Chemical Inactivation of Cyclopropenoid Fatty Acids in Cottonseed Meals and Their Physiological Evaluation

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Abstract

Chemical inactivation of cyclopropenoid fatty acids in commercial cottonseed meals was explored with three classes of compounds: anhydrous gases, organic acids and sulfhydryl compounds. Of the reagents screened, sulfur dioxide reduced the cyclopropenoid content by over 90% while free cottonseed fatty acids and thioglycollic acid reduced the cyclopropenoid fatty acid content by over 30%. Large batches of the above three selected meals, as well as a control commercial screw-pressed meal, were then incorporated at 20 wt % levels in the rations of laying hens. A negative control containing 25% soybean meal and a positive control containing a 2% refined cottonseed oil of known CPA content were also employed. During a four-week feeding period, eggs were collected during the third and fourth week and stored at 35 F for periods of 3 and 6 months. Overall egg quality and the fatty acid distribution of the yolk lipids were determined after the 3 and 6 months' storage periods.

Introduction

In a previous investigation, the removal of residual cyclopropenoid fatty acids (CPA) from commercial cottonseed meal by solvent extraction techniques was reported (1) and the meals evaluated by incorporation into the rations of laying hens (2). The results yielded a marked decrease in the biological responses associated with the presence of CPA in the rations of laying hens (3,4). The data described here are a continuation of this study, designed to explore the chemical inactivation of the residual cyclopropenoid fatty acids, in situ, in cottonseed meals. As in the first study, commercially available screw-pressed and direct solvent extracted cottonseed meals were employed and techniques considered which might be adaptable as an adjunct to presently employed commercial processing methods (5). However, the latter condition was not the sole criterion on which the various treatments were based. The main emphasis was directed toward those reagents which would most effectively react with the cyclopropenoid acids, based on the knowledge regarding the reactivity of this grouping. The chemistry of this grouping has been described for the addition of hydrogen halides (6), methyl mercaptans (7), acetic acid (8) and polymerization and ozonolysis reactions (9).

The fact that such reagents are potentially applicable toward the inactivation of CPAs has been demonstrated in cottonseed oils by several investigators. For instance, Deutschman et al. (10) showed that the positive Halphen reaction associated with CPA compounds did not occur when the cottonseed oils were treated with hydrogen chloride or sulfur dioxide (SO_2). Rayner et al. (11) described a process for eliminating the Halphen test response in cottonseed oils by heating them in the presence of organic and mineral acids. More recently, Eaves et al. (12) reported a simplified procedure for inactivating the CPA grouping in a conventional deodorizer at about 450 F where the organic acids were either added or generated in situ.

Experimental Procedures

Analytical Methods

Chemically treated meals along with appropriate controls were analyzed for residual CPA by the procedure of Levi et al. (13). Appropriate methods of the American Oil Chemists' Society (14) were used for the determination of moisture, lipids, nitrogen, free and total gossypol and crude fiber. Epsilon amino free lysine was determined by the method proposed by Rao et al. (15), total sulfur by the standard method of the Association of Official Agricultural Chemists (16) and iron by the method of Cluley and Newman (17). All the above analyses except CPA content were carried out by Barrow-Agee Laboratories, Memphis, Tennessee.

Use of Gaseous Reagents

Preparation of Meals. Moisture was added to the cottonseed meals in order to solubilize the gases. Moisture was added by steaming 250 g of the meal while it was tumbled in a 1 liter round-bottom flask on a Rotavapor flash evaporator to prevent lumping and caking and to ensure a uniform distribution of the water throughout the meal. The flask was frequently removed to check the gain in weight of the meal. Analysis of the original meal indicated a moisture content of approximately 7% and sufficient moisture was added to increase the weight of the meal by about 13% to bring the total moisture content to about 20%. The entire procedure took only about 10 min. The meal obtained was easy to handle and contained only a few small lumps, which were readily broken with a spatula.

Addition of Sulfur Dioxide and Carbon Dioxide. A 95 cm long by 20 mm ID glass chromatographic column was fitted with a fritted disc and a stopcock at the bottom and a large spherical expansion bulb having a standard ball-joint connection at the top.

About 200 g of the meal was loosely packed into the column so that the top level of the meal was just below the expansion bulb. Either SO_2 or CO_2 gas from a supply tank was bled through a bubbler counter to the bottom of the column at a rate of 150–200 ml/min. Unreacted gas issuing from the top of the column was piped to an absorption trap consisting of a three-necked flask filled with a saturated solution of sodium hydroxide. A safety valve was provided by inserting a vented stopper into one of the necks of the absorption flask.

A narrow band within the column warmed shortly after the SO_2 flow was started. This warm band slowly traveled up the column and the gas flow was continued until 30 min after the band had reached the top. The process took 2 hr for dry meal and 2.5 hr for moisturized meal. For the CO_2 treatment, no

temperature change was noted during the CO_2 flow, which was maintained for 3 hr.

