VITELLINE MEMBRANE STRENGTH
AND EGG YOLK MOTTLING

By

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CHAPTER I

INTRODUCTION

Eggs objectionable to the consumer due to mottled yolks have resulted in an economic loss to the poultry industry, and have curtailed the use of cottonseed meal as a protein source in layer rations. Considerable research has been done in this area in an attempt to pinpoint the actual cause of yolk discoloration and to find a suitable answer to the mottling problem.

The purpose of this study was to incorporate varying levels of $FeSO_4$ into cottonseed meal rations in an attempt to eliminate mottling, and also to test the strength of the vitelline membrane of these eggs which were stored for varying amounts of time.

Factors of concern in this study were:

1. Determining the effect of $FeSO_4$ in cottonseed meal layer rations on mottling and vitelline membrane strength.
2. Determining the effect of days storage on mottling and vitelline membrane strength, and
3. Determining if there is a variation in the strength of the vitelline membrane at different loci.

It is hoped that the data from this study will help to eliminate the loss of eggs due to mottling, and help explain the role of the vitelline membrane in this phenomenon.

CHAPTER II

LITERATURE REVIEW

The inclusion of cottonseed meal in the ration of laying hens and the production of mottled yolks by these hens has been a source of frustration to the poultry nutritionist and to the poultry products technologist for many years.

Although cottonseed meal contains a high quality protein it is not generally recommended as a feed for laying hens because the eggs develop objectionable colors during storage. Description of such eggs can be found in the reports by Roberts and Rice (1891), Sherwood (1928 and 1931), and Kempster (1930).

Schaible, et al., (1946) reported that commercial candlers placed these abnormal storage eggs in the lowest grades, or discarded them as rots because of the dark yolk shadow cast, despite the fact that bacteriological studies had shown no difference between them and non-cottonseed stored eggs. The eggs were not unfit for food, but were objectionable in appearance and, therefore, not marketable.

The substance responsible for the olive green to chocolate brown colored yolks in laying hens fed cottonseed meal was first identified as gossypol by Schaible, et al., (1934). Gossypol, a yellow solid, is the principal intraglandular pigment of the cottonseed.

Kemmerer, et al., (1961) indicated that the discoloration attributed to gossypol from cottonseed meal was not due to free gossypol alone, but a combination of gossypol and cyclic fatty acids such as sterculic and other acids with similar structures. Kemmerer speculated from their research that the egg yolk discoloration caused by gossypol was due to the formation of a ferrous iron, gossypol complex, and that formation of the complex was considerably augmented by an alkaline pH.

In the same publication, Kemmerer reported that two factors were responsible for the appearance of the discoloration in the yolk. These two factors were: A) pH change of the egg at oviposition and storage, B) osmotic pressure differences across the vitelline membrane. At the time of oviposition the albumen of eggs have a pH of approximately 8.5 and the yolks approximately 6.0. During cold storage the albumen will increase to as high as 9.8 and the yolks only to about 6.6. Sterculia foetida, or sterculic acid, has been reported to increase the permeability of the vitelline membrane to water (Schneider, et al., 1960). The more permeable vitelline membrane allow rapid transmission into the yolk sac of the alkaline fluids in the albumen. The yolk becomes alkaline and the gossypol in the yolk forms a complex with iron which is responsible for the discoloration. The formation of a complex of iron and sodium gossypolate in solution has been previously reported by

Jonassen and Demint (1955).

Kemmerer further stated in the report that the introduction of the alkaline fluids of the albumen into the yolk was accomplished by a difference of about 1.8 atmospheres in osmotic pressure which existed across the vitelline membrane. The movement of fluids between the egg components was the result of this osmotic gradient. Since the osmotic pressure of the yolk was greater, the initial direction of diffusion was from albumen to yolk.

The effects of storage temperature and time of storage on cottonseed meal eggs was discussed by Heywang, et al., (1954). From their data the trend among eggs stored one month was for both the percent of eggs with yolk discoloration and the degree of yolk discoloration to increase as the dietary level of free gossypol increased, and at any dietary level of free gossypol for both to increase as the length of the storage period and storage temperature increased.

In a report of yolk defects resulting from various feed additives, including cottonseed meal, Berry, et al., (1968) presented a comprehensive review of the literature on this subject. Their data indicated that mottled yolks were evident within two days after oviposition and that the expression of the defect reached a plateau after 15 days of storage. Their data showed that the contour of the lines expressing the percentage of defective egg yolks versus days of storage was similar whether the eggs were stored at 2^{o}

or 25°C. The magnitude of the response was quite different with the higher percent of mottled yolks being found at the higher temperature.

All the studies reviewed would tend to indicate that individual hens differ in their ability to absorb gossypol from the feed. Individual hens vary with respect to the effect of free gossypol on discoloration of their egg yolks, and that all layers do not consistently lay eggs with discolored yolks.

