

## GOSSYPOL TOXICITY

# Effect of Cooking and Calcium and Iron Supplementation on Gossypol Toxicity in Swine

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Studies were carried out to determine whether cooking and the addition of calcium and iron protect swine against gossypol toxicity. Gossypol in the diet resulted in lower weight gains and feed efficiencies and a high mortality rate. These effects were completely eliminated by the addition of 1%  $\text{Ca(OH)}_2$  and 0.1%  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . Serum proteins, albumin, globulin, and urea nitrogen concentration remained the same under the different treatments, but hemoglobin and hematocrit values were lower in animals fed cottonseed meal diets. Liver weight was lower in animals fed raw or cooked cottonseed meal and liver fat highest in the group fed raw meal. Histopathological studies of the heart indicated that cottonseed meal fed without addition of calcium and iron resulted in dilation of both auricles and ventricles. This lesion, of a degenerative type, which resulted in cardiac insufficiency and edema of the lungs, is probably muscular, since no abnormalities were observed in the valves.

**T**HE fatal outcome which often follows the prolonged feeding of large amounts of cottonseed meal to monogastric animals, but particularly to swine, is well documented (1, 28). It is also known that the adverse physiological effects of cottonseed meal are due to a large extent to gossypol (1, 8, 20, 29).

The toxicity of cottonseed meal due to the presence of gossypol can be reduced by the processing conditions used during the extraction of oil from the seed (1, 9, 13, 22). Since at the same time there is a decrease in available lysine content (4, 10, 24), the deficiency of this amino acid is further aggravated by the presence of gossypol, because it binds lysine, reducing the protein quality of the meal (24, 25). Newer developments in cottonseed processing have made possible the production of cottonseed meal practically free from gossypol (22), with minimum destruction of the protein quality.

Attention has also been given to dietary factors which reduce the toxicity

of gossypol. Hale and Lyman (14) showed that gossypol tolerance in swine could be increased by increasing the protein level in the diet. The beneficial effects of protein level in the diet have been attributed to either a higher availability of amino acids, particularly lysine, or the presence of specific components in the supplements (7, 9, 12, 16, 23). Hale and Lyman (15) also demonstrated that lysine supplementation of sorghum-cottonseed rations for swine greatly improved the daily gains and feed efficiencies despite the increased gossypol intake.

The addition of ferrous salts to diets containing cottonseed meal has been shown by Gallup (12), Barrick (5), Withers and Carruth (31), Hale and Lyman (16), and Clawson and coworkers (8) to reduce the toxicity of gossypol to varying degrees. It is believed that the beneficial effects of the added iron are due to the formation of an insoluble iron compound of gossypol and to the oxidation of gossypol, thus decreasing its

concentration. Bressani and coworkers (6) have shown recently that the addition of iron in the presence of calcium salts, with and without cooking, reduces to very low levels the concentrations of free gossypol in food mixtures containing cottonseed flour. It was, therefore, of interest to determine whether the addition of calcium and iron would protect swine against gossypol toxicity when fed a raw or cooked cottonseed meal diet with a relatively high gossypol content and to determine the biochemical and histopathological changes in the blood and organs of the animals studied.

## Materials and Methods

**Experimental Procedure.** The experiment consisted of five treatments, one of which was a control, and each treatment was replicated three times. In the first replication, 15 half-bred Duroc Jersey pigs were used, allotting three pigs per treatment, and in the second and third, 15 and 20 pure-bred

Duroc Jersey pigs were used, allotting three for the former and four for the latter, respectively. The animals were from 8 to 10 weeks of age when placed on the experiment and weighed between 6.4 and 14.1 kg. The average initial weight per dietary treatment was essentially the same, 9.3 kg., and each had five male and five female pigs.

**Care and Feeding of Animals.** Throughout the experiment, the pigs were kept in individual pens in order to obtain individual data on feed consumption. Water was given *ad libitum*, while the daily amount of feed to be offered was calculated from the weight of each pig, which was obtained every week. The floors of the pens were cleaned daily. The animals were weighed every 7 days and the uneaten food was collected in plastic bags and dried in a convection oven at 80° C. At no time during the experiment did the pigs lack food, and the amounts left were always small and easily collected for drying and weighing.

