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THE EFFECT OF COTTONSEED DIET ON THE CHEMICAL COMPOSITION OF THE EGG

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Eggs have become one of the major classes of foods in all countries. In the United States we eat 210 eggs per capita per year. They contain

every constituent needed by man for growth and for health. Because of the seasonal fluctuation in production and a corresponding variation in price, enormous quantities of eggs, estimated at 13 per cent of all eggs produced annually, are placed in storage each year. For the year 1930 this number would be near the three billion mark.

Of this number there are always a considerable number lost because of spoilage brought about by the presence of bacteria and other factors. It has been noticed in many cases that egg yolk changes color on storage. This was once thought to be caused by hens eating an abnormally large quantity of green feed in the spring of the year. In recent years it has been observed that the yolks of many of the eggs placed in storage in the southern states developed a peculiar color which has been quite definitely proved to be due to the use of large quantities of cottonseed meal in the diet of the hen. These peculiar discolorations have been described in detail by Thompson (1).

An examination of eggs such as he describes disclosed the fact that the eggs were not bad in that they were not decomposed, had not become watery, had not produced foul odors, and were entirely edible. The consumers, however, object to such eggs because of the so-called olive-colored yolks, and the packers must necessarily accept a considerable depreciation on such sales. Such a loss discounts the value of southern eggs, and if it were possible to discover the cause of such discoloration and thereby prevent the same, a tremendous saving of money would be brought about, not only for the packers but for the producers as well. It occurred to us that possibly these discolorations might be due to a different chemical composition of the egg produced by the use of rations high in cottonseed meal.

For this reason, endeavor has been made to make a chemical analysis of various lots of eggs which have been produced simultaneously under comparable conditions by hens kept in the poultry department of the Oklahoma A. and M. College. Comparisons were made of the composition of the eggs from hens receiving low protein diets with that of eggs from hens receiving a diet similar in all other respects except that the protein level was greatly increased. Similar studies were also made of the eggs laid by hens which had received larger amounts of the meal. Both fresh and stored eggs of the above mentioned lots were analyzed. The results obtained in this investigation are the average values of many determinations, and no results have been used which were obtained by only one determination. A total of more than 2,000 determinations were made in the investigation. The eggs from hens that received ordinary feed will be designated as fresh non-cottonseed eggs, those from hens that received a high percentage of cottonseed meal will be designated as cottonseed eggs, and those from hens that received high or low percentages of proteins will be designated as high or low protein eggs.

The methods used in the chemical analyses were ones previously proved and applied by various workers in similar lines of work. The analyses include proteins, ether soluble extracts of yolks, moisture ammonia, pH value, lipoids, P_2O_5 of fats, iodine number, coefficient of spread, and the nitrogen distribution. When an analysis was started, the egg was first weighed, broken, and the white, yolk, and shell weighed separately.

The ammonical nitrogen was determined by a modification of the aeration method used by Hendrickson and Swan (2). For the lipoid determinations a ten gram sample was weighed and analyzed by the accepted A. O. A. C. method. The iodine number was found by the Hanus method (3), the coefficient of spread by a method devised by Sharp and

Powell (4). In this procedure the width and height of the yolk were measured and the height divided by the width. The distribution of nitrogen was determined by a method used by Plimmer and Rosedale (5). The method used for determining the pH of the whites and yolks is one worked out in this station and similar to that later published by Sharp (6). The whites and yolks were separated and the pH run on each separately. The yolks were diluted with an equal volume of water and placed in the cell and shaken for about three minutes to establish an equilibrium. The potential of this cell was then measured against that of a .1 normal KCl Calomel half cell by the use of a standard type K Potentiometer. The pH value was then calculated by a standard equation, corrections being made for temperature and pressure.

