Physiological Evaluation of Solvent-Treated Cottonseed Meals in Rations for Laying Hens[1,2]

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Abstract

Two commercial cottonseed meals, a direct solvent-extracted meal and a screw-pressed meal, were subjected to successive solvent-extraction procedures by using an acetone/hexane/water azeotrope to reduce their cyclopropenoid fatty acid content. The two original commercial meals, as well as a double- and triple-extracted portion, 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 2% refined cottonseed oil of known CPA content were also employed. During a four-week feeding period the eggs were collected during the third and fourth week and stored at 35F for periods of three and six months. Over-all egg quality and the fatty acid distribution of the extracted yolk lipids were determined after the three- and six-month storage period.

Introduction

UTILIZATION OF COTTONSEED MEAL as a protein supplement in the ration of laying hens has been found somewhat limited because of certain unique biological effects on laying hens and on the storage quality of their eggs (1). These over-all effects can be attributed to the presence of gossypol, a pigment associated with the cottonseed pigment glands, and to cyclopropenoid fatty acids (CPA), which are associated with the residual cottonseed oil in the meal. Some evidence has also indicated that the cyclopropenoid moieties might be associated with the phospholipid fraction of the meal because of the difficulty with which they are removed by successive solvent-extractions (2).

The effect associated with the presence of gossypol, i.e., a brown or olive discoloration of the yolk in stored eggs, has been minimized by breeding a glandless strain of cotton (3) or by the addition of certain mineral salts to the ration (4).

The effects associated with the presence of CPA in the rations of laying hens have been shown to involve fatty acid metabolism, as indicated by altered fatty acid patterns of yolk lipids (5), pinkish discoloration of stored egg whites (6,7), pH changes of stored egg yolks and whites (7), decreased egg hatchability (8), and a delay in the sexual maturity of hens (9). The levels of CPA required to exhibit these various effects vary, and the exact mode of involvement of CPA still remains uncertain.

Kircher (10) has shown that the cyclopropenoid structure can react irreversibly with sulfhydryl compounds and that this reaction might possibly be responsible for its observed physiological properties. Since the coenzymes involved in fatty acid metabolism are sulfhydryl in nature, competition for the coenzyme would certainly be possible. More recent findings reported by Allen et al. (11) have, in fact, shown that cyclopropene fatty acids are specific, irreversible inhibitors to the dehydrogenase system and that sterculic acid was a more effective inhibitor than malvalic acid. It is also believed that the presence of CPA decreases the permeability specificity of the vitelline membrane surrounding the yolk sac. Whether or not these are the sole factors responsible for inducing the pH, discoloration, and fatty acid alterations is not known.

The present study was undertaken to evaluate the effect of various solvent-treated commercial cottonseed meals (12) on the storage quality of eggs when such meals were incorporated at 20 wt % levels in the rations of laying hens.

Experimental Procedures

Meals and Rations

The meals employed in this study were commercial, direct hexane-extracted (DSE) and screw-pressed meals (SP). Portions of each meal were subjected to a double and also a triple percolation extraction with an acetone/hexane/water azeotrope. Details on the preparation of the meals have been reported by Reilich et al. (12), and the composition of the original and extracted meals is listed in Table I.

Two control diets were used. The CPA negative control included 25% soybean meal as the 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 six experimental rations 80% of the soybean meal was replaced by cottonseed meal, as indicated in Table II. The remaining composition of the meal was identical for each ration.

Feeding Studies

For the feeding studies sufficient feed was prepared to maintain six individually caged hens on each ration for one 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 the second two-week period were collected and placed in storage for three months at 35F. A few eggs from each category were also stored for six months at 35F. The eggs from each hen, in each ration group, were evaluated individually for over-all quality.

Upon completion of the storage period, the eggs were examined for yolk and albumen discoloration, pH, yolk color (as measured by the Hoffman-LaRoche color scale), and standard egg-quality measurements such as weight, Haugh units, USDA grade, and score. Yolk lipids within each dietary treatment were pooled and analyzed for fatty acid patterns.

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[3] So. Utiliz. Res. Dev. Div., ARS, USDA.

