FOLIC ACID
In Fresh and Stored Shell Eggs

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The folic acid content of fresh shell eggs and of eggs stored for 3, 6, and 12 months was determined to see if losses of folic acid occur during the storage of shell eggs under ordinary storage conditions. Most of the folic acid of fresh eggs was in the yolk, and only a very small amount in the white. One fresh egg contained on the average 4.59γ of folic acid, with 0.51γ in the white and 4.09γ in the yolk. There was a loss of folic acid from the egg yolk and some transfer of folic acid from the yolk to the white during 12 months of cold storage. Twelve-month-old eggs contained 3.37γ of folic acid with 0.62γ in the white and 2.75γ in the yolk. Eggs lost 16% of their folic acid during 6 months of cold storage and 27% during 12 months of cold storage. The smaller amount of folic acid in stored eggs than in fresh eggs indicates that stored eggs not only do not look as good as fresh eggs, but they also have a lower nutritive value.

Several million cases of shell eggs are stored in this country during periods of high production and low costs, to be used later when production is lower and prices are higher. Any changes in nutritional quality of stored eggs are important, but very little is known about the relative nutritive values of fresh and stored shell eggs. The poultry industry has been more concerned with physical changes in eggs which affect their appearance, such as firmness of the egg white and strength of the egg membranes.

Studies of losses of nutrients from shell eggs during cold storage were started at Michigan State College several years ago. Results published to date indicate losses of protein (*10*), riboflavin (*7*), niacin (*5*), vitamin B_6 (*6*), and pantothenic acid (*11*), but not of choline (*8*) nor biotin (*9*). The purpose of the present experiment was to determine whether folic acid is lost from stored shell eggs.

Experimental Work

The eggs used in this experiment were obtained from White Leghorn hens which were kept in individual laying cages and fed a ration composed 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 (400 AOAC chick units of vitamin D and 3000 USP units of vitamin A per gram) 0.4%. Eggs from ten hens were used. Eggs were gathered daily during March 1951, marked with hen number and date, and placed in cold storage at 0° C. or assayed for folic acid. Fifteen eggs were saved from each hen. Three were used for immediate folic acid assay, and twelve were stored in a carton holding one dozen eggs. The ten cartons of eggs were placed in a fiber egg case and stored at 0° C. in a walk-in refrigerator. Eggs were removed for assay after 3, 6, and 12 months of storage. Three eggs comprised a sample from each hen for each period.

Raw eggs were used rather than boiled ones such as were used by Sunde *et al.* (*15*), because boiling destroyed either most of the folic acid in the eggs or some enzyme present in the raw egg yolk and white that supplemented takadiastase in the liberation of bound folic acid in a form available for *S. faecalis* R. Eggs from eight hens were analyzed raw and after boiling. The yolks from the raw eggs contained 4.12γ of folic acid per yolk and those from the eggs boiled for 20 minutes contained 0.53γ. Little or no folic acid was found in the egg white of boiled eggs. Hanning and Mitts (*12*) observed a 20 to 50% loss of folic acid in cooked eggs.

The raw eggs were broken out and the whites and the yolks were separated. The three yolks from each hen's eggs were put together, as were the three whites. Each group of yolks or whites was weighed and was homogenized in a Waring Blendor with two parts by weight of distilled water. Five milliliters of each homogenate were weighed and transferred into a 50-ml. Erlenmeyer flask, and 10 ml. of McIlvain's disodium phosphate–citric acid buffer of pH 4.5 containing 400 mg. of takadiastase were added. Each sample from this point on was incubated and prepared for assay exactly as described by Sunde *et al.* (*15*), and all were assayed for folic acid by the procedure of Teply and Elvehjem (*16*) using *S. faecalis* R as the assay organism and titrating the acid formed.

The folic acid values reported are not necessarily measures of "total" folic acid, because no folic acid conjugase preparation other than that present in the eggs was used. However, Sunde *et al.* (*15*) obtained no further liberation of folic acid by hog kidney and chick pancreas preparations over that obtained with the takadiastase alone.

Results and Discussion

The results calculated as micrograms of folic acid per egg are given in Tables I to III. From the data obtained for each group of eggs the folic acid concentration per gram of white or yolk was calculated. The micrograms of folic acid per egg were calculated by multiplying the micrograms of folic acid per gram by the average weight per egg of the three egg whites, yolks, or whole egg contents used to prepare the egg homogenate.