Immediately after the gas flow stopped, the meal was transferred to a large crystallizing dish and weighed. It was then placed in a vacuum desiccator and evacuated using a water aspirator. The meal was left under vacuum for a minimum of 24 hr. A slight odor of SO_2 remained in the SO_2 treated meal, therefore the meal was spread on a plastic sheet and allowed to air dry for an additional 24 hr, after which the odor of SO_2 was barely noticeable. The same procedure was followed for the CO_2-treated meals, although, of course, no odor was noticeable.

Addition of Hydrogen. The experiment was carried out in a 1 liter Parr hydrogenation reactor with stirring. The hydrogen pressure was maintained at 50 psi for 2 hr and the temperature in the vessel rose from 25 to 30 C during this time. The meal was removed from the reactor and air dried for 24 hr.

Use of Dilute Organic Acids

Citric acid, cottonseed fatty acids (Neo-Fat 140, Armour & Co.), capric acid (commercial grade, Neo-Fat 10, Armour & Co.), oleic acid, stearic acid, lactic acid and crude cottonseed oil fatty acids were all screened as reagents for CPA reduction in screw-pressed cottonseed meal.

To determine the most effective use of these solid and liquid reagents, several experimental methods were screened. Each reagent was not tested with all the methods surveyed, but to provide a valid comparison of the relative effectiveness of the methods as compared to the relative effectiveness of the various reagents, each compound was tested by at least two of the methods. This procedure permitted the simultaneous variation of two parameters but did not seriously affect the comparability of the data. In these experiments, solutions of the organic acids at acid concentrations of either 0.1 or 1.0 wt % of the meal were used. Except as otherwise noted, petroleum ether (30–60 C) was used as the solvent for the acid.

Room-Temperature Storage With 0.1 wt % Acid. Solutions containing 0.1 g of acid per 100 ml of petroleum ether (PE) were prepared except in the case of citric acid, when methanol was used to dissolve the acid. Approximately 100 g of cottonseed meal was mixed thoroughly with 100 ml of the reagent solution and stored for 24 hr at room temperature in amber jars. After storage, the solution was filtered off and the meal was air dried. The filtrate was stripped of solvent and analyzed for CPA content. The total reduction of CPA due to chemical deactivation was then calculated on the basis of the residual CPA remaining in both the meal and the filtrate.

Reflux With 0.1 wt % Acid at 40 and 70 C. The meals were mixed with 100 ml of the reagent solutions, prepared as above, and refluxed for 2 hr in PE at approximately 40 C. The meals were then stripped of solvent and aliquots of the dried meal were immediately analyzed for CPA.

Since the nonvolatile acids remain in contact with the meal during the period when the solvent is being stripped off, it was necessary to determine whether these acids would continue to deactivate CPA by prolonged contact with the meal. Portions of the meals, which had been treated with the cottonseed fatty acids, stearic acid and oleic acid were analyzed after standing for periods of time ranging from 72 hr to 3 months at room temperature.

To determine the effect of reflux temperature on the rate of deactivation, a series of experiments was carried out by using 65–110 PE at a reflux temperature of approximately 70 C for 2 hr instead of the lower-boiling point solvent.

Room-Temperature Storage With 1.0 wt % Acid. A 1.0 wt % acid concentration was deposited on the meal from PE solution. The solution was mixed thoroughly with the meal by rotating the mixture on the Rotavapor for several minutes. The solvent was then stripped off the meal. The dried meal was stored for 72 hr at room temperature in amber bottles. At the end of this period, portions of the meal were analyzed and the remainder was returned to the amber bottles for CPA analysis after 1–2 month storage.

Drying at 50 C With 0.1 wt % Oleic Acid. A 0.1 wt % oleic acid concentration was dissolved in 65–110 C PE and thoroughly mixed with the meal. The meal was placed in a vented oven at 50 C and allowed to dry at a natural rate for 72 hr. The meal was slightly moist after 24 hr. At the end of the 72 hr period, the meal was allowed to cool and then analyzed.

Soaking in Solvent Containing 0.1 wt % Oleic Acid at 50 C. This series of experiments with oleic acid was similar to that described above, except that the excess solvent was maintained throughout a 72 hr period by occasionally adding fresh solvent. Samples were withdrawn for analysis after 24, 48 and 72 hr soaking in the presence of solvent and oleic acid.

Soaking in Solvent Containing 0.1 wt % Acid and Drying at 50 C and at Room Temperature. A portion of the meal used in the above experiment was removed from the oven after 24 hr and allowed to dry. It was stored at room temperature for 72 hr before being analyzed.

Soaking in 0.1 wt % Acid at Room Temperature and Storage at Room Temperature. The meal was soaked 1 hr in the reagent solution. The solvent was stripped off and the dried meal was stored 72 hr at room temperature.