**Preparation of Rations.** In each replication, diets 1, 2, and 4 (Table I) were moistened with water and fed as a homogeneous paste. Diets 3 and 5 were cooked in boiling water for 10 minutes before feeding. To avoid a possible interaction when cooking between the cottonseed meal and the other ingredients of the diets in rations 3 and 5, the following procedure was used. In the case of treatment 3, all the cottonseed meal in the ration was cooked with a suitable amount of corn and after cooking all the other constituents of the ration were added to give the composition shown in Table I. The same procedure was followed for treatment 5 and the supplements of calcium and iron were added to the cottonseed-corn mixture before cooking.

**Analytical Procedures.** The rations were analyzed for protein and free and total gossypol by the method of the A.O.C.S. (2) and available lysine by the method of Conkerton and Frampton (10) before and after cooking.

Blood samples taken from the jugular vein were obtained from each pig at the start, middle, and end of the experimental period, which lasted 84 days. Total protein, albumin, and urea nitrogen were determined in the blood serum, and hemoglobin and hematocrit were determined in oxalated blood samples. At the end of the experimental periods, 15 pigs, three from each diet, were sacrificed and the livers removed. One lobe of each liver was immediately packed in a plastic bag and frozen until analyzed.

## Results

The growth performance and mortality of pigs fed the different rations are presented in Table II. Also shown is the free gossypol concentration of the rations before and after the addition of water and cooking in diets 3 and 5.

The animals fed the cooked cottonseed meal gained significantly more than those fed the water-treated but raw cottonseed meal. The addition of cal-

**Table I. Composition of Rations**

Treatment <sup>a</sup>	Ration No.				
	1 Control, %	2 Raw, %	3 Cooked, %	4 Raw, %	5 Cooked, %
Ace-Hi <sup>b</sup>	39.5	...	...	...	...
Cottonseed meal <sup>c</sup>	...	42.0	42.0	42.0	42.0
Ground corn	54.3	51.8	51.8	50.7	50.7
Ca(OH) <sub>2</sub>	...	...	...	1.0	1.0
FeSO <sub>4</sub> ·7H <sub>2</sub> O	...	...	...	0.1	0.1

<sup>a</sup> Other ingredients added were: 3.0% alfalfa leaf meal; 3.0% salt mixture; 0.2% Aurofac (1.8 grams aureomycin per pound).

<sup>b</sup> Commercial protein concentrate, Riverside Feeds, Guatemala.

<sup>c</sup> Cottonseed meal, a screw-press meal from Nicaragua, with the following analysis: 7.6% moisture; 10.4% fat; 40.8% protein; 6.2% ash; 8.7% crude fiber; 1.39%  $\epsilon$ -amino lysine; 100 mg. free gossypol, and 1.085 grams total gossypol per 100 grams.

**Table II. Effect of Cooking and of Addition of Calcium Hydroxide and Ferrous Sulfate to 42.0% Cottonseed Meal Ration on Performance of Growing Swine**

Diet Fed	Free Gossypol in Diet, <sup>a</sup> Mg./100 Grams		Weight, Kg.		Feed Efficiency <sup>b</sup>	Mortality
	Before	After	Initial	Final		
Control			9.4	38.6	2.98	0/10
Untreated cottonseed meal <sup>c</sup>	40	21	9.3	20.7	7.50	8/10
Cooked cottonseed meal <sup>d</sup>	42	18	9.2	28.9	4.62	7/10
Untreated cottonseed meal + 1.0% Ca(OH) <sub>2</sub> + 0.1% FeSO <sub>4</sub> ·7H <sub>2</sub> O	26	7	9.2	35.0	3.62	1/10 <sup>e</sup>
Cooked cottonseed meal + 1.0% Ca(OH) <sub>2</sub> + 0.1% FeSO <sub>4</sub> ·7H <sub>2</sub> O	30	7	9.3	34.0	3.86	0/10

<sup>a</sup> Content before and after treatment.

<sup>b</sup> Average feed consumed/average weight gained.

<sup>c</sup> Water added but no heat applied.

<sup>d</sup> Water added, heat applied.

<sup>e</sup> Mortality of one animal was due to pneumonia as diagnosed by a veterinarian.

cium hydroxide and ferrous sulfate caused greater weight gains, compared to the groups fed only cottonseed meal. No significant difference in weight gain was found between groups fed diets 4 and 5, but none of the animals fed cottonseed meal gained as much as those in the control group. Feed efficiency was better for the control group, followed by groups 4 and 5. The mortality data show that 80 and 70% of the pigs fed diets 2 and 3, respectively, died from the seventh week on but only one out of 10 pigs died in group 4, at the eleventh week of the experiment. None of the pigs died in the control group or in the group fed ration 5.

