The Effects of Dietary Gossypol on Animals[1]

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Abstract

The pathological effects of gossypol in animals and the effects of protein quality and quantity on its toxicity are described. The method used for the isolation of gossypol from the liver of gossypol-intoxicated pigs and its identification are presented. Wide distribution of gossypol in swine is shown by determining the free and bound gossypol in the various organs. The levels of iron necessary to overcome toxicity and to lower its accumulation in pigs were investigated. Effects of copper, zinc, manganese and iron, alone and in combination, on the gains and the accumulation of gossypol in the liver of rats were studied. Iron was definitely the most effective detoxifying agent.

Introduction

The worldwide demand for protein in the diet of man and monogastric animals is continually increasing. To meet these requirements, investigators are seeking new protein sources, and efforts are in progress to improve the quality of the available sources. Much research has been done and is now being directed both toward improvement of cottonseed protein quality and its more efficient use.

Literature Review

Cottonseed supplies a substantial amount of protein for the livestock industry; however, early use of the seed or meal led to nutritional problems. Dinwiddie (1) stated that all experimentors found that cottonseed meal-fed pigs die more surely and more quickly when confined to pens than when on pasture. Withers and Carruth (2) reported that the deleterious effects associated with cottonseed or cottonseed meal resulted from gossypol, a toxic polyphenol, which the seeds contained. These investigators found that rabbits were very susceptible to gossypol. The animals lost their appetite and postmortem observations showed edema, excessive abdominal fluid, hemorrhagic intestines, congested livers and kidneys. These authors (3) showed gossypol to be toxic to pigs, with the autopsy showing congestion of lungs, edema and fluid in the pleural and abdominal cavities. Hale and Lyman (4) reported that swine fed a 15.5% protein diet containing 0.01% or less of free gossypol showed no symptoms of gossypol toxicity, and gain was as good or almost as good as that for the control group where the protein was supplied by soybean meal. At a free-gossypol level of 0.015%, toxicity symptoms but no deaths were observed. Deaths occurred at 0.019% free gossypol and above. They observed no symptoms of toxicity at 0.03% free gossypol when the diet contained 30% protein. Smith (5) made pathological examination of the pigs which died from gossypol toxicity in the Hale-Lyman studies. He found the outstanding symptoms in the live pig to be labored breathing. The gross and microscopic lesions in 18 pigs, believed to be the result of accumulative gossypol poisoning, were: edema, hydrothorax, congestion and edema of lungs, hydropericardium, edema of the gallbladder, edema of lymph nodes, congestion of the liver and kidney. The edema was believed to result from venous stasis attributed to a failing heart. The hearts were generally flabby and dilated. [Changes were observed in the cardiograms of gossypol intoxicated pigs by Albrecht et al., (6)]. The livers were congested grossly. Microscopic examination revealed almost no viable parenchymal cells and only a narrow rim of hepatic cells remaining at the periphery of each lobule.

The effect of gossypol on chicks were studied by Couch, et al. (7). The adequately supplemented diets contained 21% protein supplied by cottonseed meal containing 0.04% free gossypol. The basal diet was supplemented with pigment glands to give a range of free gossypol from 0.02% to 0.25%. The growth rate, mortality and feed efficiency were not affected by 0.06% or less of free gossypol. The growth rates were improved by the inclusion of 1% of NL-lysine but it did not change the gossypol tolerance level. Narian et al. (8) fed chick diets containing 17%, 21% and 42% protein supplied by isolated soybean protein and 0% to 0.15% free gossypol supplied by cottonseed pigment glands. A reduction in growth rate occurred at each protein level as the level of gossypol increased. Performance improved with increasing levels of protein.

Hollon et al. (9) fed calves (four per treatment) diets containing 28–29% protein supplied by mixtures of cottonseed meal, soybean meal and rolled oats blended to give four diets having free gossypol levels of 0.107%, 0.071%, 0.035% and 0.023%, respectively. The four calves that consumed the 0.107% free gossypol diet, and two of those receiving the 0.071% and the 0.035% free gossypol diets died between 49 and 120 days. There was a positive correlation ($P < .05$) between free gossypol intake during the first 48 days and subsequent mortality. The autopsy findings were very similar to those reported by Smith (5) for pigs. Large amounts of serous fluid were observed in the thoracic and abdominal cavities; the blood was bright red and extremely slow in coagulation.