Hydrolyzed Crude Cottonseed Oil. A 5 ml sample of hexane-extracted crude cottonseed oil was dissolved in 25 ml % PE and refluxed with 5 ml of con-

TABLE I

Analyses of Chemically Treated Cottonseed Meals[a]

Sample	Cyclopropenoid fatty acids[b] (CPA), ppm	Moisture, %	Crude fiber, %	Fat, %	Total nitrogen, %	Epsilon free amino-lysine, g/16 g of nitrogen	Free gossypol, %	Total gossypol, %	Sulfur, %	Iron, %
Commercial screw-pressed (SP) meal	136	6.50	12.8	3.06	6.85	3.57	0.03	1.01	0.49	0.0095
SO_2-treated SP meal	1.9	7.40	11.9	2.54	6.68	3.60	0.04	1.00	1.66	0.0095
Oleic acid-treated DSE meal	26	6.42	11.4	4.26	6.78	3.88	0.13	0.83	0.54	0.0035
Thioglycollic acid-treated DSE meal	24	6.04	11.4	3.08	6.76	3.78	0.11	0.83	0.73	0.0037

[a] All analyses except CPA content determined by Barrow-Agee Laboratories, Memphis, Tennessee.
[b] CPA values obtained immediately after treatment by method of Levi et al. (6).

TABLE II
Composition of Diets[a]

Component		Percentage
Ground yellow corn		59.81
Soybean oil meal[b] (44%)		25.00
Stabilized lard[c]		2.00
Meat and bone meal (50%)		2.50
Dehydrated alfalfa meal (17%)		2.50
Ground limestone		7.25
Dicalcium phosphate (18%)		0.50
Salt		0.25
Trace mineral premix[d]		0.05
Manganese (as sulfate)	12.20%	
Iron (sulfate)	5.40%	
Copper (sulfate)	0.73%	
Cobalt (sulfate)	0.20%	
Iodine (calcium iodide)	0.38%	
Zinc (oxide)	10.00%	
Calcium (carbonate)	5.68%	
Vitamin premix[e]		0.14
Vitamin A	4,897 units/lb	
Riboflavin	1.8 mg/lb	
Niacin	25.4 mg/lb	
Pantothenic acid	5.3 mg/lb	
Choline	730. mg/lb	
Vitamin E	27.4 mg/lb	
Vitamin B_{12}	0.002 mg/lb	
Vitamin D	225 units/lb	
Total		100.00

[a] Analysis of the diets showed an average content of protein 18%, fat 4.48%, fiber 4.2%, ash 11.4%, calcium 3.23%, phosphorus 0.52% and an average caloric value of 931.4 cal/lb.
[b] All diets were identical except for replacement of 20 wt % of soybean meal for experimental rations as identified in Tables I and III.
[c] Positive control ration contained 2% refined cottonseed oil (25 ppm CPA) in place of stabilized lard.
[d] Composition of mineral premix as indicated.
[e] Values for vitamins are total amounts in diet.

centrated sulfuric acid for 15 min. The mixture was then separated and the organic phase was washed with several portions of water. In a second experiment, an equal weight of glacial acetic acid was added directly to a portion of the crude oil. The mixture was allowed to stand overnight at room temperature. It was used the following day without removal of the acetic acid. Solutions (0.1 wt %) of the acidified crude cottonseed in PE were mixed with the meal samples. Mixing continued for 1 hr. The solvent was stripped, and the dried meal samples were stored for 72 hr. An experiment using only acetic acid was carried out as a control.

Sulfhydryl Compounds

Addition of Liquid and Solid Compounds. The liquid and the solid sulfhydryl compounds were used in essentially the same manner as described for the dilute organic acids. Solutions of each compound were prepared at 0.1 wt % with respect to the amount of meal to be treated. The mercapto compounds, which were not soluble in PE, were dissolved in the acetone-hexane-water (AHW) azeotrope solvent used for the analytical extraction of the meal, since this solvent has little effect on the CPA content in the meal. The reagent solution was thoroughly mixed with the meal and the mixture stored for 24 hr in a glass-stoppered round bottom flask. The solvent was then stripped and the meal was evacuated on a rotary evaporator at 50 to 60 C for several hours until most of the sulfhydryl odor had disappeared. In all cases, a slight reagent odor remained even after a 6 hr period. To avoid further deactivation, portions of each of the meals were extracted and the extracts were stored in the refrigerator for analysis the next morning.

Sulfhydryl compounds tested by the above procedure included glutathione, 3-mercaptopropionic acid, ammonium thioglycollate and thioglycollic acid. Since only thioglycollic acid significantly deactivated CPA in the meal, a follow-up experiment was conducted in which 1 wt % of the acid was deposited on the meal by using the same procedure described for the dilute acids. The meal was allowed to remain in contact with the thioglycollic acid for 72 hr. The meal was then transferred to a round bottom flask, evacuated for 6 hr at 50 to 60 C and immediately extracted and analyzed.