Data are presented on an individual hen basis. A hen fed a fixed diet will produce eggs that are uniform in composition, but eggs from different hens may differ in composition. Three eggs from each hen were used for each assay to give plenty of sample and to average out any small differences among individual eggs from the hen. The large differences among hens in folic acid content per egg are apparent from a glance at Table I. Fresh eggs from hen 6632 contained 3.92γ of folic acid per egg and from hen 6623, 5.82γ.

The average fresh whole egg contained 4.59γ of folic acid (Table I). Of this 0.51γ was in the white (Table II), and 4.09γ was in the yolk. Egg yolk contained nearly eight times as much folic acid as the white.

Average values in millimicrograms of folic acid per gram of egg are presented in Table IV. The average fresh whole egg contained 94 mγ of folic acid per gram (Table IV). Cheldelin and Williams (*2*) reported a high value in 1942 of 860 mγ per gram of whole egg. No other investigators have reported such a high value, probably because pure folic acid was not available at that time, and a folic acid concentrate was used as the standard. Schweigert *et al.* (*13*) found 80 to 170 mγ of folic acid per gram of whole egg and Hanning and Mitts (*12*) found 80 to 270 mγ. Individual values between 77 and 126 mγ per gram were obtained in this study.

There was an average of 16 mγ of folic acid per gram of white (Table IV). Charkey *et al.* (*1*) found 15 mγ per gram.

The average egg yolk contained 232 mγ of folic acid per gram with a high of 276 mγ per gram and a low of 182 mγ per gram (Table IV). The above values compare favorably with the 226 mγ per gram of Charkey *et al.* (*1*), the 282 mγ per gram of Sreenivasan *et al.* (*14*), the 162 mγ per gram of Sunde *et al.* (*15*), and the 318 mγ per gram of Waibel *et al.* (*17*), but they are lower than the 810 and 750 to 800 mγ per gram observed by Couch *et al.* (*3*, *4*).

The differences between the folic acid content of fresh eggs observed in this study and those in others might very well be caused by differences in the folic acid content of the diets fed, because the folic acid content of the egg is dependent upon the folic acid content of the diet. Different values would have been obtained if diets of higher or lower folic acid content had been fed. Couch and German (*3*) found about 50 mγ of folic acid per gram of yolk in eggs from hens fed diets containing 20γ of folic acid per kilogram of diet and about 850 mγ per gram in eggs from hens fed diets containing 820γ per kilogram. Sunde *et al.* (*15*) fed folic acid–deficient diets to which 0.25 to 3.0 mg. of pteroyl-

Table I. Changes in Folic Acid Content of Whole Eggs during Storage of Shell Eggs

Hen No.	Fresh Eggs, γ	3-Month-Old Eggs, γ	6-Month-Old Eggs, γ	12-Month-Old Eggs, γ
6602	4.48	4.51	2.39	2.57
6603	4.01	3.78	3.06	2.16
6604	3.95	5.26	5.17	2.83
6611	4.68	4.58	3.89	4.09
6614	4.58	4.43	3.67	3.29
6615	4.88	4.28	3.56	3.46
6616	4.07	4.41	4.10	3.18
6622	5.53	5.37	4.77	4.45
6623	5.82	5.69	4.37	5.48
6632	3.92	3.54	3.45	2.18
Av.	4.59	4.59	3.84	3.37

Table II. Changes in Folic Acid Content of Egg Whites during Storage of Shell Eggs

Hen. No.	Fresh Eggs, γ	3-Month-Old Eggs, γ	6-Month-Old Eggs, γ	12-Month-Old Eggs, γ
6602	0.12	0.41	0.29	0.29
6603	0.37	0.45	0.33	0.29
6604	0.36	1.30	0.96	0.92
6611	0.41	0.59	0.65	1.00
6614	0.67	0.78	0.61	0.67
6615	0.42	0.33	0.35	0.43
6616	0.44	0.49	0.42	0.44
6622	0.77	0.56	0.40	0.48
6623	0.72	1.23	0.93	1.24
6632	0.78	0.38	0.35	0.41
Av.	0.51	0.65	0.53	0.62

Table III. Changes in Folic Acid Content of Egg Yolks during Storage of Shell Eggs

Hen No.	Fresh Eggs, γ	3-Month-Old Eggs, γ	6-Month-Old Eggs, γ	12-Month-Old Eggs, γ
6602	4.35	4.10	2.10	2.28
6603	3.64	3.33	2.73	1.88
6604	3.59	3.96	4.21	1.90
6611	4.27	3.99	3.24	3.08
6614	3.91	3.65	3.06	2.61
6615	4.46	3.95	3.21	3.03
6616	3.63	3.92	3.68	2.74
6622	4.76	4.81	4.37	3.97
6623	5.10	4.46	3.44	4.24
6632	3.15	3.16	3.10	1.77
Av.	4.09	3.93	3.32	2.75