The addition of ferrous sulphate to a ration of cottonseed meal to prevent mottled yolks was first suggested by Schaible, et al., (1934). Swenson and co-workers (1942) indicated that the addition of soluble ferric salts to a cottonseed meal ration prevented the absorption of the gossypol by the hen and the subsequent formation of olive colored yolks in stored eggs.

Kemmerer, et al., (1966) found that the use of iron sulphate reduced the expression of yolk discoloration from hens fed cottonseed meal. The defect was not completely eliminated, particularly at high levels of cottonseed meal nor was the iron sulphate uniformly effective among cottonseed meals from several manufacturers.

Campos (1952) found that the effect of the iron salts upon gossypol had also been determined in swine and guinea pigs which had their tolerance for gossypol increased when these salts were added to the ration.

The role of the vitelline membrane in the process of discoloration is evident since it controls diffusion between the internal egg components.

The composition of the vitelline membrane was first studied and reported by Liebermann (1888) who reported that the membrane consisted of keratinous material. Lacaillon (1910) found three layers, the middle layer being cellular in structure, whereas, the external layers were fibrous. Moran and Hale (1936) also reported three layers and concluded that the two external layers consisted of mucin, while the middle layer was keratinous. McNally (1943) found that the vitelline membrane of the freshly ovulated yolk was formed from the collagenous membrane which lines the inner surface of the follicular epithelium.

A study on the structure of the vitelline membrane by Doran and Mueller (1961) confirmed the conclusion of McNally (1943) that the yolk membrane of the completed egg consists of a collagen and a mucin layer. They found no evidence of a cellular layer as reported by Lacaillon (1910). The Doran and Mueller (1961) study concluded that the yolk membrane consisted of a layer of collagenous fibers formed in the ovarian follicle and a layer of mucin fibers formed in the magnum. They concluded that the collagen layer was the true vitelline membrane and that the mucin layer corresponds to the chalaziferous membrane.

Doran and Mueller (1961) postulated that in yolks which become mottled the binding of the mucin fibers to one another and the adhesion of the chalaziferous membrane to the vitelline membrane might be weaker than normal when they were formed. Subsequent movement of water across the vitelline membrane could then force the two membranes further apart and increase separation of the mucin fibers, thereby causing the appearance of a mottled spot.

Several methods for determining the vitelline membrane strength of the hen's egg yolk have been used. Haugh (1933) used the direct application of pressure to the yolk until rupturing of the yolk occurred. A vacuum principle wherein the yolk contents were removed and the force in mm. Hg. required to rupture the membrane in a given section was employed by Munro and Robertson (1935). The yolk was placed in an isotonic sucrose solution by Moran (1936) and a tube introduced into the surface of the yolk. As pressure was applied by lowering the tube, the membrane rose in the tube until rupturing occurred. The force required to rupture the yolk was calculated using the height of the membrane in the tube just prior to bursting. Each of these methods requires the contents of the yolk to be removed before or after the measurements are made. Thus, only one position on each yolk can be measured and the yolk loses its physical identity after such measurements are made.

Fromm and Matrone (1962) devised a method for determining the vitelline membrane strength that required the use of predetermined vacuum pressures and recording the time required to rupture the membrane. This method is not completely satisfactory in that some eggs will not rupture under the predetermined pressure, and also the maintenance of constant pressure is rather difficult.

Holder, et al., (1968) used a plastic template, three inches by three inches with eight numbered holes equally spaced around a circle closely approximating the circumference of the yolk and the ninth hole was in the center of the yolk. Each egg was weighed to the nearest half-ounce per dozen, and then broken in such a way that the orientation of the yolk could be identified. Then Haugh units were measured after which the yolk was separated from the albumen and placed under the template.

An aspirator was used for applying a vacuum or negative pressure on the vitelline membrane through a capillary tube which had an inside diameter of 1.2 mm. The template and the dish holding the yolk were positioned so that the top of the yolk was approximately 1.5 inches from the top of the template.

After the yolk was placed in the proper position, the pressure at each locus was measured by placing the capillary tube through the proper hole and letting it rest on the yolk. The vacuum was applied slowly until the vitelline membrane broke. The pressure, thus, required was measured through the use of an open-mercury manometer.

Fromm (1964) and Holder, et al., (1968) both recorded variations in the vitelline membrane at different loci on the yolk. Both studies showed a stronger vitelline membrane at the lower portion of the yolk (area near chalazae closest to the pointed end of shell).

Holder, et al., (1968) also found the vitelline membrane on yolks from hens fed a ration containing cottonseed meal required more negative pressure to rupture than did the yolks from hens fed a control ration.

The present study was undertaken to investigate the use of iron sulphate in cottonseed meal rations, to investigate the effect of days in storage on degree of mottling, and to investigate the strength of the vitelline membrane at nine loci on the yolk surface, and study the relationship of these readings with days of storage and mottling.