Experimental Procedures

The Effect of Protein Quality on Gossypol Toxicity

The effects of different proteins on the toxicity of free gossypol were evaluated with weanling rats over a four-week period. The proteins constituting 16% of the diets were (a) solvent-extracted soybean meal, (b) ether-extracted cottonseed meats, (c) commercial cottonseed meal and (d) same as diet (c) except for the addition of 1% of L-lysine. The rats receiving the diets containing the ether-extracted cottonseed meats and the commercial meal plus 1% of L-lysine without added gossypol made the best and about equal gains (Table I). However, under the stress of 0.075% free gossypol, the gains from the soybean meal were markedly superior to the ether-extracted cottonseed meats which was much better than the commercial cottonseed meal. The lysine had little effect. At

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TABLE I
Mean Weight Gains of Weanling Rats at Different Levels of Dietary Free Gossypol, Protein 16%

Diets[a]	Gossypol, per cent of meal		
	0, %	0.075, %	0.150, %
Soybean meal	87.7	71.3	23.6
Ether-extracted cottonseed[b]	97.7	45.0	1.7
Cottonseed meal[c]	70.3	20.5	......
Cottonseed meal + 1% L-lysine	98.3	27.3	......

[a] Adequately supplemented with minerals and vitamins.
[b] Flaked cottonseed meats, exhaustively extracted with ethyl ether.
[c] Commercial cottonseed meal.

0.150% free gossypol, only soybean meal produced gains. All rats on the cottonseed meal and cottonseed meal-lysine diets died. These data indicate that high quality cottonseed protein can produce a good growth response when gossypol does not interfere. This study showed that lysine as the free amino acid improved gains from the commercial meal but had little or no effect on overcoming gossypol toxicity.

Isolation of Gossypol From Porcine Liver

Although the effects of gossypol had been known for many years, it had not been isolated from organ tissue. Clawson et al. (10) extracted a yellow pigment from livers of pigs which had consumed high levels of free gossypol that reacted with aniline to give a more intense yellow color which could be read spectrophotometrically at the same wavelength as dianilinogossypol and gave a spectral curve similar to that of dianilinogossypol. Smith (11) attempted to isolate gossypol as the dianilino derivative in crystalline form from the livers of pigs intoxicated with gossypol. Samples of liver (140–230 g) from four pigs which had been fed cottonseed meal high in free gossypol were homogenized with 300 ml of 95% ethanol. The homogenates were heated in a boiling water bath for 15 min then filtered through a Buchner funnel with vacuum. The residues were washed with 95% ethanol and then with ether. The washed residues were heated for 3.5 hr on the steam bath with 50 ml of 82% ethanol and 15 ml of aniline and then homogenized with 300 ml of chloroform for 3 min in the Waring blendor. The mixtures were filtered through a Buchner funnel, and the residue was washed with chloroform. The combined ethanol-ether extract and washings contained 53 mg of gossypol, and the aniline-chloroform extract contained 84 mg of gossypol as measured spectrophotometrically. No precipitate could be obtained from the ethanol extract, probably due to the large amounts of other constituents. From the aniline-chloroform extract, 88.8 mg of yellow crystalline material was recovered. The precipitate was dissolved in chloroform and was slowly recrystallized on a warm steam bath after adding 8 ml of 95% ethanol and 10 ml of ethyl ether. Comparison of the UV and IR spectra of the aniline derivative of the purified product was essentially identical with the spectra obtained for pure dianilinogossypol, thus definitely proving that gossypol does accumulate in the liver of swine consuming free gossypol.

TABLE III
Distribution of Gossypol in the Organs of Pigs Consuming 600 ppm Dietary Gossypol for Four Weeks

Tissue	Gossypol ppm (dry basis)		
	Free	Bound	Total
Liver	323	270	593
Kidney	99	132	231
Spleen	38	77	115
Lymph nodes	35	142	177
Lungs	35	56	91
Heart	53	52	105
Pancreas	25	26	51
Diaphram muscle	35	28	63
Serum	46	379	425
Bile	441	69	510

