paper Electrophoresis

	Fresh Eggs	*6-Mo.-Old Eggs*
	Egg White	
Ovalbumin A_1	−87.4	−87.9
Ovalbumin A_2	−75.8	−76.0
Ovalbumin A_3	−65.2	−66.2
Ovomucoid + ovoglobulin	−25.3	−26.3
Conalbumin	− 8.0	− 7.8
Lysozyme	36.3	32.2
	Egg Yolk	
Ovalbumin	−85.1	−82.8
Livetin	−43.8	−47.6
Lipovitellenin	− 7.6	−21.1
Lipoprotein F	3.7	3.3
Protein G	11.0	14.6

times as far as lipovitellenin in fresh eggs, but in 12-month-old normal eggs it migrated nearly twice as far (*8*). The change during storage occurs more rapidly in eggs from pullets fed cottonseed oil than from pullets fed a normal ration.

Changes in the percentage composition of egg white proteins during 6 months of cold storage of eggs from pullets fed crude cotonseed oil are given in Table III. They did not occur in normal eggs (*8*). Changes in conalbumin concentration were similar in normal and cottonseed eggs, probably caused by the selective migration of ovalbumin and lysozyme from the white to the yolk of eggs from pullets fed cottonseed oil, because there was no similar migration in normal eggs.

Changes in percentage composition of egg yolk proteins were more complex than those of white proteins (Table III). Ovalbumin concentration in the yolk nearly trebled because of transfer of ovalbumin from white to yolk. Livetin and protein C concentrations decreased in both normal (*8*) and cottonseed eggs, the latter showing a greater change. It was caused in both by lipovitellenin migrating during paper electrophoresis into the protein C region thus causing inclusion of part of the protein C with the lipovitellenin in the stored eggs (Figure 1). Lipovitellenin

Table III. Percentages of Different Proteins from Hens Fed Crude Cottonseed Oil

	Fresh Eggs	6-Mo.-Old Eggs
	Egg White	
Ovalbumin		
A_1	41.5	39.5
A_2	17.7	17.6
A_3	4.8	4.9
Total	64.0	62.0
Ovomucoid + ovoglobulin	10.9	13.9
Conalbumin	18.4	18.0
Nonmobile protein	2.3	3.0
Lysozyme	4.4	3.1
Total	100.0	100.0
	Egg Yolk	
Ovalbumin	2.8	7.5
Livetin	5.7	3.6
Protein C	3.2	1.6
Lipovitellenin	37.5	45.7
Lipovitellin	38.0	30.8
Lipoprotein F	11.4	9.0
Protein G	1.3	1.8
Total	99.9	100.0

Table IV. Transfer of Egg White Proteins to Yolks in Eggs from Hens Fed Crude Cottonseed Oil

All eggs, av.	Total Protein	Ovalbumin	Ovomucoid + Globulin	Conalbumin	Nonmobile	Lysozyme
Fresh eggs, grams	3.53	2.26	0.39	0.65	0.08	0.15
6-mo.-old eggs, grams	3.36	2.09	0.46	0.60	0.10	0.11
Protein transferred, grams	0.17	0.17	−0.07	0.05	−0.02	0.04
%		65.4		19.2		15.4
Eggs with pink whites						
Fresh eggs, grams	3.61	2.31	0.40	0.65	0.09	0.16
6-mo.-old eggs, grams	3.31	2.03	0.50	0.58	0.10	0.09
Protein transferred, grams	0.30	0.28	−0.10	0.07	−0.01	0.07
%		66.6		16.7		16.7

from stored cottonseed eggs migrated much farther than that from normal stored eggs; hence the protein C decreased more. Both normal (*8*) and cottonseed eggs (Table V) displayed changes in lipovitellenin and lipoprotein F contents during storage with greater changes in cottonseed eggs.

Weights of individual egg white proteins transferred to the yolk were determined by calculating the decrease in grams of each in the egg white protein for each pullet during storage of the eggs from that pullet. All calculations were made on an individual pullet basis, but average values are presented in Table IV. Altogether 32 samples were studied from the 11 pullets. Only 10 samples showed the typical pink white and large salmon yolk discoloration. A greater percentage of the cottonseed eggs are usually discolored after 6 months of cold storage than reported herein.