TABLE I

Properties of Cottonseed Meals Used in Feeding Studies

Meal No.	Type of meal	AHW extractions [c]	Properties of Meals: CPA ppm	Moist, %	Lipids, %	Crude fiber, %	Total N, %	Gossypol, % Free	Gossypol, % Total	*epsilon* Free amino lysine g/16 g N
3a	DSE [a]	None	70	7.46	2.8	11.9	7.05	0.18	0.88	3.9
3b	DSE	2	8	6.84	0.52	13.1	6.69	0.11	0.82	3.5
3c	DSE	3	5	8.60	0.55	10.6	7.01	0.13	0.84	3.5
4a	SP [b]	None	170	6.92	3.48	12.3	7.23	0.05	1.07	2.5
4b	SP	2	29	7.54	0.81	12.9	6.86	0.04	0.86	2.6
4c	SP	3	3	8.36	0.15	13.0	6.95	0.05	1.03	2.5

[a] Direct hexane-extracted commercial meal.
[b] Commercial screw-pressed meal.
[c] Number of supplementary percolation extractions with acetone:hexane:water solvent (12).

Analysis of Yolk Lipids

After quality evaluation of the stored eggs, yolks in each ration category were pooled and mixed. Duplicate aliquots were dried by lyophilization for 16 hr and ground in a mortar. Yolk lipids were extracted with chloroform:methanol (1:1), as suggested by Frampton et al. (5). Lipid content of the yolks ranged from 65 to 78% of the dry powder. One gram of each of the concentrated yolk lipids was converted to methyl esters according to the methanolysis procedure of Luddy et al. (13) and made up to known concentrations.

Response values were determined by using standard mixture K-108 (Applied Science Laboratories) and the National Institute of Health standard mixture D (14). Methylheptadecanoate was employed as an internal standard. The analyses were performed on a Loenco model 70 gas chromatograph with a dual hydrogen flame-ionization detector. Over-all operating parameters included a coiled copper column $\frac{3}{16}$ in. × 8 ft containing 12 wt % diethylene glycol succinate on Anakrom ABS (60–70 mesh). The column, preheater, and detector temperatures were maintained at 180, 230, and 250C respectively.

Results and Discussion

Quality evaluations of eggs after a three-month storage period at 35F are given in Table IV for each of the experimental diets. Although six hens were alloted to each treatment, some did not produce eggs during the two-week collection period. It may be noted that the positive CPA control (Diet 1, Table IV), delivering 26 ppm of CPA, did not produce discolored yolks or whites or any alteration in the pH values of the yolks and whites. In contrast to this, the original, direct solvent-extracted commercial meal (Diet 3a), which delivered 14 ppm of CPA, produced a high incidence of yolk and albumen discoloration although the pH of yolks and whites, 7.0 and 8.5 respectively, were only slightly different from the negative control (Diet 2).

The major incidence of yolk and white discolorations was observed for the two untreated commercial meals (Diets 3a and 4a, Table IV). However only the untreated screw-pressed meal (Diet 4a) produced pH values of yolks and whites significantly different from those of the negative control (Diet 2). A double AHW extraction of either the direct solvent or screw-pressed meals produced a marked decrease in the incidence of yolk and white discoloration (Diets 3b, 4b) while a triple extraction (Diets 3c, 4c) produced no discolored yolks and only three discolored whites in 108 eggs (2.8%).

From the above data it appears that a double solvent-extraction with the AHW azeotrope is sufficient to substantially reduce the incidence of discoloration of both yolks and whites in eggs stored at 35F for three months even at the high ration level of 20 wt %.

From the data accumulated earlier (12) on the effectiveness of CPA removal with successive AHW extractions, it would appear that a single-extracted cottonseed meal might serve as well in the final ration as a double-extracted meal. For instance, the CPA content found for a single-extracted DSE cottonseed meal amounted to 25 ppm and, when incorporated at a 20 wt % ration level, would constitute approximately 5 ppm CPA. This value would be comparable with the CPA level in ration 4b, the eggs from which also

TABLE II

Composition of Diets[a]

Component		Percentage
Ground yellow corn		59.81
Soybean oil meal[b] (44%)		25.00
Stanbilized 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	
		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 in place of stabilized lard.
[d] Composition of mineral premix as indicated.
[e] Values for vitamins are total amounts in diet.

TABLE III

Experimental Rations

Diet No.[a]	Type of meal used	Meal in Diet, % Soybean	Meal in Diet, % Cottonseed	CPA in diet, ppm
1	Soybean +2% CS oil (positive control)	25	0	26
2	Soybean (negative control)	25	0	0
3a	Commercial DSE meal	5	20	14
3b	Meal 3a extr. 2x with AHW	5	20	1.6
3c	Meal 3a extr. 3x with AHW	5	20	1.0
4a	Commercial SP meal	5	20	34
4b	Meal 4a extr. 2x with AHW	5	20	5.8
4c	Meal 4a extr. 3x with AHW	5	20	0.6

[a] Diet numbers of cottonseed meals refers to meal numbers in Table I.