Figure 1 shows growth curves of the animals on experiment. The rate of weight gain of the animals fed the different cottonseed meal rations did not reach that of the control group.

The mean values for the blood composition analysis are shown in Table III. No difference in blood composition was observed among the pigs at the start of the experiment. The second analysis was made at the end of the sixth week; no major change was found in total protein, albumin, or globulin concentration when compared to the initial values. Serum urea values increased in all groups. A significant ( $P < 0.01$ ) decrease in hemoglobin concentration and

hematocrit values occurred in the animals fed the uncooked and cooked cottonseed meal without the addition of Ca(OH)<sub>2</sub> and FeSO<sub>4</sub>·7H<sub>2</sub>O.

At the end of the experiment, no major changes were observed in total serum protein, albumin, and globulin concentration in the animals fed the different rations. Serum urea values increased slightly, except for the group fed the untreated cottonseed meal with the addition of Ca(OH)<sub>2</sub> and FeSO<sub>4</sub>·7H<sub>2</sub>O. Both hemoglobin concentration and hematocrit values decreased slightly in all groups fed the cottonseed meal ration with or without cooking, and with or without additives.

The chemical composition of the livers of three animals from each diet group is shown in Table IV. Liver weight was essentially the same in all groups, except in the animals fed the untreated and the cooked cottonseed meal. Nitrogen content was similar in all groups, while fat content was highest in the group fed the untreated meal and essentially the same in all other groups.

The autopsy findings showed that organ weight was lower in those animals receiving the raw cottonseed meal than in the control group or the groups fed the cooked or raw cottonseed meal with the mineral supplements. Histopathological studies of the organs of some of the

