Acknowledgment

The authors wish to thank D. A. Cook and other members of the Silicate, Detergent, Calcium Division of Diamond Alkali Company for their helpful suggestions.

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[Received November 2, 1956]

Incorporation of Gossypol Into Eggs of Hens Fed Gossypol Schiff Bases

J. M. DECHARY, Southern Regional Research Laboratory,[1] New Orleans, Louisiana, and G. WAKABAYASHI and C. R. GRAU, University of California, Davis, California

IT HAS BEEN REPORTED (4) that gossypol fed to laying hens is deposited in the yolks as gossypol-cephalin and as gossypol-protein complexes. The amount of gossypol present in the yolk is reported to be a sensitive measure of dietary gossypol that is available to the hen, and it forms the basis for a useful definition of available gossypol units (AGU):

$$AGU = \frac{(Abs_{400} - Abs_{445})\text{diet egg} - (Abs_{400} - Abs_{445})\text{pre-diet egg}}{\%\text{ of material tested in diet}} \times 100$$

Where Abs_{400} = Absorbance at 400 mμ of a crude cephalin solution (see Experimental)

Abs_{445} = Absorbance of solution at 445 mμ.

This definition of available gossypol was proposed by Grau and coworkers (1) in their studies of the distinctive yolk component of eggs from hens fed gossypol. The gossypol was administered in cottonseed meal and also as gossypol added to purified diets.

Eggs with absorbance differences of less than 0.03 (that is, diet egg absorbances minus pre-diet egg absorbances) have been found not to discolor either on exposure to an ammonia atmosphere or during storage.[2]

There are two promising approaches to the gossypol-in-egg problem. These are either destruction of gossypol in the cottonseed meal, or its binding through nondigestible linkages. Cooking and screw-pressing the cottonseed reduce the available gossypol, but usually these operations are not sufficiently effective in reducing the gossypol to the point where significant amounts of processed meal may be fed to laying hens. Phloroglucinol can be used to destroy the gossypol in cottonseed meal, but the treatment may reduce the nutritive value of the meal (2). Preliminary experiments show that aniline does not bind the gossypol in cottonseed meals tightly enough to prevent some incorporation into the yolk.

In the present study gossypol Schiff bases prepared from aromatic and aliphatic amines were fed to laying hens at 0.1–0.5% equivalent dietary gossypol levels, and the eggs were examined by spectrophotometry for gossypol-cephalin content. Some of these compounds are apparently stable enough to preclude gossypol incorporation and subsequent discoloration of the eggs.

[1] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] This specification applied only to cottonseed meals and to gossypol derivatives, not to apogossypol and its oxidation products.

Experimental

The examination of the eggs used in this study was restricted to the determination of the available gossypol units as defined above.

The crude cephalin solution was obtained by extracting 10 ml. of yolk exhaustively with acetone, then extracting the residue once with hexane-acetone (3:1), and bringing the volume to 15 ml. The absorbance of this solution was determined with a Beckman [3] Model B Spectrophotometer (1).

Each hen was its own control in that eggs were taken for comparison before the gossypol-containing diet was fed. These were the prediet eggs. The birds were then fed the gossypol-containing diet for at least 10 days before a "diet egg" was taken and examined for the presence of the gossypol-cephalin complex.

AGU values for a series of gossypol Schiff bases are recorded in Table I. Some of the gossypol Schiff bases listed in Table I have not been previously reported and are described in Table II. These compounds were prepared by heating for a few minutes under reflux an isopropyl alcohol solution containing one mole of gossypol and two and one-half moles of the amine (3). The reaction mixture was chilled in the ice-box over-night, and the precipitated base was collected, washed thoroughly with cold solvent, and air-dried. Some of the products were recrystallized from the appropriate solvent before analysis.

[3] It is not the policy of the Department to recommend the products of one company over those of any others engaged in the same business.

TABLE I

Available Gossypol in Eggs from Hens Fed Gossypol Schiff Bases

Gossypol Schiff base	Millimoles equivalent gossypol [c]	% of the diet as gossypol	AGU
Bis(phenylimino)gossypol	38.6	0.5	10.3
(Dianilinogossypol)	12.9	0.17	4.0
Bis(p-carboxyphenylimino)	11.2	0.15	35.5
Bis(benzylimino)	12.4	0.16	.....[b]
Bis(furfurylimino)[a]	12.4	0.16	.....[b]
Bis(cyclohexylimino)[a]	38.6	0.50	4.2
Bis(n-octylimino)[a]	38.6	0.50	0.0
Bis(n-decylimino)	38.6	0.50	0.0
Bis(n-dodecylimino)	38.6	0.50	0.0
Bis(n-tetradecylimino)	38.6	0.50	0.0
Bis(n-octadecylimino)	38.6	0.50	0.2
Bis(dehydroabietylimino)[a]	38.6	0.50	26.2–34.7
Bis(oleylimino)[a]	6.2	0.08	.....[b]

[a] New compounds not previously reported and described in Table II.

[b] Dietary gossypol level was too low to permit a reliable measure of the gossypol-cephalin content of the egg.

[c] Amount ingested per hen per test period.