TABLE IV

Evaluation of Interior Egg Quality Criteria on Eggs Stored Three Months at 35F

Diet[a]	CPA[b] in diet ppm	Gossypol %[b] Free	Gossypol %[b] Total	Hen No.	Total number of eggs produced	Number with discolored yolks	Number with discolored whites	Yolk color (Hoffman-LaRoche)	pH of Yolk	pH of Whites	Haugh units	Average egg weight, g
1	26	.....	.....	1	8	0	0	6.2	6.7	8.5	37.9	47.7
				2	3	0	0	6.8	6.7	8.7	64.0	54.4
				3	8	0	0	6.7	6.9	8.5	68.4	52.8
				4	9	0	0	7.0	6.7	8.4	68.3	59.0
				5	10	0	0	7.2	6.8	8.5	65.2	57.2
2	0	.....	.....	7	12	0	0	7.2	6.6	8.6	69.3	53.3
				8	8	0	0	7.0	6.6	8.6	67.6	56.1
				9	12	0	0	7.3	6.7	8.7	70.3	56.6
				10	11	0	0	6.2	6.8	8.6	69.2	50.8
				11	3	0	0	7.0	6.6	8.4	61.7	50.1
3a	14	0.036	0.18	13	9	9	7	c	7.5	8.2	70.5	54.4
				14	9	9	3	c	6.8	8.5	74.6	50.9
				15	4	4	1	c	6.5	8.6	75.8	51.9
				16	10	10	3	c	6.8	8.5	70.8	50.6
				17	10	10	3	c	6.9	8.6	68.7	51.7
				18	9	8	6	c	7.4	8.5	70.3	60.1
3b	1.6	0.022	0.16	19	5	0	0	7.3	6.7	8.5	66.4	50.5
				20	10	1	0	7.2	6.6	8.4	69.4	53.0
				21	9	0	0	7.5	6.7	8.4	67.7	58.6
				22	4	0	0	7.0	6.6	8.5	64.3	55.1
				23	7	0	0	7.6	6.6	8.6	68.0	58.8
				24	8	1	0	7.8	6.4	8.5	71.6	57.9
3c	1.0	0.026	0.17	25	12	0	0	7.1	6.5	8.4	71.6	52.8
				26	10	0	0	7.4	6.4	8.5	71.1	49.5
				27	3	0	9	7.3	6.5	8.3	69.3	51.1
				28	12	0	0	7.5	6.5	8.4	62.4	52.4
				29	8	0	0	7.4	6.5	8.4	72.0	56.8
				30	9	0	0	7.3	6.5	8.4	67.5	56.9
4a	34	0.010	0.23	31	6	4	5	c	7.4	8.4	69.3	56.8
				32	10	10	9	c	8.2	8.2	66.3	57.3
				33	10	10	10	c	8.2	8.5	67.9	54.2
				34	10	10	9	c	8.4	8.4	66.5	55.4
				35	8	7	8	c	8.3	8.4	69.1	55.5
				36	4	4	4	c	8.4	8.4	65.5	54.4
4b	5.8	0.008	0.17	37	6	1	4	7.6	7.1	8.6	69.7	59.4
				38	3	0	0	7.3	6.4	8.6	71.7	63.3
				40	8	0	0	6.8	6.3	8.4	69.5	57.8
				41	8	0	0	7.3	6.4	8.4	62.7	61.7
				42	8	0	0	6.7	6.4	8.5	69.1	56.8
4c	0.6	0.010	0.21	43	3	0	0	6.8	6.6	8.5	66.3	54.1
				44	10	0	0	7.2	6.6	8.6	70.0	52.8
				45	10	0	2	7.3	6.6	8.4	65.1	56.8
				46	10	0	1	7.2	6.7	8.5	68.7	56.9
				47	9	0	0	7.1	6.6	8.5	69.2	57.5
				48	12	0	0	7.7	6.4	8.4	69.3	58.4

[a] Diet No. same as in Table III.
[b] Calculated on basis of 20 wt % C/S meal in diet.
[c] Color too dark to evaluate on Hoffman-LaRoche scale.

showed a marked improvement in quality over the original meal.

Eggs stored for six months were also evaluated for egg quality. On the basis of the relatively small number of eggs evaluated, it appears that no significant changes occurred as a result of the additional three-month storage period. The major exception is the value of the Haugh units, which, as would be expected, was for all groups considerably lower than for the eggs which were three months in storage.