Ovalbumin and conalbumin migrated in about the same proportion as they occurred in fresh white protein. Ovalbumin has a molecular weight of 45,000, conalbumin 87,000, ovomucoid 27,000, and the molecular weight of lysozyme is 14,800 (*12*). The low molecular weight of lysozyme accounts for its greater quantity and mobility through the weakened vitellin membrane.

Evans, Bandemer, Davidson, Bauer, and Butts (*6*) calculated that the protein which migrated from white to yolk during storage of eggs from hens fed crude cottonseed oil contained 79% ovalbumin, 3% conalbumin, 15% ovomucoid, and 3% ovoglobulin. The present data are more reliable because direct measurements were made. Values obtained from the literature may not be the same as the actual values for the eggs used, because eggs from different hens differ in protein distribution in both the white and yolk and in the proportion of the two in the egg (*8*). Schaible and Bandemer (*10*) isolated conalbumin from the yolks of salmon-colored eggs; 18% conalbumin in the migrating protein (Table IV) appears more probable than 3% (*6*).

In the method used to obtain the corrected values given in Table V for 6-month-old eggs, the total weight of egg yolk protein was adjusted so that the weight of yolk protein plus white protein was equal to that of the fresh eggs. It was necessary to subtract 0.12 gram from the total yolk protein weight obtained for 6-month-old eggs to obtain the corrected weight. Individual yolk protein weights were also corrected by subtracting the values given on line 4 from the observed values. The values of line 4 were obtained by multiplying 0.12 by percentage of each protein given in Table III for fresh eggs divided by 100. Fresh egg percentages were used, because corrections were made on the basis of protein distribution in fresh yolks and not in the yolks after the white protein had migrated to the yolk. Corrected values are given on line 5. Similar corrections were made for the 10 samples with pink white discoloration, except that larger corrections were needed, because the 6-month-old eggs contained 0.23 gram more protein than the fresh ones.

The increases in ovalbumin and protein G of the yolk are related to the transfer of ovalbumin and lysozyme from white to yolk. The increase in lipovitellenin resulted from the transfer of conalbumin from white to yolk, from the overlapping of its electrophoresis band over the protein C band (they are grouped together), and from its change to a protein that migrated on the paper electrophoresis strip in the same way as lipovitellenin. The relationship between lipovitellin and lipovitellenin in stored cottonseed eggs is not known, but they seem to be related to loosely bound lipide that is easily removed by ether extraction (*5*).

A schematic representation of the migration during storage of the various proteins between white and yolk of eggs from hens fed crude cottonseed oil is given in Figure 2. The line between the white and the yolk represents the vitellin membrane. The curved arrows show that part of a protein was probably included and measured with another.

The migration diagram for 10 eggs

Table V. Transfer of Egg Yolk Proteins in Eggs from Hens Fed Crude Cottonseed Oil

Av. all eggs, grams	Total Protein	Ovalbumin	Livetin	Lipovitellenin	Lipovitellin	Lipoprotein F	Protein G
Fresh eggs	2.36	0.07	0.21	0.88	0.90	0.27	0.30
6-mo.-old eggs	2.65	0.21	0.14	1.21	0.81	0.23	0.05
Yolk correction[a]	0.12	0.00	0.01	0.05	0.05	0.01	0.00
Corrected 6-mo.-old eggs[a]	2.53	0.21	0.13	1.16	0.76	0.22	0.05
Protein transferred	0.17	0.14	−0.08	0.28	−0.14	−0.05	0.02
Eggs with pink whites, grams							
Fresh eggs	2.39	0.06	0.22	0.89	0.91	0.27	0.03
6-mo.-old eggs	2.92	0.35	0.12	1.35	0.84	0.20	0.06
Yolk correction[a]	0.23	0.01	0.02	0.09	0.09	0.03	0.00
Corrected 6-mo.-old eggs[a]	2.69	0.34	0.10	1.26	0.75	0.17	0.06
Protein transferred	0.30	0.28	−0.12	0.37	−0.16	−0.10	0.03

[a] Calculation of corrected values in text.

with pink whites and large salmon-colored yolks (Figure 3) differs from the average of all the eggs in the weight of migrated protein. A questionable feature was that more lipovitellin (0.29 gram) was found with lipovitellenin in these 10 eggs than in the average of all eggs.