EXPERIMENTAL

The eggs used for the analyses were obtained from the poultry department of the A. and M. College. These eggs were laid by White Leghorn hens of the same age, same strain, and which had been given the same kind of feed with the exception of the amount of cottonseed meal and protein which was fed to certain pens of the hens. For non-cottonseed eggs the usual college laying mash was fed. For the cottonseed eggs, a like amount of the protein supplements was replaced with cottonseed meal. The hens from which we obtained eggs known as high protein eggs received a ration consisting of the A. and M. ration supplemented with meat scraps and milk. A scratch feed of oats, corn and kafir was given them once a day. The hens producing eggs known as low protein were fed a mixture containing wheat bran, barley, kafir, and corn in equal parts. The protein of this mixture is very low compared to that of the high protein lot. All the hens received water *ad libitum*, oyster shell and grit. The eggs were collected daily, and some of them were kept in a commercial storage plant six months before analysis.

WEIGHTS OF WHITES, YOLKS, AND SHELLS OF VARIOUS CLASSES OF EGGS

It is interesting to note the relation of the weight of the white to the weight of the yolk of the eggs that had been stored and those that were fresh. It will be seen that this ratio is much larger in the fresh eggs than in the stored ones. In the case of the fresh cottonseed eggs, the ratio is 1.820 while in the stored cottonseed eggs it is only 1.235. In the other cases the same relation holds, though not so great. This difference is due to the passage of water from the white to the yolk during the storage. Table I shows the data that were obtained, given in average values for all eggs taken.

MOISTURE CONTENT

The moisture content of the whites and yolks of the fresh and stored lots yield little information that would indicate any difference in cottonseed and non-cottonseed eggs, save that the low protein eggs have somewhat greater moisture content. The migration of water from white to yolk as suggested in the weights of whites and yolks in Table I is proved in Table II. In the case of the cottonseed egg an increase of 8 per cent in moisture is observed.

COEFFICIENT OF SPREAD

The market value of eggs is determined to a certain extent by the condition of the yolk. Consumers not only demand a good color, odor and taste, but a yolk that stands up well is desired. When eggs of poor

quality are broken the yolks flatten out. The flattening of the yolk and its decreased resistance to breaking are attributed largely to the

TABLE I

Weights of the Whites, Yolks, Shells, and Total Weights of Eggs

Type of Egg	Total Weight	Weight of White	Weight of Yolk	Weight of Shell	Ratio White Yolk
Fresh Cottonseed	49.3548	28.4755	16.0818	5.7939	1.820
Stored Cottonseed	49.0270	23.5841	19.3139	5.9005	1.235
Fresh Non-Cottonseed	45.7426	28.6413	13.7850	5.6288	2.082
Fresh High Protein	54.7125	31.8362	16.5845	6.2936	1.925
Stored High Protein	53.6939	29.9467	17.8713	5.8758	1.675
Fresh Low Protein	54.9141	31.1139	17.8715	5.9261	1.765
Stored Low Protein	53.4573	30.2307	17.3671	5.8594	1.762

passage of H_2O from the white to the yolk during storage. There results a decreased viscosity of the yolk and the yolk tends to assume a flattened shape. The changes finally proceed so far that the egg cannot be broken and the yolk kept intact. Sharp and Powell found this average index in many eggs to be 0.411. An examination of Table II will show the indices determined by us. Here it is demonstrated that the index falls for stored

TABLE II

Comparison of Chemical Analysis of Various Types of Fresh and Stored Eggs

Classes of eggs	Moisture in Whole egg Per Cent	Moisture in Yolk Per Cent	Height of Yolk Cm	Width of Yolk Cm	Coefficient of Spread	Protein of White Per Cent	Protein of Yolk Per Cent	Fat of Yolk Per Cent
Fresh Cottonseed	87.8	46.9	1.62	3.92	.412	11.45	15.69	26.67
Stored Cottonseed	86.8	54.5	1.64	4.31	.379	11.16	15.43	21.50
Fresh Non-Cottonseed	87.5	49.4	1.55	3.78	.420	11.13	16.09	24.90
Fresh High Protein	86.8	44.4	1.67	4.02	.420	11.28	14.98	28.90
Stored High Protein	85.3	52.2	1.55	4.20	.350			26.19
Fresh Low Protein	88.1	47.7	1.65	4.00	.410	11.05	15.09	27.05
Stored Low Protein	87.2	51.7	1.47	4.42	.330	13.98	12.85	26.93

eggs but the cottonseed eggs do not make a fall comparable to the non-cottonseed eggs. In this respect the cottonseed eggs correspond more nearly to fresh eggs despite the fact that the color of the yolks make them appear less desirable. There is no doubt that the yolk is more viscous in the cottonseed egg.