Distribution of Gossypol in the Organs of Swine

After the development of methods for determining free and bound gossypol in animal tissues (12), Sharma et al. (13) did an experiment to determine the distribution of gossypol in the organ tissues of swine consuming 600 ppm for 28 days in diets containing either 14% or 28% protein. The diets are shown in Table II. The organs of the pigs were analyzed, and the distribution of the free and bound gossypol in the organs of the pigs receiving the 14% protein diet is shown in Table III. The highest levels were found for both free and bound gossypol in the liver, bound in serum, and free in the bile. These data for the different organs indicate that gossypol is widely distributed in the animal. All of the tissues from the pigs receiving 28% protein were significantly lower ($P < .01$) than the corresponding tissues from the pigs receiving the diet containing 14% protein. The lower level of gossypol in the tissues of pigs receiving 28% protein is at least a partial explanation of the nontoxic effects observed by Hale and Lyman (4) when the diets contained 30% protein and 0.03% free gossypol. This probably results from a combination of the free gossypol with the free amino groups of the high protein diet and excretion in the feces as bound gossypol. It is of interest that the major portion of the gossypol in serum is bound and that in the bile is free. The high level of free gossypol in the bile suggests the bile as a route of excretion. It

TABLE II
Composition of Diets for Pigs With 600 ppm Free Gossypol Added

Ingredients	14% Protein, %	28% Protein, %
Corn	40.00	40.00
Soybean meal	20.75	48.20
Starch	20.44	4.96
Glucose monohydrate[a]	15.50	4.00
Dicalcium phosphate	2.00	1.00
Calcium carbonate	0.25	0.80
Trace mineral salt[b]	0.50	0.50
Vitamin antibiotic mix[c]	0.50	0.50
Gossypol crystalline[d] (600 ppm)	0.06	0.06

[a] Cerelose, Corn Products Company, Argo, Illinois.
[b] Composition of trace mineral salt: sodium chloride, 97.00%; zinc, 0.800%; manganese, 0.400%; iron, 0.330%; copper, 0.048%; cobalt, 0.022%; and iodine, 0.011%.
[c] Vitamin and antibiotic supplement per kilogram of diet: stabilized vitamin A palmitate, 2200 IU; vitamin D, 1100 IU; DL-α-tocopherol, 1.1 IU; and riboflavin, 4.4 mg; pantothenic acid, 10.2 mg; niacin, 22.0 mg; choline chloride, 55.1 mg; vitamin B_{12}, 0.022 mg; and oxytetracycline, 0.022 mg.
[d] Crystalline gossypol was ground in a mortar with a portion of the starch.

TABLE IV
Rations Used to Obtain Graded Levels of Gossypol

Ingredients	Cottonseed meal and lysine, %	Cottonseed meal and fish meal, %	Soybean meal control, %
Ground yellow corn	79.18	81.40	83.40
Cottonseed meal (41%)[a]	12.50	12.50	
Fish meal		3.90	
Soybean meal	4.50		14.00
L-Lysine supplement	1.02		
Deflourinated phosphate	0.80	0.30	1.00
Ground limestone	1.00	0.90	0.60
Trace minerals[b]	0.50	0.50	0.50
Vitamin supplement[c]	0.50	0.50	0.50

[a] Two cottonseed meals: (a) a prepress solvent-extracted meal with 0.07% free gossypol; (b) a direct solvent-extracted meal with 0.32% free gossypol.
[b] Contained in per cent: sodium chloride, 94–97%; zinc, 0.8%; manganese, 0.6%; iron, 0.2%; copper, 0.06%; iodine, 0.016%; and cobalt, 0.016%.
[c] Supplied per kilogram of diet: vitamin A, 2200 IU; vitamin D, 220 IU; and roboflavin, 1.1 mg; pantothenic acid, 5.5 mg; nicotinic acid, 16.5 mg; choline chloride, 110 mg; vitamin B_{12}, 0.0176 mg; and butylated hydroxy toluene, 250 mg.

TABLE V
Gossypol and Iron Content of Livers of Pigs Fed Rations Containing Varying Ratios of Iron to Gossypol

	Gossypol level, mg/kg									Corn-soybean meal control
	80	244	400	80	244	400	80	244	400	
	Iron level, mg/kg									
	0	0	0	40	122	200	80	244	400	
Liver gossypol[a,b]										
Free	157	226	203	145	248	244	114	168	162	14
Bound	162	263	253	153	275	265	121	176	203	8
Liver iron,[c] µg/g	341	646	960	466	482	500	561	553	560	419

[a] Expressed as mean for 12 pigs per treatment in micrograms of gossypol per gram of dry matter.
[b] LSD for comparing means of iron to gossypol ratios are as follows: for free gossypol, 24 and 31; bound gossypol, 26 and 35; and for iron, 110 and 145, respectively, for the 0.05 and 0.01 levels of significance.
[c] LSD for comparing mean gossypol levels within iron to gossypol ratios are as follows: for free gossypol, 41 and 55; for bound gossypol, 45 and 60; and for iron 60 and 79, respectively, for the 0.05 and 0.01 levels of significance.

is possible that the gossypol in the bile is in combination with some constituent to form a product which is soluble in the solvents used to extract free gossypol.