TABLE III
Experimental Rations

Diet no.	Type of meal used	Meal in diet, %		CPA in diet, ppm[a]	Consumption, gm/Hen[b]
		Soybean	Cottonseed		
1	Soybean (negative control)	25	0	0	1700
2	Soybean + 2% cottonseed oil (positive control)	25	0	26	1613
3	Commercial SP meal	5	20	27.2	1906
4	SO_2-treated SP meal	5	20	0.4	2203
5	Oleic acid-treated DSE meal	5	20	5.2	1771
6	Thioglycollic acid-treated DSE meal	5	20	4.8	1090

[a] Calculated on basis of analysis of the cottonseed meal.
[b] Mean value ration consumed per hen. Based on 6 hens.

Addition of Methanethiol. Methanethiol (MeSH), a gas at room temperature, was used in the gaseous form in the column method described above for the use of anhydrous gases.

Liquefied MeSH contained in a 100 g glass ampul was cooled to about −30 C to ensure safe cutting and removal of the neck of the ampul. The meal (100 g) was loosely packed into the column, the ampul neck was broken and the bottom of the column was directly connected to the ampul while being maintained in the cold bath. The coolant was then replaced with a water bath maintained at about 1 C above the boiling point (5.9 C) of MeSH by the addition of ice. Evolution of MeSH vapor at this temperature was extremely slow. The water-bath temperature was gradually raised to about 15 C so that vaporization of the reagent could be maintained at a fairly moderate rate.

Although the amount of MeSH used was about fourfold greater than the molar amounts of the other gases used, it was believed that if the deactivation efficiency of MeSH were relatively low, the maximum effects would thus be observed. Also, if MeSH proved to be an excellent deactivating reagent, scaling down to an optimum reagent concentration would probably require fewer experiments than scaling up from relatively low degrees of deactivation.

The excess unreacted gas from the top of the column was fed into an absorption trap containing concentrated aqueous sodium hydroxide and into a second trap containing an approximately 1 M solution of mercurous oxide in dilute acetic acid. After treatment, air was blown through the column for 72 hr. Since the MeSH odor was still very strong, the column was heated for a short time with a hot air gun. After about 30 min, no decrease in odor was noted.

The meal was transferred to a round bottom flask and placed on a rotary evaporator and evacuated for 5 hr as described for the liquid and solid sulfhydryl compounds. A strong MeSH odor still remained and the water-bath temperature was raised to its boiling point. After about 15 min at this temperature, the meal turned dark brown and was removed from the bath. Although the MeSH odor had disappeared, the toasted meal had a strong unpleasant odor. No further attempts to deodorize the meal were made.

Since it was difficult to eliminate the unpleasant MeSH odor without heating the meal, a control meal was also prepared for comparison. The CPA concentration obtained for the control was used as the reference value for this experiment. It was assumed

TABLE IV
Chemical Deactivation by Gases

Meal		Reagent	Crude extract, % of meal	Methyl esters, % of meal	CPA, ppm	Reduction of CPA, %
Type	Moisture, %					
Screw-pressed	7	Control	4.9	3.5	142.8	
		SO_2	6.4	3.5	11.8	92
Screw-pressed	20	Control	6.9	4.3	186.0	
		SO_2	5.9	4.1	4.9	97
		CO_2	8.0	3.7	194.5	0
		H_2	5.7	4.3	188.7	0
Direct solvent extracted	20	Control	4.6	1.9	50.3	
		SO_2	4.2	1.4	Trace	99
		CO_2	4.5	1.8	51.7	0

that the extent of CPA reduction, due to heating, would be additive and that any significant differences between the two meals would be attributed to the chemical alteration of the CPA.

Preparation of Rations

Specific details describing the incorporation of the experimental meals into the rations has been reported (2). In the present study the screw-pressed meal (SP) was treated with dry sulfur dioxide, while the direction hexane-extracted meal (DSE) was treated with oleic acid and thioglycollic acid. The composition of the original and treated meals is shown in Table I. Two control diets were used. The CPA negative control included 25 wt % soybean meal as a protein supplement (Table II) and the positive CPA control contained 2% refined cottonseed oil of known CPA content in place of the stabilized lard. For the three experimental rations, 80% of the soybean meal was replaced by the cottonseed meal as indicated in Table III. The remaining composition of the diet was identical for each ration.