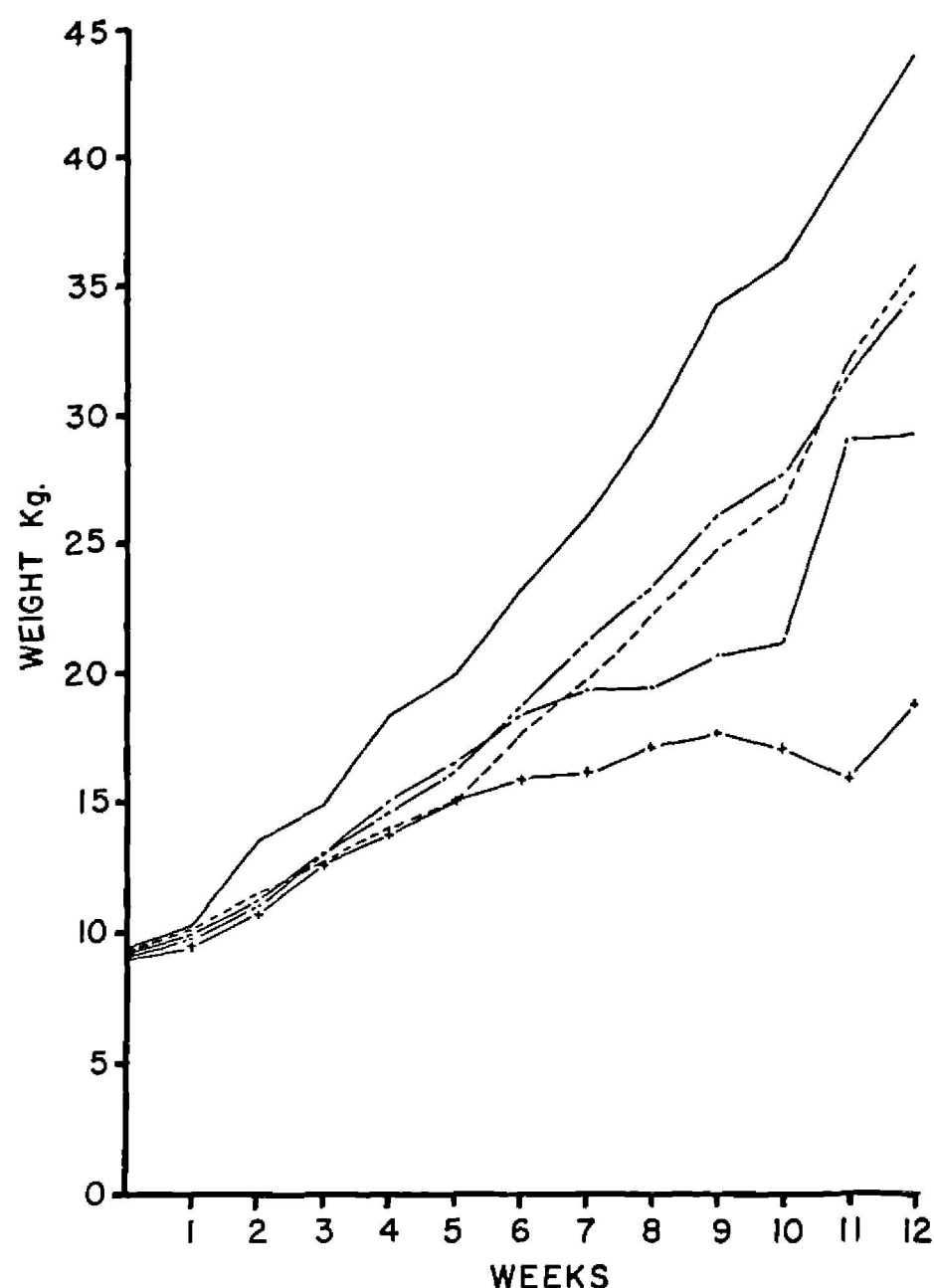


Figure 1. Growth curves of swine

— Control group  
 +—+ Group fed 42.0% uncooked cottonseed meal with water  
 ···· Group fed 42.0% cooked cottonseed meal with water  
 - - - - Group fed 42.0% uncooked cottonseed meal + 1.0% Ca(OH)<sub>2</sub> + 0.1% FeSO<sub>4</sub>·7H<sub>2</sub>O  
 ···· Group fed 42.0% cooked cottonseed meal + 1.0% Ca(OH)<sub>2</sub> + 0.1% FeSO<sub>4</sub>·7H<sub>2</sub>O

Table III. Average Blood Composition at the Beginning and Sixth and Twelfth Weeks of Experiment

Group No.	Protein, %	Albumin, %	Globulin, %	Urea N, Mg. %	Hemoglobin, G. %	Hematocrit, %
BEGINNING						
1	6.32	2.52	3.90	16.0	11.5	35
2	6.19	2.17	4.02	14.5	11.6	36
3	6.48	2.18	4.30	13.6	10.9	33
4	6.14	2.35	3.79	15.2	11.1	34
5	6.37	2.32	4.05	11.0	11.2	34
6TH WEEK						
1	7.14	2.91	4.23	22.6	14.5	45
2	5.87	2.27	3.60	22.0	9.7	26
3	6.50	2.51	3.99	21.1	9.9	28
4	6.52	2.58	3.90	24.2	13.2	37
5	6.61	2.70	3.92	19.4	13.4	38
12TH WEEK						
1	7.28	3.10	4.08	27.1	15.7	42
2	6.27	2.58	3.69	24.1	7.4	20
3	6.89	2.88	4.01	27.0	6.2	18
4	6.90	2.98	3.94	21.4	11.9	33
5	6.81	3.16	3.64	21.6	11.8	31

animals of group 2 showed necrosis of the liver and atrophy of the acini of the pancreas and of the Malpighian bodies of the spleen. These nonspecific findings are probably the outcome of the general state of undernutrition of these animals. The heart, however, showed more specific symptoms characterized by a

dilation of both the auricles and the ventricles, with the left ventricle being more affected. Since no abnormalities were observed in the valves, this lesion, of a degenerative type, is probably muscular, resulting in the cardiac insufficiency and edema of the lungs, observed in these animals, and eventually death.

Several workers (16, 17, 30) have shown that pigs are highly sensitive to gossypol, a pigment present in cottonseed. Therefore, the levels of cottonseed flour or meal used in swine rations very seldom exceed 15% or levels providing more than 0.01% free gossypol (1).

Several dietary factors have been shown to decrease the toxicity of gossypol; among them, increased protein in the ration appears to overcome the dyspnea and mortality attributed to the pigment (14, 17). Likewise, the source of supplemental protein influenced gossypol toxicity (7, 9, 19, 23, 27). Other dietary factors affecting the toxicity of gossypol are lysine supplementation (9, 15, 26) and the addition of iron (11, 12, 16, 18, 31). The results were, however, obtained with diets containing low levels of cottonseed meal. In the present investigation, diets containing 42% cottonseed meal with a free gossypol level of 100 mg. per 100 grams were used.