Effect of Dietary Iron on Gossypol Toxicity and the Accumulation of Gossypol and Iron in the Liver

After learning that the liver is the primary site of gossypol accumulation (13,14), it seemed that an assay of this organ for gossypol should yield information on the effectiveness of efforts to overcome gossypol toxicity.

To determine the effects of varying levels of iron, which had been shown to alleviate gossypol toxicity (15–19), a total of 114 pigs, six replications per treatment, were fed diets containing 80, 244 and 400 ppm of free gossypol and varying levels of iron. The desired levels of gossypol were obtained from two cottonseed meals containing 0.07% and 0.32% free gossypol used singly or as a mixture of equal parts of each at a level of 12.5% of the diet. Soybean meal or fish meal was added to bring the protein level to 15.5%. Additional lysine was obtained by the substitution of 3.9% of fish meal for soybean meal which gave a dietary level of 0.6%, or by supplemental L-lysine to provide a dietary level of 1%. Iron was added as ferrous sulfate monohydrate at the expense of corn in weight ratios of elemental iron to free gossypol at 0 to 1, 0.5 to 1, and 1 to 1. A soybean meal diet was used as a control. The diets are shown in Table IV.

The pigs had an initial average weight of 55 lb. and were group-fed until they either reached market weight or died. Since the lysine and fish meal showed no significant differences, the data were grouped according to gossypol and iron level, giving 12 replications per treatment. The mortality was three for no iron and 244 ppm gossypol, nine for no iron and 400 ppm gossypol, and one for 200 ppm iron and 400 ppm gossypol. The weight gain for the pigs was significant for gossypol level and for iron level ($P < .01$). However, the gains for the pigs on 80 ppm gossypol without iron and those on the higher levels of gossypol with 1 to 1 ratios of iron to gossypol were not significantly different; nor were they different from the soybean control. The results of the liver analysis are shown in Table V. The free and bound gossypol were lower ($P < .01$) in the livers of pigs fed 80 ppm dietary gossypol than in those fed 244 ppm, and for those receiving the 1 to 1 vs. 0.5 to 1 ratio of iron to gossypol. When no supplemental iron was fed, the liver iron varied with the dietary gossypol ($P < .01$). The possible explanation for this could be that the high level of gossypol in the liver combines with the iron, thus concentrating it in that organ. This was also indicated by increased levels of gossypol in the liver and spleen of pigs injected with iron (14). Pigs that died from gossypol intoxication had lower levels of gossypol and dry matter in the liver. The mortality noted in this study indicates that a 0.5 to 1 ratio of iron to gossypol does not completely detoxify the gossypol.

TABLE VI
Basal Diet for Determining the Effects of Varying Levels of Iron on the Performance and on the Accumulation of Gossypol in the Livers of Pigs Fed 400 ppm of Dietary Free Gossypol[a]

Diet	Percentage
Ground yellow corn	77.73
Cottonseed meal[b]	11.75
Soybean meal	7.40
Deflourinated phosphate[c]	1.00
Limestone	0.87
Trace mineral salt[d]	0.50
Vitamin supplement[e]	0.50
Lysine supplement[f]	0.25

[a] Iron was supplied as ferrous sulfate monohydrate.
[b] Contained 40.8% protein, 0.7% fat, 14.8% fiber, 76.4% soluble nitrogen, 3.54 g/16 g of nitrogen of free ε-amino lysine, 0.38% free and 0.74% total gossypol.
[c] Contained 34% calcium and 18% phosphorus.
[d] Same as in Table IV.
[e] Same as in Table IV.
[f] Fifty per cent L-Lysine.