Feeding Studies and Techniques. For the feeding studies sufficient feed was prepared to maintain 6 individually caged hens on each ration for 1 month. This period included a preliminary two-week equilibration period prior to the two-week test period during which eggs were collected. The eggs laid during this second two-week period were collected and placed in storage for 3 months at 35 F. A few eggs from each category were also stored for 6 months at 35 F. Upon completion of the storage period, the eggs were examined for yolk and albumen discoloration, pH of the yolks, yolk color and the fatty acid composition of the pooled yolk lipids.

Results and Discussion

Anhydrous Gases

After degassing and drying, duplicate samples of each meal were obtained by quartering and analyzed for residual CPA. The data are presented in Table IV. Of the three gases tested, only SO_2 proved effective in reducing the CPA content of the meals.

Although the crude extract weights of all the treated meals varied greatly, the values calculated for the recovered methyl ester in the analytical procedure were fairly consistent and duplication of the CPA results was excellent. Some of the variation of the crude extract values was probably due to the unusually large amount of water extracted from the meals, droplets of which became evident when the samples were stripped of solvent. This excess water apparently

TABLE V
Chemical Deactivation by Dilute Organic Acids[a]

Reagent		Method	CPA			Recheck time after initial analysis	CPA		
Type	Conc. wt %		Control,[b] ppm	Exptl, ppm	Change from control, %		Exptl, ppm	Change from control, %	Change from initial change, %
Cottonseed fatty acids	0.1	2 hr Reflux at 40 C	133	102	−23	72 hr	56	−58	−25
	0.1	2 hr Reflux at 70 C	87	55	−37	1 week	71	−18	+19
						2.5 months	85	0	+37
	1.0	1 hr Soak + 72 hr dry storage	101	69	−31	1 month	88	−22	+ 9
Stearic acid	0.1	2 hr Reflux at 70 C	87	27	−70	3 months	61	−29	+41
	1.0	1 hr Soak + 72 hr dry storage	101	60	−40	1 month	93	− 8	+32
Oleic acid	0.1	2 hr Reflux at 70 C	87	14	−84	3 months	22	−75	+ 9
	1.0	1 hr Soak + 72 hr dry storage	101	59	−41	1 month	77	−24	+17
	0.1	72 hr at 50 C	103	53	−49				
	0.1	24 hr at 50 C in solvent	103	70	−32	72 hr[c]	96	− 7	+25
	0.1	48 hr at 50 C in solvent	103	78	−22				
	0.1	72 hr at 50 C in solvent	103	99	− 4				
Lactic acid	0.1	1 hr Soak + 72 hr dry storage	133	86	−35	2 months	94	−29	+ 6
	1.0	1 hr Soak + 72 hr dry storage	133	84	−37	2 months	91	−32	+ 5
H_2SO_4-treated crude cottonseed oil	0.1	1 hr Soak + 72 hr dry storage	133	99	−27				
Acetic acid-treated crude cottonseed oil	0.1	1 hr Soak + 72 hr dry storage	133	99	−27				

[a] Screw-pressed cottonseed meal used for organic acid deactivation.
[b] Control carried through experimental procedure.
[c] Dry storage.

TABLE VI
Chemical Deactivation by Sulfhydryl Compounds

Reagent		Method	CPA		
Type	Conc, wt %		Control, ppm	Exptl, ppm	Change from control, %
Glutathione	0.1	24 hr Storage in solvent	134	135	0
3-Mercaptopropionic acid	0.1	24 hr Storage in solvent	134	137	0
Ammonium thioglycollate	0.1	24 hr Storage in solvent	134	140	0
Thioglycollic acid	0.1	24 hr Storage in solvent	132	83	−37[a]
	1.0	Drying and 72 hr standing at room temperature	101	69	−32[b]
MeSH		See text	18[c]	19[c]	0

[a] Recheck of an aliquot of this sample after 2 months showed an additional 19% decrease, so the net change from the control was −55% (40 ppm CPA).
[b] Recheck of an aliquot of this sample after 1 month showed an additional 14% decrease so the net change from the control was −46% (55 ppm CPA).
[c] Toasted meal.

removed non-lipid materials that precipitated when the water was azeotroped from the sample. The amount of extracted water varied noticeably from sample to sample; practically no free water appeared in some samples, while others contained large droplets. When large amounts of water were present, the water was azeotroped by using a mixture of hexane and acetone in the proportion normally used for the AHW azeotrope. In most cases, a yellow solid was found in the extract after the water had been removed. Neither PE nor methanol dissolved the solid and it was discarded as a solid during the regular cleanup procedure.

Determining the amount of gas adsorbed on the meal during treatment proved difficult because of the tendency of the meal to lose the excess moisture very rapidly. Of the meals weighed immediately after treatment, only those treated with SO_2 showed a gain in weight (about 10%); those treated with CO_2 showed a loss in weight. The degree to which the loss of moisture was compensated by adsorption of the reactant could not be immediately determined. When the meals were weighed after degassing, all samples showed a loss in weight. However, this decrease in weight never exceeded the weight accounted for by loss of excess moisture. In the dry meal treated with SO_2, the net change in weight was negligible, although before degassing it had showed a weight increase of 13%.