Table IV. Changes in Folic Acid Concentration in Eggs during Storage of Shell Eggs

(Average values for eggs from ten hens)

	Fresh Eggs, Mγ/Gram	3-Month-Old Eggs, Mγ/Gram	6-Month-Old Eggs, Mγ/Gram	12-Month-Old Eggs, Mγ/Gram
Whole eggs	94	93	80	74
Egg white	16	22	19	24
Egg yolk	232	221	167	142

glutamic acid were added per kilogram of diet. After receiving the diets for 10 weeks, the hens fed the low level produced eggs containing 23 mγ of folic acid per gram of yolk, and those the high level 242 mγ per gram.

No loss of folic acid occurred during the first 3 months of cold storage of shell eggs. During the next 3 months 16.4% of the folic acid was lost, and 26.6% was lost in 12 months of total storage (Table I). The loss was all from the egg yolk. The folic acid content of the white increased slightly (Table II). The 21.6% increase of total folic acid in egg white and the 50% increase in micrograms of folic acid per gram of egg white (Table IV) is not unexpected. A much larger increase more nearly to equalize the concentration of folic acid in the yolk and white would be expected from the work of Evans *et al.* (*11*), who found that the pantothenic acid concentration in egg white more than doubled and that in the yolk decreased so as to approach a more equal distribution of pantothenic acid between white and yolk. Apparently pantothenic acid moves through the vitelline membrane more freely than folic acid.

Total folic acid in the egg yolk decreased by 32.8% and the folic acid concentration by 38.8% during 12 months of storage (Tables III and IV). Part of the folic acid was in some way lost and the rest migrated to the white. The method of folic acid loss is unknown and will not be speculated on at this time. The folic acid concentration in the white and the yolk changed to a greater extent than did the total folic acid content of these parts of the egg. Evans and Davidson (*8*) observed a migration of water from the white to the yolk of stored shell eggs and also an additional loss of water from the whole egg, probably from the white through the shell membrane. The loss of water from the white combined with the transfer of folic acid from the yolk to the white can explain why a 21.6% increase in total folic acid in the white would cause a 50% increase in folic acid concentration. And the transfer of some water from the white to the yolk at the same time that folic acid migrates from the yolk to the white explains why the folic acid concentration of the yolk can decrease by 38.8% when the total folic acid decrease is but 32.8%.

Total folic acid in the whole egg decreased by 26.6%, and the folic acid concentration decreased by 21.3% during 12 months of cold storage. The reason for the smaller percentage decrease in the folic acid concentration than in the total folic acid of whole stored eggs is that the loss in folic acid is accompanied by a loss of water that tends to increase the folic acid concentration and partially counteract the folic acid loss.

Summary

Eggs from ten White Leghorn hens kept in laying cages and fed a diet of constant composition were used in an experiment to study changes in the folic acid content of fresh and stored shell eggs. The eggs were studied on an individual hen basis. Whites and yolks of fresh eggs and of eggs stored for 3, 6, or 12 months at 0° C. were assayed for folic acid with *S. faecalis* R. Fresh eggs contained on the average 4.59γ (94 mγ per gram) of folic acid with 0.51γ (16 mγ per gram) in the white and 4.09γ (232 mγ per gram) in the yolk. There was a loss of folic acid from the egg yolk and some transfer of folic acid from the yolk to the white during 12 months of cold storage. Twelve-month-old eggs contained 3.37γ (74 mγ per gram) of folic acid with 0.62γ (24 mγ per gram) in the white and 2.75γ (142 mγ per gram) in the yolk.

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NUTRIENT COMPOSITION

Corn in the United States

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THE COMMITTEE ON FEED COMPOSITION of the National Research Council, Agricultural Board, Division of Biology and Agriculture, was formed in August 1946 at the suggestion of the Committee on Animal Nutrition to compile complete and accurate tables on the composition of all feeding stuffs (*3–5*).

Because corn is used in livestock feeding to a greater extent than any other single grain, a survey of the 1946 crop was undertaken for the purpose of evaluating the effect of hybrid corn varieties on the composition, particularly the protein content. The survey also had for its purpose the evaluation of all the readily measurable nutritive constituents in this important feed crop on a nation-wide basis that would be representative of that crop as marketed and fed to animals.

The success of the first year's survey encouraged the committee to repeat it in 1947, in order to obtain the benefit of two seasons. By chance, the two years were markedly different in character, 1946 being a normal year and 1947 a "soft corn" year.

Plan of the Surveys

The extent of the surveys was limited