Data on the fatty acid distribution (Table V) of yolk lipids after three months of storage agreed with the previously established findings regarding alterations in yolk fatty acid patterns (increase in stearic acid, reduction in oleic acid) owing to ingestion of CPA (5,15). For example, Sample 2, which represents the negative control ration (soybean meal), exhibited the lowest stearic acid value (10.5%) of the eight rations studied. Increased stearic acid values and increased stearic(S)/oleic(O) acid ratios (S/O) were found for all other groups. The largest stearic acid value (19.3%) was obtained for the commercial screw-pressed control ration (4a), which also contained the highest level of CPA (34 ppm) in the ration. Both the yolks and whites of the eggs from hens fed this ration were also discolored, and, as mentioned earlier, hens fed this commercial screw-press ration also produced eggs exhibiting a marked increase in yolk

TABLE V

Fatty Acid Distribution of Yolk Lipids from Eggs Stored Three and Six Months at 35F

Diet[a] No.	CPA[b] in diet, ppm	Storage period, months	Average Fatty Acid Distribution,[c] % C_{14}	C_{16}	C_{16-1}	C_{18}	C_{18-1}	C_{18-2}	Fatty Acid Ratio Stearic: oleic	Palmitic: palmitoleic
1	26	3	0.4	26.7	1.6	14.1	38.4	18.8	0.37	17
		6	0.3	26.6	1.4	13.8	38.2	19.7	.36	19
2	0	3	0.3	26.0	2.9	10.5	42.8	17.5	0.25	9
		6	0.2	25.4	2.4	10.3	44.0	17.7	.23	11
3a	14	3	0.4	26.7	1.6	14.4	38.7	18.2	0.37	17
		6	0.4	28.0	1.2	13.9	39.5	17.0	.35	23
3b	1.6	3	0.5	25.8	2.4	12.6	41.6	17.1	0.30	11
		6	0.2	24.7	1.9	12.4	42.2	18.6	.29	13
3c	1.0	3	0.5	26.1	2.5	12.0	40.8	17.8	0.29	10
		6	0.4	27.4	1.9	10.9	41.7	17.7	.26	14
4a	34	3	0.5	27.4	1.3	19.3	33.3	18.2	0.58	21
		6	0.3	29.1	1.2	19.0	32.3	18.1	.59	24
4b	5.8	3	0.4	27.1	2.2	12.5	40.4	17.4	0.31	12
		6	0.4	27.3	1.9	10.4	43.0	17.0	.24	14
4c	0.6	3	0.4	27.1	2.0	13.1	40.0	17.4	0.33	14
		6	0.3	28.1	1.8	11.4	41.6	16.8	.27	16

[a] Diet No. same as in Table III.
[b] Calculated on basis of 20 wt % C/S meal in ration.
[c] Average values, based on analysis of two samples of pooled yolks.

pH. Sample 3a, which represents the direct solvent-extracted control, also exhibited discloration of both yolks and whites and has the second largest stearic acid value (14.4%). This sample however did not exhibit an increased pH value above the negative control.

The other groups, including the positive control sample (2% cottonseed oil), all showed increased stearic acid values, but none was as pronounced as the original screw-pressed control ration. Also the remaining groups, as mentioned previously, did not exhibit any color changes in yolks or whites, and their respective pH values were not altered. The ration representing the positive control (2% cottonseed oil) had a high CPA value of 26 ppm but did not show discoloration. Also the average stearic acid value for this sample (14.1%) was similar to that obtained for the direct solvent-extracted control ration (14.4%), which did not discolor, even though the CPA level in the latter ration (14 ppm) was substantially less.

These facts may tend to support the belief that a synergistic relationship exists between CPA's and other components (e.g., gossypol, etc.) in the ration and suggests that higher levels of CPA can be tolerated when these other components are reduced or absent. The fatty acid pattern of the yolk lipids however is affected whether color or pH changes are observed or not and apparently represents a much more sensitive and reliable criterion for low levels of CPA in the ration. Apparently the metabolic involvement of the CPA to block the desaturation of fatty acids is sufficiently sensitive to exhibit distinct and measurable changes even at extremely low CPA values. A similar alteration in the palmitic and palmitoleic acid values was also observed in the yolk lipids, but since the level of palmitoleic acid in yolk lipids represents only a few per cent (1 to 3%) of the total fatty acid content, the accuracy and usefulness of this relationship are rather limited.

The stearic:oleic acid ratios ranging from 0.30 to 0.33 for the AHW-extracted meals (Diets 3b, 3c, 4b, 4c) are of the same order of magnitude as those reported by Rayner et al. (16) in feeding studies with treated Halphen-negative cottonseed oils in the diets of laying hens.

The fatty acid patterns of the eggs opened after six months of storage exhibited no evidence of further reduction in egg quality because of the longer storage period and supported the detailed statistical analyses advanced by Frampton et al. (5), which indicated that there were no further alterations in fatty acid patterns of yolk lipids on account of storage.

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