CHAPTER III

MATERIALS AND METHODS

This study is concerned with the effect of $FeSO_4$ on layer rations containing cottonseed meal and the variations in strength at nine different loci of the vitelline membrane of cottonseed meal and non-cottonseed meal eggs.

Hens used in the experiment were secured from Parkin Farm and Hatchery in Shawnee, Oklahoma, and were hybrid white Leghorns (H & N). The pullets were delivered to the Oklahoma State University Poultry Farm after March 15, 1967, and fed a commercial layer ration until the initiation of the study on May 12, 1967. The birds were confined to single cages (18" x 10") in a windowless, environment-controlled house and kept on an artificial lighting program which varied from 14 hours and 15 minutes of light on September 25 to 17 hours of light from December 11 until the study concluded on April 12, 1968.

Eggs were collected each afternoon after 4:30 p.m., identified as to hen and date of oviposition, and then placed in a cooling unit on the Oklahoma State University Poultry Farm for periods of from one to three days before being placed in storage at the Poultry Industries Building at 4°C. The storage periods were set at 28, 33, 38, 42, 44, and 48 days.

Twelve hens were randomly placed in each of twelve different ration treatments. The ration was a layer diet formulated by the Poultry Science Department of Oklahoma State University. Substitutions and additions were made in order to incorporate the desired ingredients in the rations. The complete ration and the amount of each of the test ingredients are shown in Appendix A and Appendix B.

The study was divided into twelve, four-week periods. At the conclusion of each period the hen's body weights were recorded in grams, and egg production records were kept by periods.

The eggs examined were delegated into two groups. The eggs laid from Saturday until the following Thursday were broken out and assigned a mottling score by visual examination. An egg breaking table was used which incorporated a mirror allowing simultaneous examination of the top and bottom of the yolk. The method used to assign mottling scores was that of Berry, et al., (1968). The scoring range was 0, 1, 2, and 3. Eggs receiving the "0" score were normal eggs, "1" indicated some slight mottling, "2" indicated large areas of mottling, and "3" indicated that the entire yolk exhibited a mottled appearance. The term "mottling" was given to the olive or brownish colored areas on the egg yolks. A mottling score of "2" or "3" would be considered objectionable to the consumer.

The second group of eggs, laid on Friday of each week, were weighed to the nearest ounce per dozen, Haugh units recorded, and assigned a mottling score by the above mentioned method. The yolk was then separated from the albumen by hand, and the yolk placed on a watch glass and oriented as to location of the blastodisc. The strength of the vitelline membrane was tested and recorded in nine different locations on the yolk surface by the method used by Holder, et al., (1968). The equipment used for the vitelline membrane portion of the study is diagrammed in Fig. 1.

If a hen failed to lay on Friday, then an egg would be taken from the date nearest to that corresponding Friday, and the storage period corrected accordingly.

Due to mortality during the experimental period the statistical portion of the study was completed on ten hens instead of the original twelve. In those treatments where none or only one unit was lost the discarded units were chosen at random.

The factorial method described by Snedecor and Cochran (1967) was used to analyze the data generated. Random selection of data from the approximately 20,000 eggs broken during the 48 week duration of the experiment was effected in such a way that there was an equal number of eggs from each of ten hens within each of the following "factors" or treatments:

4 levels of cottonseed meal 0, 4.5, 9, and 18 percent of the ration

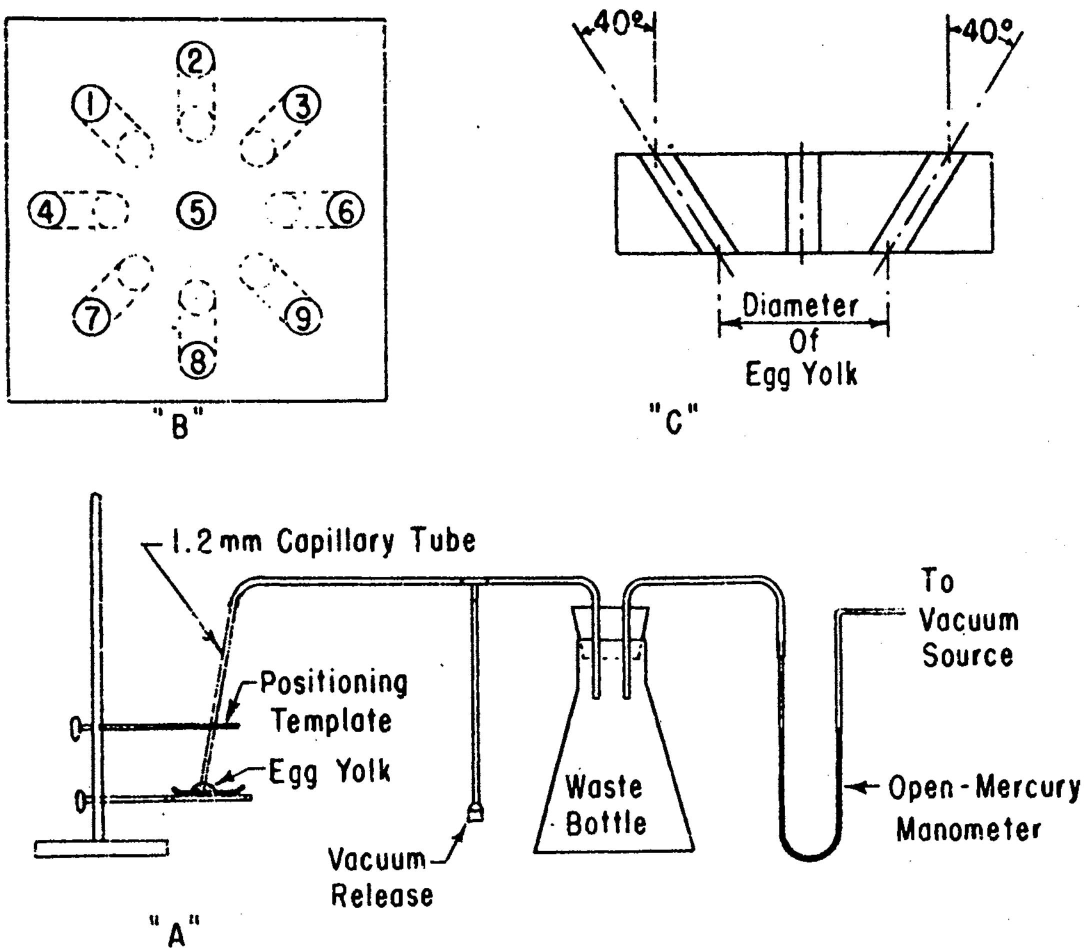

Figure 1. Schmatic drawings of equipment used for negative determinations. A - overall sketch of device showing relative positions. B - top view of positioning template. C - cross-section of positioning template showing angle of holes through template. (From Holder, et al., 1968).

3 levels of $FeSO_4$ — 0, 177, and 531 gms. $FeSO_4$ per 45.5 Kg. of ration

6 levels of storage periods - 28, 33, 38, 42, 44, and 48 days after oviposition

3 levels of production periods - 1. (May - Aug.); 2. (Sept. - Dec.); 3. (Jan. - April)

As a result of this selection, the data from 2,160 eggs were used for the analysis of a 4 x 2 x 3 x 6 x 3 factorial arrangement. The data were analyzed using the facilities of the Statistics Department of the Oklahoma State University campus.

CHAPTER IV

RESULTS AND DISCUSSION

Ration Supplementation

As was to be expected, the closest cause and effect relationship found, within the data generated, was between levels of cottonseed meal supplementation and yolk mottling. These data are shown in Tables I and II and are illustrated in Figure 2. An increase in the percent of cottonseed meal, particularly when included at a level of 18 percent of the ration, caused a dramatic increase in average mottling score, e.g., .044 to 1.867. The fact that this mottling score did not increase such that all eggs exhibited the maximum score possible indicated that there is considerable individual hen reaction to the inclusion of cottonseed meal in the ration.

In spite of the abnormally high level of this ration ingredient, some eggs were produced which were near normal in appearance and were assigned a low score. In fact, of the approximately 2,000 eggs produced by the hens receiving 18 percent cottonseed meal with no $FeSO_4$ supplementation, 24, 10, 1, and 65 percent of the eggs were assigned scores of 0, 1, 2, and 3, respectively. Some effect on the scor-

TABLE I

THE EFFECTS OF VARIATION IN RATION ON RESPONSE VARIABLES

Level of Cottonseed Meal (Percent of ration)	Level of $FeSO_4$ (Gm. per 100 lbs. ration)	Vacuum Reading (mm of Hg)	Mottling Score	Haugh Units
0	0	71.2	.044	76.5
0	177	73.2	.006	76.3
0	531	73.7	.011	77.0
4.5	0	72.4	.006	77.8
4.5	177	73.0	.011	75.8
4.5	531	74.4	.028	78.5
9.0	0	74.1	.344	75.0
9.0	177	75.0	.044	77.6
9.0	531	78.7	.056	78.2
18.0	0	75.9	1.867	73.9
18.0	177	78.7	1.150	80.0
18.0	531	75.6	.750	78.1
	AVERAGE	74.6	.360	77.0

TABLE II

ANALYSIS OF VARIANCE FOR THE EFFECT OF RATION VARIATION ON RESPONSE VARIABLES

Source of Variation	DF	Vacuum MS	F	Mottling Score MS	F	Haugh Units MS	F
Cottonseed meal	3	18,439.67	3.79*	194.65	166.16**	75.82	.26
$FeSO_4$	2	8,042.62	1.65	24.33	20.77**	870.50	3.04*
CSM X $FeSO_4$	6	3,865.19	.79	12.87	10.98**	542.83	1.90
Error	108	5,010.10		1.17		286.22	

