determinations in parallel. Since no unusual equipment is required, the fallout cesium separation can be made in a general-purpose laboratory. It is expedient, however, not to conduct any part of the procedure in a laboratory used for radiotracer studies or to use glassware used for radioactive materials. The counting equipment used is commercially available. The size of the initial sample is set only by the Cs^{137} level expected and the precision of measurement desired. A sample containing 25 picocuries of Cs^{137} was necessary to give a count rate equal to background, when the equipment previously described was used and an energy width of ± 5 k.e.v. was counted.

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COTTONSEED MEAL IN POULTRY FEED

Collaborative Study of the AGU Method of Grading Cottonseed Meals for Laying Rations

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The results of a collaborative study of the available gossypol unit (AGU) method of grading cottonseed meals for laying rations indicate that significant differences exist in AGU values among eggs, birds, and meals. The correlation between the AGU values of cottonseed meals and coloration in yolks of stored shell eggs produced by the meals is virtually zero. The AGU method may not be relied upon for grading cottonseed meals for laying rations.

A LARGE proportion of yolks from shell eggs from hens on cottonseed meal-containing diets develop a brown coloration when the eggs are stored under refrigeration conditions. Cottonseed meals vary widely in their ability to induce brown coloration in yolks of stored shell eggs. It was presumed by earlier workers that coloration develops because of the "free" gossypol present in the meals (*6, 7, 10, 11*).

Apparently cottonseed meals cannot be graded for laying rations on the basis of their free gossypol contents, however, since the correlation between intensity of coloration in yolks and free gossypol content of the meals fed is poor (*3*). A method for grading cottonseed meals for laying rations proposed by Grau (*4, 5*) depends upon a greater concentration in the yolks from eggs laid by cottonseed meal-fed hens of some acetone-hexane soluble material than that occurring in yolks from hens fed other rations. This acetone-hexane soluble material has an absorption maximum at 440 mμ. The absorption at this wave length was ascribed by Grau to a condensation product of gossypol and cephalin. The increased absorptivity of the preparation obtained from cottonseed meal-produced yolk over that obtained with control yolk was proposed by him as a measure of the available gossypol in the cottonseed meal fed. More specifically, the AGU of a cottonseed meal was defined by the relationship:

$$\text{AGU} = \frac{[A'_{400} - A'_{450}] - [A''_{400} - A''_{450}]}{\% \text{ of material tested in ration}} \times 100$$

where A' has reference to the absorbance of the extract from cottonseed meal-produced egg and A'' has reference to the absorbance of the extract from the control egg. Subscripts refer to the wave lengths at which the absorptivities are measured. A cottonseed meal having an AGU of 0.30 or less was reported by Grau to be suitable for laying rations in amounts up to 10% of the total ration.

Large quantities of cottonseed meals have been used in laying rations for hens during the past 2 years, where the meals incorporated into the rations were selected on the basis of the AGU testing method. The eggs produced were sold on the fresh egg market. A collaborative test of the AGU method became imperative because of the wide interest engendered by this use of cottonseed meals. These collaborating in the test were: V. P. Entwistle, Calif. Dept. Agr., Sacramento, Calif.; C. R. Grau, Univ. of Calif., Davis, Calif.; A. A.

Table I. Per Cent Composition of Rations for Laying Hens

Constituent	Amount, %
Cottonseed meal	10
Soybean meal	15
Ground yellow corn	15
Ground milo	49.4
Alfalfa meal, dehydrated	2.5
Ground limestone	3.5
Bone meal	3.5
Manganized salt	0.5
Source of riboflavin equivalent to 500 unit/gram	0.3
Source of vitamin A, 2,250 units; and vitamin D, 300 units/gram	0.3

Heidebrecht, Paymaster Feeds, Abilene, Tex.; B. W. Heywang, U. S. Department of Agriculture, Glendale, Ariz.; A. R. Kemmerer, Univ. of Ariz., Tucson, Ariz.; C. H. Koons, Swift and Co., Chicago, Ill.; H. L. Wilcke, Ralston Purina Co., St. Louis, Mo.; A. B. Watts, La. State Univ., Baton Rouge, La.

Experimental

Six cottonseed meals which differed widely in their ability to induce egg yolk discoloration in stored shell eggs were distributed to each of seven stations, where they were incorporated into laying rations and fed to laying hens. The ration formula is given in Table I. The control ration was prepared in an identical manner, except that soybean meal completely replaced cottonseed meal.