Two distinct changes occurred in the proteins of eggs from hens fed cottonseed oil when the eggs were stored for 6 months at 0° C. A transfer of white proteins except ovomucoid and ovoglobulin from the white into the yolk was accompanied by movement of livetin from yolk to white. If ovomucoid and ovoglobulin were transferred in the same proportion as the other egg white proteins, about 0.03 gram of the two would have migrated to the yolk, and 0.10 gram or nearly half of the livetin would have been transferred from yolk to white. The second change was a partial conversion of the lipovitellin to a protein that behaved like lipovitellenin under the conditions of filter paper electrophoresis used.

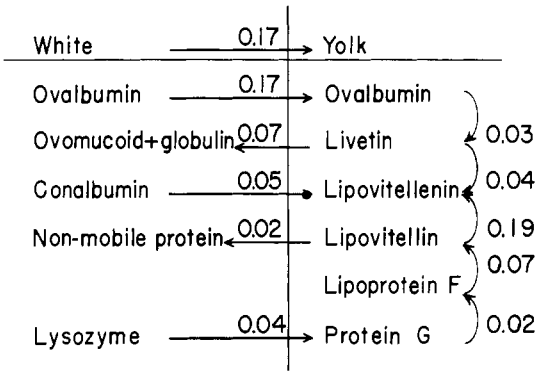

Figure 2. Migration of egg proteins between white and yolk of eggs from hens fed cottonseed oil

Average values for all eggs used as calculated in Tables IV and V. Protein transferred given in grams

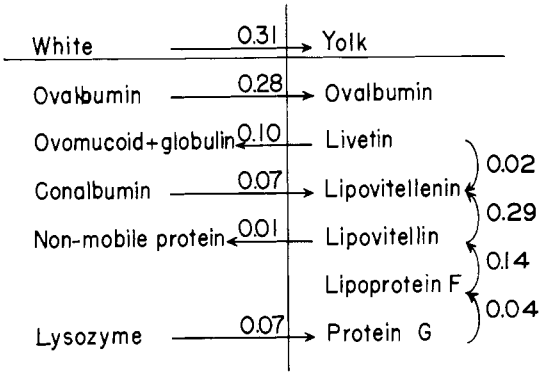

Figure 3. Migration of egg proteins between white and yolk of eggs with pink whites

Average values for 10 eggs which developed pink whites and large salman colored yolks during storage as calculated in Tables IV and V were used. Eggs produced by hens fed crude cottonseed oil. Protein transferred given in grams

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ALCOHOL IN FEEDS

Effect of Ethyl Alcohol and Starch on Digestibility of Nutrients and on Nitrogen Retention at Two Levels of Urea Feeding

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This work is based on a study of whether ethyl alcohol is more effective than starch in increasing the utilization of urea nitrogen on high molasses rations for cattle. Nitrogen retention by steers was increased by addition of ethyl alcohol to a mixture of molasses and urea to about the same extent as by an equal number of calories from starch. Both were much greater than on the basal ration without either starch or ethyl alcohol. Better results, however, were obtained with soybean meal and corn. Ethyl alcohol would appear to be an effective supplement to a ration of poor quality, low-protein roughage, liberal molasses and urea, and when corn or other cereal grains are not fed.

FEW controlled experiments on the use of ethyl alcohol in ruminant nutrition have been reported. Thomson (*13*) described the use of pot ale (an alcoholic beverage) in the nutrition of nursing mothers and high-producing dairy cows. In Italy and France surpluses of cheap wine were sometimes fed to farm animals (*10*). Currently ethyl alcohol is added to a liquid cattle feed consisting of molasses, urea, minerals, and vitamins. In trials involving this liquid feed (*5*, *8*), a favorable effect of ethyl alcohol was observed when it was added to a basal diet of corn cobs, molasses, and urea. Richardson, Smith, Koch, and Cox (*11*) in Kansas have studied the value of ethyl alcohol in molasses mixtures for beef cattle. Steers fed a high roughage ration during the winter consumed more feed on a molasses mixture containing ethyl alcohol, but gained less weight than similar steers fed soybean oil meal and sorghum grain. In the fattening period that followed, the steers receiving alcohol gained faster than the controls, but for the total feeding period there was practically no difference. Other steers fed the same molasses mixture without ethyl alcohol gained as rapidly during both the wintering and the fattening periods as those receiving alcohol. Starch or starchy con-