**Significant at 1% level

*Significant at 5% level

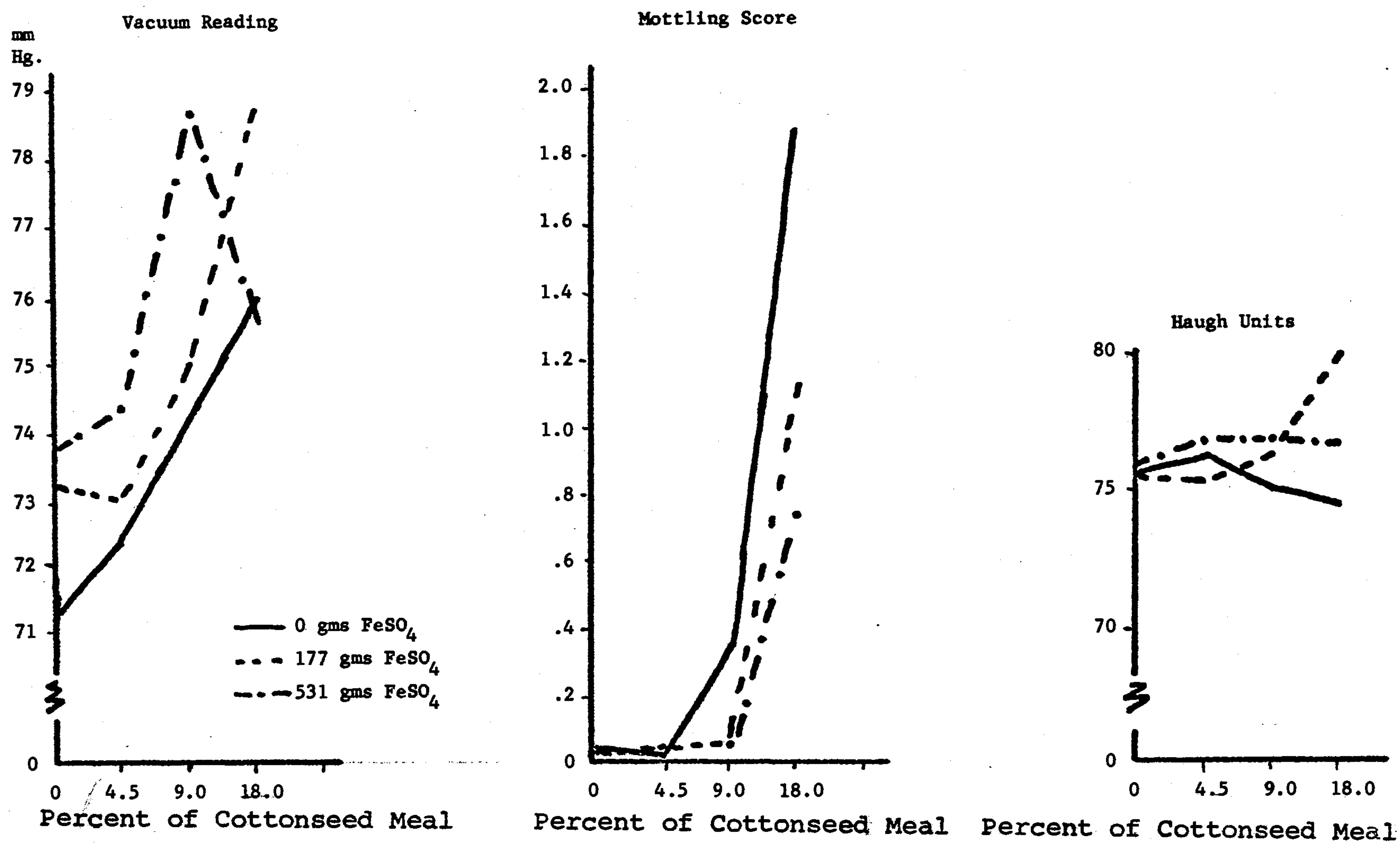

Figure 2. The effects of variation in ration on response variables.

ing might be attributed to storage times; however, more than 95% of all eggs broken were stored in excess of 21 days. From most reports in literature, this is more than enough time for any mottling to manifest itself.