Effect of Increased Levels of Iron and a Depletion Period on the Performance of Pigs and the Accumulation of Gossypol in the Livers of Pigs Fed 400 ppm of Free Gossypol

Since the preceding study showed that a ratio of iron to gossypol of 0.5 to 1 did not completely detoxify the gossypol, it seemed desirable to increase the iron level fed. A study was done to determine if higher levels of iron and changing the pigs from a diet containing 400 ppm of free gossypol to a gossypol-free diet for a depletion period just before slaughter would further reduce the accumulation of gossypol in the liver of the pigs and affect their performance. The gossypol was supplied by a cottonseed meal containing 0.38% free gossypol. Soybean meal was added to bring the protein level to 15.5% (Table VI). Ferrous sulfate monohydrate, to give 400, 800 and 1200 ppm of iron, was blended with the cottonseed meal prior to mixing with the other ingredients. It was added at the expense of corn.

Seventy-two pigs weighing about 46 lb. each were randomly assigned, 24 pigs to each iron level. They were housed by pairs in pens with concrete floors.

TABLE VII
Effect of Increased Levels of Iron and the Replacement of a Diet Containing 400 ppm Free Gossypol With a Gossypol-Free Diet on the Accumulation of Gossypol in the Liver of Pigs and on Their Performance

Ratio of iron to gossypol	No. of pigs	Average daily gain	Average daily feed	Feed/ lb. gain
1:1	24	1.62	5.33[a]	3.28
2:1	24	1.63	5.53	3.40
3:1	24	1.65	5.69	3.45

[a] Average daily feed intake increased with increasing levels of iron ($P < .05$).

TABLE VIII
Performance of Pigs for Entire Period, Including Those Fed the Gossypol-Free Soybean Meal Diet for Three Weeks

Diet	Iron-gossy. ratio	Pigs per treatment	Average daily gain, lb.	Average daily feed, lb.	Feed/lb. gain lb.
Cottonseed meal	1:1	8	1.60	5.50	3.45
Soybean meal[a]	0:0	7[b]	1.62	5.51	3.40
Soybean meal	1:0	8	1.66	5.96	3.60
Cottonseed meal	2:1	8	1.59	5.67	3.57
Soybean meal	0:0	8	1.68	6.04	3.59
Soybean meal	2:0	8	1.63	5.75	3.53
Cottonseed meal	3:1	8	1.63	5.88	3.60
Soybean meal	0:0	8	1.61	5.83	3.63
Soybean meal	3:0	8	1.72	6.16	3.59

[a] Gossypol-free depletion diet.
[b] One pig died after transfer to depletion diet, apparently from gossypol toxicity. Performance was not significantly different for treatment.

Feed and water were supplied ad lib. Three weeks prior to an anticipated market weight of 215 lb., the pigs on each iron treatment were divided into three groups with eight replications. Of each iron treatment, one group continued to receive the same diet; a second group was changed to a 15.5% protein corn-soybean meal diet containing neither gossypol nor iron; and the third group was placed on the corn-soybean meal diet to which iron was added at the level used before the dietary change. All pigs were slaughtered three weeks later. Livers were analyzed for free and bound gossypol (12) and for iron (20). The performance of the twenty-four pigs, per iron treatment, prior to the dietary change, is shown in Table VII. Feed intake increased significantly with increasing iron levels ($P < .05$). The average daily gains were not affected significantly. The performance of the pigs during the entire period was not significantly different for the different treatments (Table VIII).

The gossypol concentration in the liver (Table IX) was reduced ($P < .01$) by feeding the pigs a gossypol-free diet for a three-week period just before slaughter. The free and bound gossypol in the livers of the pigs fed the gossypol-free diets were reduced approximately two thirds and one half, respectively, of that in the pigs continued on the original diets. Iron added to the gossypol-free diet had no effect. One pig receiving the lowest level of iron died after transfer to the depletion diet. This was attributed to gossypol toxicity and indicates that a 1 to 1 ratio of iron to gossypol was inadequate for complete protection against gossypol toxicity in this experiment. The performance of the pigs and the reduced liver gossypol observed in this study indicates that levels of iron to gossypol in ratios of 2 to 1 or 3 to 1 are effective in overcoming the toxicity and reducing the level of gossypol in the liver. Liver gossypol was further reduced by feeding a gossypol-free diet for three weeks before slaughter.