As was expected, all the animals fed the uncooked meal showed signs of toxicity due to the pigment. Furthermore, cooking alone gave only slight protection against toxicity, while the presence of calcium and iron with or without cooking resulted in a full protection of the animals. This was apparent not only in the general appearance and weight of the animals but in the serum chemistry, liver analysis, and general gross pathology of liver, heart, lungs, and other organs.

Figure 2 shows the food and gossypol intake and mortality in the different groups. Mortality started after the sixth week on the study, when gossypol intake had reached approximately 2.4 grams from the initiation of the experiment, while feed intake started to decrease in groups 2 and 3 almost after the second week, but decreased significantly after the sixth week of the study.

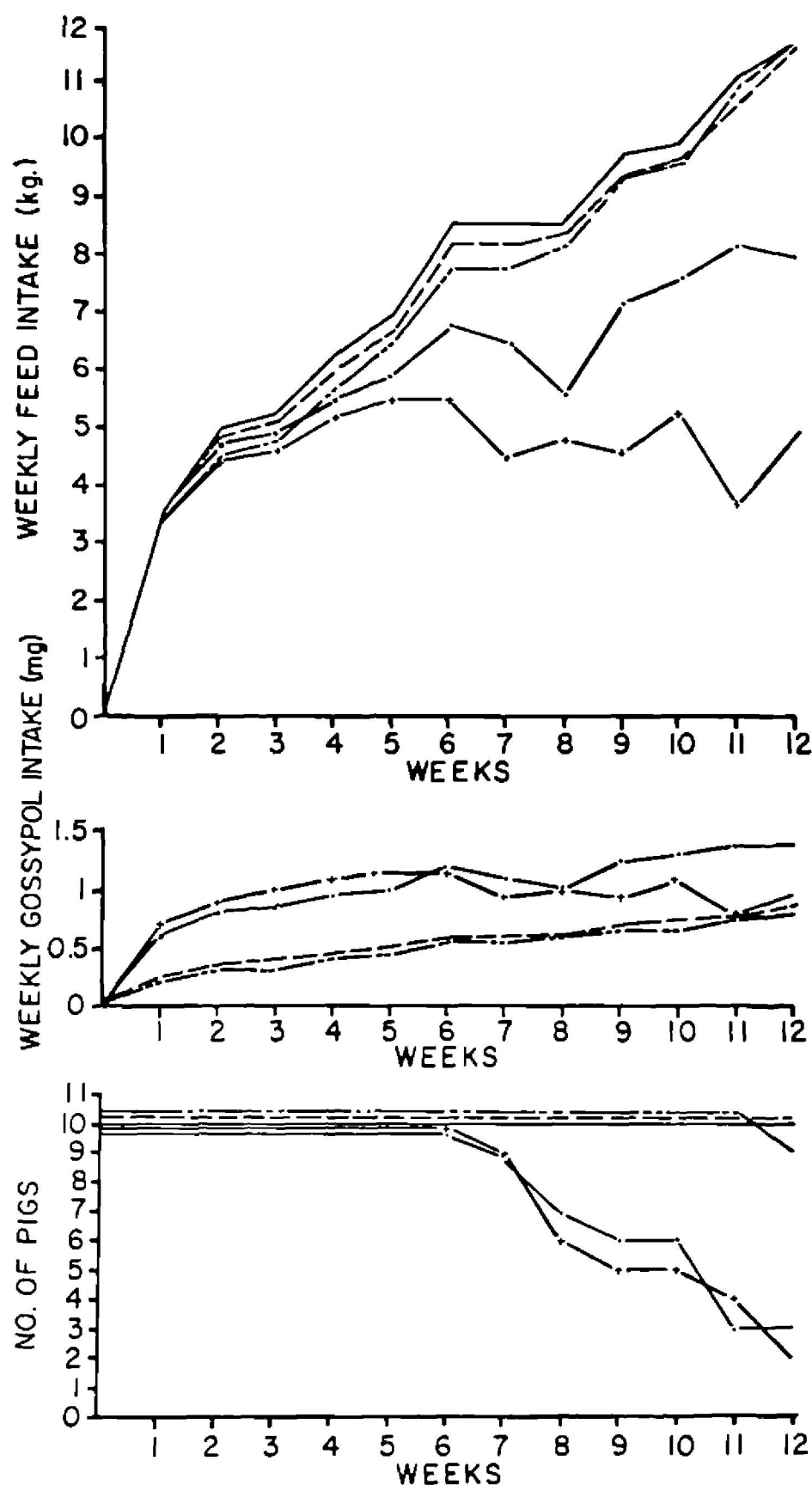
The mechanism of the protection by calcium and iron against gossypol toxicity is still not fully known. Bressani and coworkers (6) recently showed that cooking cottonseed flour-containing mixtures with calcium and iron added results in a highly significant decrease in free gossypol and some decrease in total gossypol concentration. This effect might very well be due to a destruction or binding, or both, of the free gossypol present in the cottonseed flour mixture. It is probable that both calcium and iron chelate the gossypol, thus decreasing its absorption by the animal. A possible role of calcium, besides those already stated, is that of increasing the absorption of iron (3). The increased iron absorbed will in turn bind gossypol, thus reducing its toxic effects. This possibility is under investigation. In any case, the effect of calcium and iron with gossypol is not