Several research workers reporting at the Conference on Inactivation of Gossypol with Mineral Salts (Kemmer, et al., Reese and Heidebrecht, 1966) suggested the use of ferrous sulphate to deter the production of mottled yolks. Certainly the findings reported herein indicate that the reduction is significant at all the levels of cottonseed meal included in the rations. However, when cottonseed meal was included at the high rate of 18 percept of the ration, even the level of 531 grams $FeSO_4$ per 100 lbs. of feed was not sufficient to completely eliminate the production of mottled yolks. It is evident that a drastic reduction in mottling score was affected as evidenced by the average scores or 1.867, 1.150, and .750 for the $FeSO_4$ levels of 0, 177, and 531 grams per 100 pounds of ration, respectively. It would certainly appear from the data shown that a ratio of approximately 20 to 1 (percent cottonseed meal to grams of $FeSO_4$ per 100 lbs. of ration) was sufficient to deter the production of mottled yolks to a negligible level at levels of cottonseed meal below 9 percent of the ration. However, when the percent of cottonseed meal was increased to 18, even a ratio of 30 to 1 was not high enough to hold the mottling score to such a low level. This suggests that perhaps there is merit to using

$FeSO_4$ as a deterrent to mottled yolk production.

At least two conditions should be suggested for consideration at this point (1) before the $FeSO_4$ is used at too high a level, the toxicity of the additive should be thoroughly tested, and (2) the addition of $FeSO_4$ to a ration might add to the cost of the ration to the point where other protein sources could be used to a better economic advantage.

Although the effect is not clearly evident from the data, the analysis of variance does indicate a significant effect of the $FeSO_4$ on interior quality as measured by Haugh units. There is a slight, but direct, positive trend to higher quality with each increase in amount of additive. Calculation shows average Haugh units of 75.8, 77.4, and 77.9 for the 0, 177, and 531 levels of $FeSO_4$, respectively. This does seem to establish rather conclusively that neither of these feed ingredients would be detrimental to albumen height within the limitations imposed by the present test.

Production Periods

The data were combined into three, four-month production periods as shown in Table III and the analysis of variance for the effect of production periods as shown in Table IV. The greatest effect found was in the vitelline membrane strength as demonstrated by the vacuum readings. The increase of approximately 10 mm of Hg between the first and second period may have been due in part to a change in

TABLE III

THE EFFECTS OF PRODUCTION PERIODS ON RESPONSE VARIABLES

Periods	Vacuum Reading (mm of Hg)	Mottling Score	Haugh Units
1 (May - Aug.)	68.0	.357	80.3
2 (Sept. - Dec.)	78.8	.364	76.0
3 (Jan. - Apr.)	77.1	.358	74.8
Ave.	74.6	.360	77.0

TABLE IV

ANALYSIS OF VARIANCE FOR THE EFFECT OF PRODUCTION PERIODS ON RESPONSE VARIABLES

Source of Variation	DF	Vacuum MS	Vacuum F	Mottling Score MS	Mottling Score F	Haugh Units MS	Haugh Units F
Periods	2	217,476.56	596.45**	0.01	0.03	5,894.54	163.66**
Cottonseed meal x Periods	6	2,532.69	6.94**	1.26	3.47**	235.55	6.54**
$FeSO_4$ x Periods	4	1,560.36	4.27	4.62	12.75**	23.79	.66
Storage x Periods	10	1,714.76	4.70**	1.72	4.74**	210.67	5.85**
CSM x $FeSO_4$ x Periods	12	1,077.20	2.95**	5.26	14.52**	78.78	2.19*
CSM x Storage x Periods	30	1,663.55	4.56**	1.84	5.06**	106.87	2.97**
$FeSO_4$ x Storage x Periods	20	705.91	1.93**	0.60	1.65*	45.28	1.26
4-way Interaction	60	565.80	1.55	0.75	2.08**	30.94	.86
Error Term	1296	364.61		.36		36.02	

*Significant at 5 percent level **Significant at 1 percent level ambient temperature; however, there was also a slight increase in a positive relationship between vacuum readings and mottling score. The author feels that much of the increase is related in this manner rather than with temperature. Certainly, the ambient temperatures were not high enough during the first period to adversely affect egg quality as indicated by the high Haugh unit readings.

The slight change in vitelline membrane strength between the second and third periods, with both mottling scores and Haugh units showing decreases, suggests several possibilities. One explanation is that over a period of time the hen's physiological system becomes refractive to the intake of cottonseed meal which causes both vitelline membrane strength and yolk mottling to reach a plateau. These two responses then remain rather static, while egg quality, as measured by Haugh units, continues to decline, throughout the hen's first year of production.

The highly significant effect of period of production on both vitelline membrane strength and Haugh units was of sufficient magnitude to cause almost all of the interactions to likewise be highly significant.

Storage

Previous work in this laboratory, reported by Berry, et al., (1968) had indicated that yolk mottling resulting from cottonseed meal was found soon after oviposition. Since the report by these authors had covered only storage periods up to 15 days, the present work extended this period to 48 days of storage. The data were combined into six selected storage days as shown on Tables V and VI. An effect of storage on this response is certainly evident, although some variation is noted particularly at 44 days of storage as evidenced by the low mottling score at that time. The fact that the F-value for the cottonseed meal x storage interaction is as close to the F-value for storage alone is clear indication that these two variables are the major causes for yolk discoloration. Although it was shown earlier in this paper that the $FeSO_4$ supplementation did have some effect in reducing yolk discoloration, the effects of the iron appeared to have been masked by the extended storage periods used in this study.