TABLE IX
Effects of Iron and a Three-Week Depletion Period on Gossypol and Iron in Livers of Pigs Fed 400 ppm Dietary Gossypol[a]

Treatment	Dietary iron	Liver: Gossypol Free,[b] µg/g	Liver: Gossypol Bound,[b] µg/g	Liver: Iron, µg/g
Cottonseed meal	400	247.6	246.9	613.6
Soybean meal	0	75.9	136.6	598.0
Soybean meal	400	80.1	144.3	627.0
Cottonseed meal	800	168.7	203.8	788.1
Soybean meal	0	65.1	106.5	723.1
Soybean meal	800	63.3	113.9	764.8
Cottonseed meal	1200	152.2	164.5	819.8
Soybean meal	0	49.0	92.9	689.1
Soybean meal	1200	55.0	100.2	836.6

[a] Soybean meal replaced cottonseed meal during the three-week depletion period.
[b] Free and bound gossypol significantly reduced $P < .01$ by dietary iron level and by three-week depletion period.

TABLE X
The Effect of Copper, Zinc, Manganese and Iron, Alone or in Combination, on Weight Gains and on Free and Bound Gossypol in the Livers of Rats Fed Diets Containing 0.075% Free Gossypol (Six Rats per Treatment)

Diet No.	Treatment	Mean gain, g	Gossypol µg/g Free[a]	Gossypol µg/g Bound[b]
1	Control	17.66 ± 2.89	43.71	92.81
2	Cu, 300 ppm	15.33 ± 4.68	34.14	79.79
3	Zn, 500 ppm	18.50 ± 4.68	35.97	62.94
4	Mn, 1600 ppm	14.17 ± 4.03	37.56	63.21
5	Fe, 1600 ppm	50.67 ± 4.90[c]	12.70	16.15
6	Cu-Zn	16.67 ± 3.06	42.93	107.24
7	Cu-Mn	9.83 ± 3.39	45.42	108.67
8	Zn-Mn	14.00 ± 3.32	40.87	66.67
9	Cu-Zn-Mn-Fe	21.83 ± 9.67	13.64	16.38

[a] Free gossypol: $LSD_{.05} = 11.26$; $LSD_{.01} = 15.08$.
[b] Bound gossypol: $LSD_{.05} = 22.10$; $LSD_{.01} = 29.58$.
[c] Significant $P < .01$.

The Effects of Copper, Zinc, Manganese and Iron, Alone and in Combination, on the Performance of Rats Consuming 0.075% Free Gossypol and Its Accumulation in Liver

The effects of iron on gossypol toxicity have been recognized for many years, but little is known about the effects of other elements. Rand (21) suggested that copper and zinc may be more strongly complexed by nitrogen compounds than with gossypol and that manganese has been overlooked as a detoxifying agent for gossypol. A study was undertaken to evaluate the effects of copper, zinc, manganese and iron, alone and in combination, using weanling rats as the test animal. The basal diet contained the following in gram per kilogram of diet: cottonseed meal, 234.4 (0.32% free gossypol); Wesson oil, 50.0; cod liver oil, 5.0; alphacel, 20.0; Wesson salt, 30.0; starch, 657.2; L-lysine, 3.4; and vitamins at an adequate level. The copper, zinc, manganese and iron were incorporated at the rate of 300, 500, 1600 and 1600 ppm of the diet, respectively, as $CuSO_4 \cdot 5H_2O$, ZnO, $MnSO_4 \cdot H_2O$, and $FeSO_4 \cdot H_2O$ at the expense of starch. The weanling rats were randomly assigned to treatment. They were individually housed and were fed ad lib. for four weeks. At the end of the feeding period, they were killed, and the livers were analyzed for free and bound gossypol.

The mean weight gains of the rats and the mean free and bound gossypol content of the livers are shown in Table X.

Iron was the only metal ion which produced a gain greater than the basal control diet. The superior gain for iron was significant at $P < .01$. Treatments were significant for both free and bound gossypol at the $P < .01$ level. Iron alone or in combination reduced both free and bound gossypol most effectively. Both zinc and manganese alone lowered bound gossypol significantly ($P < .01$), but lowered it less in combination ($P < .05$). The copper and zinc, and copper and manganese treatments showed a significant interaction increasing the bound gossypol above that of either element alone ($P < .01$) and even above the value for the control. This interaction probably results from the concentration of these elements in the liver where they either form chelates with the gossypol, or alter the liver constituents to a more favorable state for binding gossypol.

This study showed iron to be the most effective of the elements tested in the detoxification of gossypol.

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