TABLE II
Gossypol Schiff Bases

Schiff base	Yield	Recrystallized from	M.P. (dec.)	Analyses[a]					
				Carbon		Hydrogen		Nitrogen	
				Calc'd	Found	Calc'd	Found	Calc'd	Found
	%		°C.	%	%	%	%	%	%
Bis(furfurylimino)gossypol	100	.....	264–265	70.99	70.94	5.96	5.98	4.14	4.19
Bis(cyclohexylimino)gossypol	100	.....	314–315	74.09	73.55	7.70	7.76	4.12	4.05
Bis(oleylimino)gossypol	97	Propanol-2	106–109	77.91	77.63	9.91	9.78	2.75	2.75
Bis(n-octylimino)gossypol	93	.....	196–198	74.56	74.72	8.71	8.57	3.78	3.80
Bis(dehydroabietylimino)gossypol	95	Abs. ethanol	212–214	79.81	79.34	8.42	8.60	2.66	2.62

[a] Analyses calculated on basis of two moles of amine condensing with one mole of gossypol and with the elimination of two moles of water.

Results and Discussion

From Table I it can be seen that aniline and p-aminobenzoic acid do not bind gossypol tightly enough to prevent its incorporation into the yolk. The bis(p-carboxyphenylimino)gossypol is alkali-soluble, which may account for the high AGU value found for eggs from hens fed this substance; this value (35.5) is about nine times as great as that obtained with bis(phenylimino)gossypol, which is not alkali-soluble.

Dehydroabietylamine and cyclohexylamine were also not effective in preventing egg discoloration when their gossypol Schiff bases were fed at the 0.5% equivalent gossypol level. It is unlikely that benzylamine, or furfurylamine, would be worthwhile studying at the 0.5% level since the aromatic group in the Schiff base is isolated from the azomethine linkage by a methylene group, and this would not be expected to change the stability of the linkage very greatly. In other words, these compounds would be expected to be similar to aniline with regard to the ease of hydrolysis of the Schiff base and the liberation of gossypol.

Some of the gossypol Schiff bases derived from the n-alkylamines of varying chain lengths, C_8 to C_{18}, were chosen for study at the higher level when it was found that little or no available gossypol was produced when these compounds were fed at the 0.2% level. n-Octyl-, n-decyl-, n-dodecyl-, n-tetradecyl-, and n-octadecylamine were particularly effective in reducing gossypol availability when their gossypol Schiff bases were fed at the 0.5% level (see Table I). Eggs from birds fed these compounds were indistinguishable from normal eggs with respect to color and structure, and no discoloration was observed in the ammonia test.

Since some of the long-chain aliphatic amines show promise as gossypol binders, extended feeding-studies with selected members of the series are being carried out. Also preliminary experiments with n-octadecylamine-treated cottonseed meal have given indication of sufficient gossypol binding capacity to permit feeding such meal to laying hens in unlimited amounts.

Summary

Eggs from hens fed gossypol Schiff bases derived from various aromatic and aliphatic amines were examined spectrophotometrically for gossypol-cephalin.

Aniline, p-aminobenzoic acid, dehydroabietylamine, and cyclohexylamine were not effective gossypol binders when the Schiff base was fed at the 0.5% equivalent gossypol level in the diet whereas the long chain aliphatic amines were very effective. Hens produced normal eggs when fed the 0.5% level of gossypol Schiff bases derived from these amines.

Acknowledgment

The authors wish to express their appreciation to L. E. Brown of the Analytical, Physical Chemical, and Physics Section (SRRL) for the elemental microanalyses of the new gossypol Schiff bases.

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[Received July 12, 1957]

ABSTRACTS . . . R. A. REINERS, Editor

ABSTRACTORS: Lenore Petschaft Africk, S. S. Chang, Sini'tiro Kawamura, F. A. Kummerow, Joseph McLaughlin Jr., and Dorothy M. Rathmann

• Oils and Fats

The determination of iodine number. A. Seher and W. Arends. (Univ. Münster/Westfalen, W. Ger.). *Mitt. Gebiete Lebensm. u. Hyg.* **48**, 1–4(1957). Methods for the determination of the iodine number are discussed and the results of the Wijs method as modified by Stähli and of Kaufmann's procedure are compared for olive, castor, rape, soybean, linseed, and cod-liver oils, and elaidinic and oleic acids. Stähli's method always gives higher values than Kaufmann's. The iodine numbers by Kaufmann's method agree with the hydrogenation numbers after a 2-hour reaction period, and after a 24-hour reaction period increase far less than the values obtained by Stähli's method. (*C.A.* **51**, 12511)

Hydrogenation of rapeseed oil. Kimitoshi Nakazawa, Shinji Mitsunaga, and Kyujiro Tada(Nihon Yushi Co., Tokyo). *Abura Kagaku* **5**, 292–6(1956). Hydrogenation was carried out with reduced nickel under the following conditions: (a) 180°, 10 lb./sq. in.; (b) 150°, 30 lb./sq. in.; (c) 130°, 30 lb./sq. in. and (d) 110°, 60 lb./sq. in., respectively. The reaction velocity