Egg quality, as measured by Haugh units, stayed unusually high throughout the present study. The author feels that this can be attributed to the excellent storage facilities. However, this fact coupled with the variation found in the mottling scores apparently had some effect on vacuum readings. The F-values do indicate that the storage periods selected did have a significant effect on each of the three responses measured, although the effect is certainly not linear. It would appear from this that some of these factors were compensatory or perhaps some factors not being measured by this project had a vectoring and as yet inexplicable effect on responses.

TABLE V

THE EFFECTS OF SELECTED DAYS OF STORAGE ON RESPONSE VARIABLES

Days Stored	Vacuum Reading (mm of Hg)	Mottling Score	Haugh Units
28	75.0	.322	76.6
33	76.1	.339	75.5
38	75.8	.464	79.0
42	74.3	.308	77.5
44	72.8	.286	76.8
48	73.8	.438	77.0
Ave.	74.6	.360	77.0

TABLE VI

ANALYSIS OF VARIANCE FOR THE EFFECT OF SELECTED DAYS OF STORAGE ON RESPONSE VARIABLES

Source	DF	Vacuum MS	Vacuum F	Mottling Score MS	Mottling Score F	Haugh Units MS	Haugh Units F
Storage	5	5,279.10	15.97**	1.94	4.80**	498.90	14.62**
Cottonseed meal x storage	15	2,219.38	6.71**	1.40	3.46**	135.76	3.98**
$FeSO_4$ x storage	10	752.08	2.27*	0.57	1.41	35.06	1.03
3-way Interaction	30	1,008.83	3.05**	.70	1.73*	62.39	1.83
Error Term	540	330.43		.40		34.12	

**Significant at 1 percent level *Significant at 5 percent level

Vacuum Loci

Holder, et al., (1968) had shown considerable variation among the various loci on the yolk with regard to vitelline membrane strength. This conclusion is definitely confirmed by the present work as examination of the data in Figure 3 will show. The orientation of the yolk for vitelline membrane testing was the same as for the earlier work; i.e. the rows are perpendicular to the long axis of the egg with row 1 toward the large end of the egg, row 3 toward the small end and row 2, the median between the other row. The germ spot was always oriented in an approximate 2 o'clock position. Thus, the germ spot was, as nearly as possible, centrally located from the sampling positions row 1 col 2, row 1 col 3, row 2 col 2, and row 2 col 3.

The range in values from 65.2 mm of Hg. to 87.6 mm of Hg. for the loci row 1 col 1 and row 3 col 2, respectively, indicates a considerable variation in vitelline membrane strength over the surface of the yolk. No record was made of whether the areas being tested were discolored or not. The fact that both of the two-way interactions between the levels of cottonseed meal and rows and column were highly significant (Table VII) would indicate that, at least part of the time, the areas sampled were mottled. The large F-value for the effect of rows is, no doubt, the reason for most of the significant relationships shown. All possible sources of variation were calculated and evaluated for

Rows	Columns 1	2	3	Ave.
		mm of Hg.		
1	65.2	67.6	68.9	67.2
2	70.7	74.0	74.0	72.9
3	80.6	87.6	83.0	83.8
Ave.	72.1	76.5	75.2	74.6

A - Location of blastodisc

Figure 3. Sampling points and average vacuum required to break the vitelline membrane at selected yolk loci.

TABLE VII

PARTIAL ANALYSIS OF VARIANCE OF VACUUM READINGS AT SELECTED YOLK LOCI.

Source of Variation	DF	MS	F
Rows	2	457,081.41	6,336.03**
Columns	2	31,997.18	443.54**
Cottonseed meal x row	6	2,121.65	29.41**
CSM x Col	6	190.30	2.63*
$FeSO_4$ x Row	4	1,112.55	15.42**
Storage x Row	10	805.78	11.16**
Period x Row	4	27,259.27	384.79**
Per x Col	4	502.90	6.97**
Col x Row	4	5,584.56	77.41**
CSM x $FeSO_4$ x Row	12	493.94	6.84**
CSM x Stor x Row	30	273.68	3.79**
CSM x Per x Row	12	213.37	2.95**
$FeSO_4$ x Stor x Row	20	139.79	1.93**
$FeSO_4$ x Per x Row	8	140.89	1.95*
Stor x Per x Row	20	346.39	4.80*
Stor x Per x Col	20	165.99	2.30**
Per x Row x Col	8	270.53	3.75**
CSM x $FeSO_4$ x Stor x Row	60	175.70	2.43**
CSM x $FeSO_4$ x Per x Row	24	237.75	3.29**
CSM x Stor x Per x Row	60	230.25	3.19**
$FeSO_4$ x Stor x Per x Row	40	154.36	2.13**
CSM x $FeSO_4$ x Stor x Per x Row	120	117.46	1.62**
Error Term	15,552	72.14	

significance. However, for the sake of brevity only those F-values less than five (5) percent probability are listed.