PROTEINS

Table II likewise shows only minor changes in the protein content of whites and yolks of each type of eggs. The changes observed are primarily the result of the water shift from white to yolk. It is interesting to find rations varying so much in protein content and producing eggs of such a similar nature. These figures indicate that if there is a change it must be found in the quality of the protein determined in a nitrogen distribution study rather than in the simple Kjeldahl determination.

FAT CONTENT OF YOLK

The fat content of the yolk shows a greater change than any single other factor. The cottonseed eggs contained more fat than the non-cottonseed eggs, while the high protein contained a larger amount than either of the others. The stored eggs all showed a decrease, that of the cottonseed eggs the greater, indicating that possibly a decomposition of the fatty material may be responsible for or associated with the liberation of a coloring material.

FREE AMMONIA CONTENT OF THE EGG

It has been observed that as an egg deteriorates in storage there is an increase in the amount of free ammonia probably due to a deamonization of the protein amino acids. Lythgoe (7) has made an extensive study of the free ammonia content of commercial stored eggs. As a result of these analyses he concludes that 95 per cent of the stored eggs give an analysis between 2.1 and 4.3 parts per 100,000 of whole egg. The average analysis of all our eggs fell within these limits, the stored non-cottonseed egg being the higher. Again no distinctive difference is exhibited, and from this determination the preference would be in favor of the cottonseed egg.

IODINE NUMBER OF FAT OF YOLKS

It is a well-known fact that the fatty acids of cottonseed oil are quite unsaturated. This factor is responsible for the unsaturated condition of hog-lard and cottonseed butter. It is equally well known by chemists that unsaturated linkage is always responsible for active chemical changes. For these reasons, it occurred to us that possibly an abnormally large amount of unsaturated fatty acids might be responsible for internal chemical changes that produce the colored yolks of cottonseed eggs. The fat of eggs was extracted by methods described for the determination of fats, redissolved in chloroform and analyzed by the Hanus method. The findings are recorded in Table III. Again we found small persistent variations but not sufficiently large to use as an explanation of the abnormal colors. Likewise, the amount of lipoid content can not be used as an explanation as the variation is small.

pH OF WHITES AND YOLKS

An interesting observation made by us in our early observations was that the pH of the white and yolk of the egg was quite different. One would not expect to find solutions of such great variation separated by a thin osmotic membrane. This condition was found accidentally while we were attempting to determine whether acidic decomposition might be greater in one type of egg than the other. This fact was originally reported by Thompson (1), and later was observed and reported by Sharp (6). These eggs on standing gradually change and the pH of white and yolk approach each other. As will be noted in Chart III, again the results of the two classes of eggs are similar.

NITROGEN DISTRIBUTION

The nitrogen distribution determination revealed a number of differences, some of them being slight, while others were more striking, as

TABLE III

Comparison of Chemical Analysis of Cottonseed and Non-Cottonseed Eggs

Classes of Eggs	Ammonia in p. p. 100,000 in Whole Egg	Iodine Number of Yolk	pH of Yolk	pH of White	Lipoids in Yolk Per Cent
Fresh Cottonseed	1.542	56.56	6.42	9.15	36.88
Stored Cottonseed	3.584	59.69	7.65	8.30	
Fresh Non-Cottonseed	1.650	55.87	6.42	9.03	33.96
Stored Non-Cottonseed	4.123		7.63	8.61	

will be observed by consulting Table IV. The eggs used in this determination were of three types: high protein, low protein, and cottonseed eggs. Some of the main differences are reviewed here briefly. The whites of the cottonseed eggs were low in the amino mono-amino nitrogen and amino