Dilute Organic Acids

A summary of the deactivation obtained with dilute organic acids is presented in Table V. The percentages of deactivation represent the actual deactivation due to the presence of the reagent and were adjusted for any decrease in CPA found for the control samples. These were exposed to identical experimental conditions, except for the organic acid.

The experimental conditions were kept relatively simple and mild for two reasons. First, one of the primary considerations was the ease and economy of possible commercial application, by the use of minimal solvent to meal ratios which would permit the inactivation treatments to be carried out commercially in a large storage tank or cooker at a relatively low cost. Second, mild conditions maintain the nutritive value of the meal. Because the meal treated at 100 C became toasted, the nutritive value of this meal was undoubtedly affected.

It was hoped that deactivation with the dilute acids could be accomplished under dry conditions, such as those used for bleaching wheat flour where the bleaching agent is mixed with the flour at the time of packaging and bleaching occurs during storage. This was not the case, however, for the dilute organic acids. Although the CPA values continuously decreased during the first 3 to 4 days of storage, data for samples analyzed after 1 month or more of storage indicated that some of the originally deactivated CPA tends to regenerate (Table V). Since an even cursory investigation into the chemical mechanism of the organic acid deactivation of CPA in the meal was not possible, we cannot speculate on the probable reason for the apparent regeneration of the CPA. The data in Table V, however, cannot be due to any error in analysis, since most of the data was generated on different days and is too consistent to be accidental. In addition, the effect of the presence of free organic acids in the Halphen reaction as well as during the sodium methoxide workup procedure was checked and

TABLE VII
Composition of Chemically-Treated Cottonseed Meals Used for Laying-Hen Feeding Studies

Meal			Components in Meal								
Treatment	Moisture	Treatment reagent	CPA ppm	Crude fiber, %	Fat, %	Total N_2, %	Epsilon-free amino-lysine, g/16 g N_2	Gossypol free, %	Gossypol total, %	S, %	Fe, %
Screw-pressed	6.50	None[a]	136	12.8	3.06	6.85	3.57	0.03	1.01	0.49	0.0095
Screw-pressed	7.40	SO_2	1.9	11.9	2.54	6.68	3.60	0.04	1.00	1.66	0.0095
Direct solvent extracted	7.46	None[a]	37	11.9	2.78	7.05	3.94	0.18	0.88	.....	.....
Direct solvent extracted	6.42	Oleic acid	26[b]	11.4	4.26	6.78	3.88	0.13	0.83	0.54	0.0035
	6.04	Thioglycollic acid	24[b]	11.4	3.08	6.76	3.78	0.11	0.83	0.73	0.0037

[a] Control meal, no treatment.
[b] Value obtained immediately after treatment.

found to have no effect at the CPA levels in these cases.

In the case of the thioglycollic acid, deactivation continued during dry storage, but very slowly. Data on the follow-up study of this compound are included in Table VI.

Sulfhydryl Compounds

Of the sulfhydryl compounds screened, only thioglycollic acid was effective, as indicated in Table VI.

The two heated meals had practically identical CPA concentrations, so under the conditions used MeSH probably did not contribute significantly to deactivation.

Based on the results from the chemical inactivation experiments above, the residual CPA, in 30 lb lots of commercial screw-pressed and direct hexane-extracted cottonseed meals, was treated with sulfur dioxide, oleic acid and thioglycollic acid. These large batches of treated meals were then used for a biological screening evaluation by incorporating them into the rations of laying hens.

Sulfur Dioxide Treatment

Because of the high deactivation efficiency of SO_2, high-CPA-content screw-pressed meal was used. Since this treatment does not require the use of solvents, it should be suitable for commercial plants not having solvent handling facilities.

A 30 lb batch of meal was placed into a 2.75 ft^3 stainless steel reactor. The meal was supported by a perforated stainless steel plate set 3 in. above the bottom of the vessel. The vessel was fitted with a gas inlet, a vacuum inlet and a safety pressure vent. A standard pressure gauge was used to measure the internal pressure and a thermometer was set so that its sensing element was about halfway between the top and the bottom levels of the meal.

Technical grade SO_2 gas at 15 psi was piped into the reaction vessel. The reaction was allowed to proceed without external heating. After 30 min, the temperature had risen to 40 C and the pressure had dropped to about 5 psi. The vessel was again pressurized to 15 psi and the reaction was continued for an additional 30 min. The temperature was then 45 C. Treatment was discontinued and the reactor was thoroughly flushed with nitrogen. Vacuum was applied for several hours and the tank was again flushed with nitrogen. Purging was repeated twice more to remove the major portion of the excess SO_2. The results are given in Table VII.