Hens that had not received cottonseed meal, oil, or soapstock for at least 60 days prior to the initiation of the experiment were used at each station for each meal. They were maintained in individual cages having hardware cloth floors and were fed *ad libitum*. Although uniform numbers of hens and eggs were not studied at all stations (cf. Table IV for degrees of freedom at each station), all of the hens were initially fed the control ration. Starting on the tenth day on the control ration a minimum of 12 eggs was collected from each hen at most stations. Six of the eggs were used in the determination of the AGU blank, and six were placed in cold storage in the shell to serve as control eggs 6 months later when all of the stored eggs were broken out to determine the degree of coloration developd upon storage.

The hens were then transferred to the cottonseed meal-containing rations and at most stations 12 eggs were collected fromeach hen, starting 13 days later. Alternate eggs were used for AGU determinations; the others were stored under commercial cold storage conditions. The identity of each hen and the date for each egg were recorded on the shell.

AGU values were determined according to the method described by Grau (*4*). At most stations, these determinations were carried out by two investigators working independently of each other. The AGU data at each station reported in this paper are average values for each meal.

Reference color photographs of yolks that varied from no coloration to intensely brown coloration were supplied to most stations. These color photographs were taken of yolks produced at Louisiana State University by feeding a ration having the formula given in Table I. The eggs were opened at New Orleans and exposed to ammonia in order to increase the pH of the yolks so that color would develop, since the chromogen in yolks is pH sensitive. The yolks were then photographed using standard color film. Color prints were prepared with magnification of unity. Values of 0 through 5 were assigned to these prints at New Orleans: 0 was for no brown coloration, 1 for very light brown, 2 for light brown, 3 for brown, 4 for dark brown, and 5 for intensely dark brown.

The degree of pigmentation in yolks of stored shell eggs produced at Glendale, Ariz., was estimated visually at Glendale, independent of the color charts supplied. Five eggs from each of seven hens on each meal were used. Here color intensities were assigned numbers of 0 through 5, as was done for the color photographs. A similar number of the eggs produced at Glendale, Ariz., were shipped to Tucson, Ariz., where an independent classification of the yolks was established through the use of reference photographs. Yolks were classified at other stations in accordance with intensity of

Table II. Chemical Properties of Cottonseed Meals Used in Laying Hen Rations

Meal No.	Meal Type	ε-Free Amino Lysine, G./16 G. N	Gossypol, % Chemically uncombined	Gossypol, % Free	Gossypol, % Total	Nitrogen Solubility, %
CM-100	Prepress solvent	3.40	0.01	0.03	0.82	58.2
101	Screw press	2.59	0.01	0.03	1.25	37.5
102	Prepress solvent	3.40	0.01	0.02	0.70	43.5
103	Acetone extd. in lb.	4.35	0.04	0.08	0.27	98.4
104	Prepress solvent	3.12	0.00	0.02	0.97	54.7
105	Prepress solvent	3.07	0.01	0.02	0.66	48.2

Table III. Average AGU Values Reported for Cottonseed Meals

Meal	Station 1	2	3	4	5	6	7	Av.
CM-100	0.47	0.31	0.53	0.40	0.26	0.47	0.47	0.42
101	0.61	0.35	0.48	0.58	0.53	0.61	0.62	0.54
102	0.29	0.20	0.40	0.34	0.16	0.34	0.13	0.26
103	0.50	0.29	0.52	0.53	0.30	0.56	0.44	0.45
104	0.35	0.20	0.38	0.34	0.24	0.27	0.31	0.30
105	0.31	0.23	0.32	0.36	0.15	0.28	0.18	0.26

Table IV. Analyses of Variance of AGU Values

Station No.	Variance, Source	Freedom, Degrees	Squares, Sum	Variance	F[a]	P[b]
1	Meals	5	2.7297	0.5459	30.50	0.0005
	Hens	30	0.5360	0.0179	1.19	0.25
	Eggs	175	2.6332	0.0150		
	Total	210	5.8989			
2	Meals	5	0.7198	0.1440	6.21	0.0005
	Hens	30	0.6976	0.0232	3.41	0.0005
	Eggs	180	1.2236	0.0068		
	Total	215	2.6410			
3	Meals	5	0.5951	0.1190	4.76	0.005
	Hens[c]	27	0.6758	0.0250	4.46	0.0005
	Eggs[d]	62	0.3470	0.0056		
	Total	94	1.6179			
4	Meals	5	1.8233	0.3647	7.151	0.0005
	Hens	36	1.8352	0.0510	8.973	0.0005
	Eggs	163	0.9410	0.0058		
	Total	204	5.2393			
5	Meals	5	0.5435	0.1087	6.84	0.0005
	Hens[c]	30	0.4757	0.0159	6.36	0.0005
	Eggs[d]	24	0.0605	0.0025		
	Total	59	1.0797			
6	Meals	5	2.6234	0.5247	6.38	0.001
	Hens	21	1.7297	0.0823	20.58	0.0005
	Eggs	128	0.5089	0.0040		
	Total	154	4.8602			
7	Meals	5	5.4984	1.0997	26.12	0.0005
	Hens[c]	27	1.1357	0.0421	6.28	0.0005
	Eggs[d]	163	1.0948	0.0067		
	Total	195	7.7289			

[a] Ratio of variances. [b] Per cent probability. [c] Hens in meals. [d] Eggs in hens in meals.

coloration through the use of the color photographs.