CHAPTER V

SUMMARY AND CONCLUSIONS

The effects of four levels of cottonseed meal ranging from 0 to 18% of the layer diet, and ferrous sulphate ($FeSO_4$ $7H_2O$) in three levels ranging from 0 to 531 grams per 45.4 kg. of ration were measured on the egg quality responses of yolk mottling, Haugh units, and vitelline membrane strength.

The method employed to assign mottling scores was that of Berry, et al., (1968). The scoring range was from "0" to "3". A mottling score of "2" or "3" would be considered objectionable to the consumer.

The strength of the vitelline membrane was tested by the method employed by Holder, et al., (1968). An open-mercury manometer was used for the negative-pressure determinations, and the values were recorded in mm. of Hg.

These effects and responses were analyzed using a complete factorial design in which the additional factors of production periods, days of storage, rows, and columns were used as applicable in the analysis. Three production periods were used in this study, and the days of storage range was from 28 to 48 days.

The data were analyzed and the following conclusions made from the analysis:

1. Some egg yolk mottling was found in all rations with highly significant effects attributable to cottonseed meal, ferrous sulphate, and days of storage. Apparently, the period of the year in which the eggs were produced did not have an effect great enough to show significance.
2. Egg quality, as measured by Haugh units, was affected by production periods and days of storage with sufficient magnitude to be highly significant. The effect of ration supplementation had little or no effect on this response variable as the iron sulphate proved to be only significant and the cottonseed meal showed no measurable significance.
3. Vitelline membrane strength was significantly affected by all factors considered except the iron sulphate addition; however, the cottonseed meal effect was at a relatively low level.
4. This study confirms the work of Holder, et al., (1968) with regard to vitelline membrane strength at nine different loci on the egg yolk.

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APPENDICES

APPENDIX A

PROJECT 1293

NEWELL'S LAYER RATION BASAL

MAY 10, 1967

Ingredients	Percent	Pounds
Fat (feed grade tallow)	3.96	35.64
Corn, ground yellow	28.35	255.15
Milo, ground yellow	47.14	424.26
Oatmill feed	3.78	34.02
Alfalfa meal (17%)	.92	8.28
Fish meal (60%)	2.68	24.12
Blood meal (84%)	.79	7.11
Distillers solubles	.92	8.28
Whey, dried	.61	5.49
Meat & bone scrap (50%)	1.40	12.60
dl-methionine	.12	1.08
Dicalcium phosphate	2.50	22.50
Calcium carbonate	5.61	50.49
VMC - 60	.61	5.49
Salt	.61	5.49
Total	100.00	900.00

RATION NO. N-6701

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	18.0 pounds

RATION NO. N-6702

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	18.0 pounds
Ferrous Sulfate	177.0 grams

RATION NO. N-6703

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	18.0 pounds
Ferrous Sulfate	531.0 grams

RATION NO. N-6704

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	13.5 pounds
Cottonseed meal	4.5 pounds

RATION NO. N-6705

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	13.5 pounds
Cottonseed meal	4.5 pounds
Ferrous Sulfate	177.0 grams

RATION NO. N-6706

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	13.5 pounds
Cottonseed meal	4.5 pounds
Ferrous Sulfate	531.0 gra,s

RATION NO. N-6707

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	9.0 pounds
Cottonseed meal	9.0 pounds

RATION NO. N-6708

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	9.0 pounds
Cottonseed meal	9.0 pounds
Ferrous Sulfate	177.0 grams

RATION NO. N-6709

Newell's Layer Ration Basal	82.0 pounds
Soybean meal (50%)	9.0 pounds
Cottonseed meal	9.0 pounds
Ferrous Sulfate	531.0 grams

RATION NO. N-6710

Newell's Layer Ration Basal	82.0 pounds
Cottonseed meal	18.0 pounds

RATION NO. N-6711

Newell's Layer Ration Basal	82.0 pounds
Cottonseed meal	18.0 pounds
Ferrous Sulfate	177.0 grams

RATION NO. N-6712

Newell's Layer Ration Basal	82.0 pounds
Cottonseed meal	18.0 pounds
Ferrous Sulfate	531.0 grams

APPENDIX B

Levels of $FeSO_4$ (2)	Levels of Cottonseed Meal[1]			
	1	2	3	4
1	0 / 0	4.5 / 0	9.0 / 0	18.0 / 0
2	0 / 177	4.5 / 177	9.0 / 177	18.0 / 177
2	0 / 531	4.5 / 531	9.0 / 531	18.0 / 531

(1) - Percent of ration.
(2) - gms $FeSO_4$ per 45.4 kilograms of ration.

Ration variation for the experiment.

VITA

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