Oleic Acid and Thioglycollic Acid Treatment

Because the deactivation efficiency of oleic and thioglycollic acids is relatively low, direct solvent-extracted meal, which has a lower CPA content than screw-pressed meal, was used. Also, the solvents used to prepare direct solvent-extracted meal create no great problems for processors using solvent extraction.

Treatments with these two reagents were carried out in the reaction vessel used for the SO_2 treatment, but the perforated steel support plate was removed and vanes were placed in the sides of the tank to cause tumbling to aid mixing. The vessel was set in a horizontal position and rotated occasionally to ensure thorough mixing of the meal with the reagent solution. Hot water was piped through the external heating coils to heat the reaction mixture. Although the water temperature was 70 C, the maximum internal temperature obtainable with this system was 43 C. The internal pressure was approximately 5 psig.

For the oleic acid treatment, 0.3 lb (1.0 wt % of the meal) of the acid was dissolved in 30 lb of PE. For the thioglycollic acid treatment, a similar amount was dissolved in about 1 liter of methanol and the methanolic solution was mixed with 30 lb of PE. In each case the meal was mixed thoroughly with the reaction solution and heated for 72 hr in the sealed vessel to allow the pressure to rise. At the end of the 3-day reaction period, vacuum was applied to strip the solvent. The meals were air dried on trays for an additional 24 hr to evaporate any residual solvent. This procedure was employed since the primary purpose was to evaluate the effect of the chemical treatment of the meal and any material incidentally extracted from the meal would be retained. In commercial practice, however, the solvent should be removed after 3 to 4 days by filtration, during which a large additional portion of the residual CPA would be extracted. The results are also given in Table IV.

The data obtained from the quality evaluations of the stored eggs from each of the experimental diets are outlined in Table VIII. As expected, the negative soybean meal control diet, which had no CPA also produced no adverse effects on white or yolk discoloration and the yolk pH was normal. Addition of 2% of refined cottonseed oil to the soybean meal control diet, providing a dietary level of 25 ppm of CPA, produced effects attributable to CPA, i.e., 26% pink whites and 14% discolored yolk, and an increase in yolk pH of the eggs stored for three months at 35 C.

Inclusion of the sulfur dioxide-treated meal in the ration at a 20% level (CPA, 0.4 ppm) was effective in improving the quality of eggs stored for 3 months, as compared to the untreated control meal (CPA, 27 ppm) at the same dietary level. The incidence of pink whites and discolored yolks, 2.0% and 7.2% respectively, in eggs from hens fed the SO_2-treated meal were markedly lower than those produced by rations containing the untreated cottonseed meal, 55% pink

TABLE VIII

Chemically Inactivated Cottonseed Meal Feeding Study: Physical Evaluation of Eggs Stored at 35 F

Type of ration	CPA[a] ppm	Gossypol %[a]		Storage period months	Number of eggs evaluated	% Discolored		Average pH	
		free	total			yolk[b]	white[c]	yolk	white
Negative control (soybean meal)	0	.....	.....	3	29	0	0	6.5	9.1
				6	11	0	0	6.8	8.6
SO_2-treated SP meal	0.4	0.008	0.20	3	56	7.2	2.0	6.7	8.9
				6	23	22.7	13.0	7.0	8.6
Thioglycollic acid-treated DSE meal	4.8	0.022	0.17	3	19	31.6	0	6.8	8.9
				6	15	100	6.7	6.9	8.6
Oleic acid-treated DSE meal	5.2	0.026	0.17	3	39	66.7	7.7	6.7	9.0
				6	19	100	31.6	7.2	8.5
Positive control (2% C/S oil)	25	0.00	0.00	3	52	13.5	62	7.9	8.7
				6	25	42.8	40.0	7.7	8.6
Commercial screw-pressed meal control	27.2	0.006	0.22	3	64	50.0	55	7.5	8.7
				6	23	81.8	60.9	7.8	8.5

[a] Amounts in diet calculated on basis of 20 wt % cottonseed meal in ration.
[b] Yolk discoloration based on color values greater than 8.0 on the Hoffman-LaRoche scale.
[c] Pink whites.