The gossypol contents of the meals (the chemically uncombined gossypol) were determined by the method described by King and Frampton (*8*). Free and total gossypol were determined by the method of the American Oil Chemists' Society (*1*). Epsilon-free amino lysine in cottonseed proteins was determined by the method of Conkerton and Frampton (*2*). Nitrogen solubility was determined by the method of Lyman, Chang, and Couch (*9*).

Results and Discussion

Chemical data for the cottonseed meals used are recorded in Table II, while the average AGU values for the six meals determined at several stations are recorded in Table III.

Analyses of variance of the AGU data are recorded in Table IV. There is, of course, a variation in AGU values as determined from egg to egg from any one given hen. However, it is obvious that at every station there were AGU differences among hens over and above such that could be expected from variability in AGU values determined from the eggs they laid. The probability that differences between hens are due to chance is about 1 in 20,000.

The analyses also show that there are differences among the meals over and above that expected from variability between hens. The probability that the differences between meals are due to chance is also of the order of 1 in 20,000.

The correlation between the AGU values for the meals determined at each station and egg yolk color values determined at the same station was very poor. There is no basis, from data obtained in this study, for concluding that the AGU value of a meal and the degree of coloration in the yolks of stored shell eggs produced by the meal are related. It was because of the extremely poor correlation that the relatively heavy computations required to assess the variance between stations were not carried out. These heavy computations would be required because of the nonuniformity in number of eggs and hens at each station. Computations to determine the minimum number of eggs, hens, and stations necessary to give a reliable AGU value for a given meal were not carried out for the same reason.

However, a study of data recorded in Table III for average values for the several meals at the different stations indicates that variance among the meals is about as great as variance among the stations, the values calculated are 0.093 and 0.038, respectively. Apparently, a large part of the variance among stations, as calculated from these data, is masked by the high degree of variance among the meals. It should be obvious, however, that in order to determine if a meal attains some arbitrarily selected AGU level, such as 0.30, the decision should not be based on results from a single station.

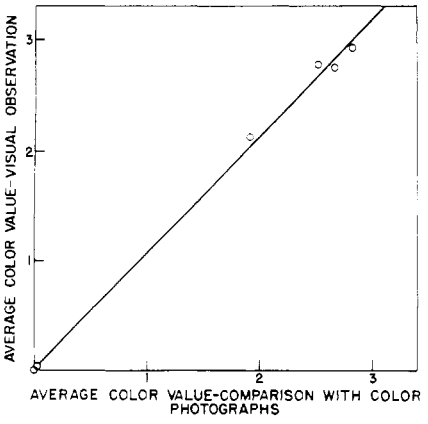

Figure 1. Agreement between degree of discoloration of yolks as determined by use of reference color photographs and by visual observation rating

The color data (average of 35 eggs for each meal) obtained at Glendale, Ariz., independently of the reference photographs, are plotted in Figure 1 *vs.* the color data (average of 35 eggs for each meal) obtained at Tucson through the use of the reference photographs. The coefficient of correlation calculated for these data is 0.97. This constitutes an independent check on the suitability of color photographs which were used by the other stations.

The average color values for the six meals as determined on the basis of the reference color photographs are recorded in Table V. The calculated coefficients of correlation between the AGU values for the meals and the average intensities of brown coloration at stations 1, 2, and 4 (the ones reporting) were 0.01, −0.25, and −0.01, respectively. Evidently, the AGU method may not be relied upon for the purpose of grading cottonseed meals for laying rations.

However, the AGU values were almost invariably positive. Apparently, the AGU measures some constituent of egg yolks that is present in higher concentrations in yolks of eggs produced by cottonseed meal-fed hens than in yolks produced on the control rations.

Table V. Average Color Values Estimated through Use of Reference Color Photographs

Meal	Station 1	2	4
CM-100	0.0	0.4	2.1
101	1.5	2.4	3.0
102	1.6	2.8	2.8
103	0.0	2.5	0.0
104	0.0	2.0	0.0
105	0.8	2.2	2.7

No satisfactory correlations were obtained between either the AGU or the degree of coloration of the yolks and the several chemical properties of the meals recorded in Table II.

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