TABLE IV

Nitrogen as Percentage of Total Nitrogen

	Whites L. P.	Whites H. P.	Whites C. S.	Yolks L. P.	Yolks H. P.	Yolks C. S.
Amide	9.44	9.44	9.52	10.44	10.28	9.79
Humin	3.88	6.51	3.04	3.44	3.35	4.25
Di-Amino	30.29	26.19	30.20	30.93	32.09	34.55
Mono-Amino	60.76	59.18	58.43	55.67	53.30	57.37
Total	104.37	101.32	101.19	100.53	99.02	105.46
Amino Mono-Amino	59.17	57.48	53.30	49.54	53.00	54.31
Non-Amino Mono-Amino	1.59	1.70	5.13	6.13	.30	3.06
Amino Di-Amino	23.06	20.12	19.92	17.04	18.91	19.21
Non-Amino Di-Amino	7.23	6.07	10.28	13.89	13.18	15.34

di-amino nitrogen, but the yolks were high in humin, total di-amino, total mono-amino, amino mono-amino, and amino di-amino nitrogen. The whites of the high protein eggs were high in humin nitrogen, but low in total di-amino nitrogen, while the yolks of those eggs were low in total mono-amino nitrogen and very low in non-amino mono-amino nitrogen. The whites of the low protein eggs were high in amino mono-

amino nitrogen and also high in amino di-amino nitrogen. The yolks of the same eggs showed several differences; namely, low in total di-amino, low in amino di-amino, low in amino mono-amino, and high in non-amino mono-amino nitrogen.

EXTRACTION OF COLORING MATTER OF RATIONS

An analysis of the eggs having failed to reveal any unusual constituent difference in composition, we resorted to biological methods to trace the cause of the color. It is well known that alcohol is a solvent for many color producing substances. The cottonseed meal was thus extracted for 10 hours in a continuous extractor. The extracted cottonseed was fed to one group of hens and the extract was added to the normal ration of another group. The eggs from these hens were collected and stored for six months. We expected by this method to isolate the coloring matter into one group of eggs. Strange as it must seem, neither set of eggs produced any colored yolks. Apparently the color producing material is subject to some chemical change, probably oxidation or hydrolysis during the extraction.

GOSSYPOL STUDY

The toxic principle of cottonseed meal that is responsible for injury to hogs and cattle is termed gossypol. This compound in the presence of iron salts turns black. Reasoning that possibly some such combination might be taking place in the egg we isolated a quantity of gossypol by the usual laborious methods. This material was placed in capsules and fed to hens; the eggs from these hens were placed in storage for six months.

Upon examination these eggs resembled cottonseed eggs in physical appearance in that the viscosity was greater than for normal eggs, and the coefficient of spread was practically that of cottonseed eggs. But the peculiar olive color was lacking, indicating that either gossypol is not responsible for the color, or else its properties had been inactivated in the isolation process.

CONCLUSIONS

1. The ratio of the weight of the white to the weight of the yolk is much larger in fresh eggs than in stored ones. This is due to the passage of water from the white to the yolk during storage.

2. The yolks of stored eggs contain a greater amount of moisture than yolks of fresh eggs.

3. The coefficient of spread of fresh eggs is almost identical, regardless of the diet of the hen, but fresh eggs have a higher coefficient of spread than stored eggs.

4. The ammonia content is greater in stored eggs than in fresh eggs, but there is little difference in the ammonia content of cottonseed eggs and non-cottonseed eggs.

5. Fresh high-protein eggs contain a higher percentage of fat than the others. The stored eggs show less fat than fresh ones.

6. Stored cottonseed eggs have higher iodine absorption values than fresh ones. There is little difference in the values of cottonseed and non-cottonseed eggs.

7. The only differences in protein were between the high and low protein eggs. The whites of stored low protein eggs are higher in protein than those of either the fresh high protein eggs or low protein eggs.

8. The pH values of cottonseed and non-cottonseed eggs are practically the same. The values of white decrease on standing, while the yolks increase, the two approaching each other in time.

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