TABLE IX
Chemically Inactivated Cottonseed Meal Feeding Study
Yolk Lipid Fatty Acid Distribution in Eggs Stored at 35 F

Type of ration	CPA[a] ppm	Storage period months	Fatty acid distribution wt %						Ratios	
			C_{14}	C_{16}	C_{18-1}	C_{18}	C_{18-1}	C_{18-2}	C_{18}: C_{18-1}	C_{16}: C_{16-1}
Negative control (soybean meal	0	3	0.3	26.2	2.5	9.9	46.4	14.7	0.21	10.5
		6	0.4	26.5	2.4	9.7	46.6	14.4	0.21	11.0
SO_2-treated SP meal	0.4	3	0.3	26.8	2.2	10.7	47.4	12.6	0.23	12.2
		6	0.3	27.2	1.8	11.0	48.2	11.5	0.23	15.1
Thioglycollic acid-treated DSE meal	4.8	3	0.2	26.0	1.6	12.0	45.4	14.1	0.26	16.3
		6	0.4	25.1	1.6	12.1	45.9	14.4	0.26	15.7
Oleic acid-treated DSE meal	5.2	3	0.3	25.9	1.6	11.9	46.4	13.9	0.26	16.2
		6	0.3	26.3	1.4	12.4	46.0	13.6	0.27	18.8
Positive control (2% C/S oil)	25	3	0.4	27.6	1.3	14.9	36.9	18.9	0.40	21.2
		6	0.4	27.1	1.2	14.6	37.9	18.8	0.39	22.6
Commercial screw-pressed meal control	27.2	3	0.4	28.3	1.4	14.4	40.3	15.2	0.36	20.2
		6	0.4	28.6	1.2	14.5	41.7	13.6	0.35	23.8

[a] Calculated on basis of 20% wt % C/S meal in ration.

whites and 50% discolored yolks. The yolk pH of the stored eggs, 6.7, was normal. Even after 6 months' storage there was only a moderate increase in the discoloration of whites and yolks.

In the case of the thioglycollic acid-treated meal, eggs from hens fed this chemically treated ration (CPA, 4.8 ppm) showed no evidence of pink white discoloration after 3 months' storage and only a small increase, 6.7%, after 6 months' storage. Moreover the yolk pH was normal and fully comparable to that of eggs from the negative soybean meal control diet. There was, however, a marked increase in the yolk discoloration in the stored eggs from hens fed the thioglycollic acid treated meal which undoubtedly reflected the fourfold increase in the free gossypol content (220 ppm) of this meal over that of the control ration (60 ppm). After 3 months' storage 32% of the yolks were discolored and after 6 months, 100% of the eggs had discolored yolks. Thus, thioglycollic acid treatment of cottonseed meal appears to be effective for preventing pink white discoloration attributed to CPA, but rather ineffective for preventing the yolk discoloration in stored shell eggs attributed to gossypol.

Oleic acid treatment of cottonseed meal (Table IV) was less effective than either sulfur dioxide or thioglycollic acid treatments for improving the quality of stored eggs. Although the incidence of pink whites, 7.7%, was moderate in 3-month stored eggs, this value increased to 31.6% after 6 months' storage. Moreover, the high incidence of yolk discoloration, 67%, after 3 months and 100% after 6 months' storage is substantially higher than the 50.0% and 81.8% yolk discolorations observed for the 3 and 6 months' storage period, respectively, for the untreated control ration. However, here again the high free gossypol content (260 ppm) of the oleic acid-treated meal is greater than that of either the thioglycollic acid treated meal (220 ppm) and the untreated control ration (60 ppm).

The moderate degree of yolk discoloration observed for the positive control meal, which is free of gossypol, can be attributed to the high CPA content in this ration. Although yolk discoloration is normally associated with the free gossypol present in the meal, it can also accompany rations high in CPA and free of gossypol. The overall effects of high CPA containing meals, both in the presence and absence of free gossypol on yolk discoloration, has been reviewed by Phelps et al. (4).

In view of the residual odor associated with the final meal rations containing the SO_2 and the thioglycollic acid treated meals, some concern was given as to the hens' acceptance of the meal. Table III includes the consumption levels of the various ration groups and, as can be seen, only the thioglycollic acid group displayed a noticeable reduction in ration consumption.

Yolk Lipids

The fatty acid distribution of the yolk lipids (Table IX) appears to be dependent on the CPA content of the meal as was observed in earlier feeding studies (2,18). That is, the ratio of stearic (C_{18}) to oleic acid (C_{18-1}) and palmitic (C_{16}) to palmitoleic acid (C_{16-1}) increase with increasing CPA level in the ration. The C_{18}/C_{18-1} value increased from 0.21 for the negative control to a value of 0.40 for the positive control delivering 25.0 ppm of CPA in the ration. The commercial screw-press control meal delivering 27.2 ppm of CPA in the meal gave a ratio of 0.36, slightly lower than the value (0.40) observed for the positive control. The three remaining chemically treated meals, having CPA contents of 0.4, 4.8 and 5.2 ppm CPA, gave C_{18}/C_{18-1} values of 0.23, 0.26 and 0.26, respectively. The values, in the order given, represent the SO_2, thioglycollic acid and oleic acid treated rations.

A comparison of the yolk fatty acid values for the 3- and 6-month stored eggs, indicate a very similar pattern. This fact was observed by Frampton et al. (18) in a previous feeding study, which demonstrated that changes in the fatty acid patterns of yolk lipids take place during egg development and do not change during storage.

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