**Table IV. Weight and Nitrogen, Fat, and Iron Content of Livers of Pigs Fed Cottonseed Meal Ratios**

Ration Fed	Average Liver Wt., Grams	N, %	Fat, %	Fe, Mg. %
Control	1323	10.69	7.62	46.7
Untreated cottonseed meal	1075	11.05	11.04	55.1
Cooked cottonseed meal	1127	9.75	8.40	39.1
Untreated cottonseed meal + 1.0% Ca(OH) <sub>2</sub> + 0.1% FeSO <sub>4</sub> ·7H <sub>2</sub> O	1293	9.44	7.10	47.0
Cooked cottonseed meal + 1.0% Ca(OH) <sub>2</sub> + 0.1% FeSO <sub>4</sub> ·7H <sub>2</sub> O	1323	9.84	7.45	43.0

<sup>a</sup> Dry weight basis.



**Figure 2. Weekly feed and gossypol intake and occurrence of death in experimental animals**

— Control group  
 +—+ Group fed 42.0% uncooked cottonseed meal  
 - - - Group fed 42.0% cooked cottonseed meal  
 - - - - Group fed 42.0% uncooked cottonseed meal + 1.0% Ca(OH)<sub>2</sub> + 0.1% FeSO<sub>4</sub>·7H<sub>2</sub>O  
 - - - - Group fed 42.0% cooked cottonseed meal + 1.0% Ca(OH)<sub>2</sub> + 0.1% FeSO<sub>4</sub>·7H<sub>2</sub>O

the same as that taking place when cotton seed is subjected to oil extraction, since in this case lysine decreases, while in the wet cooking in the presence of calcium and iron, lysine concentration remains essentially the same, as determined chemically and biologically (6).

The symptoms of gossypol toxicity were not the same for all the animals that died, and death did not occur at the same time after the initiation of the study. In most cases the signs of toxicity began to appear about the seventh week after the initiation of the study. In this case, the animals showed anorexia and weight loss and died almost from starvation. On the other hand, the death of a few pigs was sudden, and the animals appeared in fair condition before death. Therefore, the reported chemical values in blood and liver are not necessarily due to gossypol, but to a significant decrease in food intake or even inanition.

Even though the animals receiving both calcium and iron looked and performed satisfactorily, they were slightly less heavy than the animals fed the control diet at the end of the study. There is no explanation available as yet for this observation, but it is possible that calories became somewhat deficient. Recently, it has been reported that high levels of gossypol decreased utilization of energy in chicks (27). Low levels of gossypol were not tested.

The question of how much iron is needed per unit of gossypol, and whether this will vary in the presence of calcium salts, is of interest. The evidence available from the present study suggests that 5 moles of iron to 1 of gossypol will eliminate gossypol toxicity in swine on the basis of 40 mg. of free gossypol and 55 mg. of FeSO<sub>4</sub> per 100 grams of ration. Most workers who have tested the addition of iron to cottonseed diets have obtained different degrees of success. It is possible that this was due to the fact that the diets were not supplemented with additional levels of calcium. In the studies reported by Bressani and co-workers (6), iron addition alone was no more effective in decreasing free gossypol levels than cooking, but a significant decrease occurred when calcium was also present.

## Conclusions

Iron and calcium exert a protective effect against gossypol toxicity to swine, but it is too early to state that iron alone has the same effect. The addition of the two ions will, therefore, permit the use of greater levels of cottonseed flour containing higher levels of gossypol in swine rations.

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