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Effect of Gossypol on the Iron-binding Capacity of Serum in Swine [1,2]

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ABSTRACT The effect of iron and calcium ions, alone and in combination, on the toxicity of gossypol for swine fed cottonseed flour rations, was studied. Weekly data on the performance of the animals were kept for 15 weeks, and blood samples were taken every 15 days and analyzed for hemoglobin, hematocrit, glutamic-oxaloacetic transaminase, total iron-binding capacity and serum iron. The results indicated that, when both chemicals were added to the ration, the animals showed better weight gains and no changes in the hair coat. The addition of calcium alone resulted in low hemoglobin, hematocrit, iron-binding capacity and serum iron levels, but there was no change in the glutamic-oxaloacetic transaminase serum levels. Iron supplementation, however, resulted in hemoglobin, hematocrit and iron-binding capacity approaching those of the control group, but the levels of glutamic-oxaloacetic transaminase were significantly elevated, indicating some liver damage. The addition of both minerals resulted in low levels of the transaminase and hemoglobin, hematocrit and iron-binding capacity levels similar to those obtained with the addition of iron alone. The results suggest that gossypol binds iron, interfering with its absorption, and consequently iron-deficiency anemia develops. The addition of calcium increases the effectiveness of the gossypol-iron complex formation, resulting in full protection from gossypol toxicity, although probably the levels of iron used were not sufficiently high to maintain a normal blood picture.

Several workers [3] (1, 2) have shown that the addition of ferrous salts to swine rations containing cottonseed meal reduces the toxicity of gossypol to varying degrees. The added iron forms an insoluble complex with gossypol, decreasing its free concentration and consequently its toxicity. In vitro studies by Bressani and co-workers (3) showed that the addition of iron in the presence of calcium salts decreases the concentration of free gossypol to a low level in food mixtures containing cottonseed flour. Jarquín et al. (4) have shown recently that the addition of 1% $Ca(OH)_2$ and 0.1% $FeSO_4 \cdot 7H_2O$ completely eliminates or inactivates the toxicity of gossypol for pigs fed high levels of cottonseed meal. Furthermore, Braham et al. (5) observed that the high levels of serum glutamic-oxaloacetic transaminase observed in animals fed high levels of cottonseed meal decreased to normal values when the ration was supplemented with both iron and calcium.

The results of Jarquín et al. (4) with swine, and those of Danke and Tillman (6) with rats, showed that the feeding of high levels of gossypol resulted in decreased levels of hemoglobin and hematocrit. The latter authors showed that the anemia thus produced was microcytic, hypochromic in character.

The present study deals with the effect of the addition of calcium and iron, alone or in combination, on the performance and certain blood constituents of swine fed high levels of cottonseed meal.

MATERIALS AND METHODS

Experimental procedure. The experiment consisted of 7 treatments, one of which was a control. Eight pure-bred Duroc Jersey piglets, 4 males and 4 females, were used per group. The animals were from 6 to 8 weeks of age when placed on the experiment and weighed between 5.4 and 12.2 kg.

Composition of rations. The composition of the rations fed is described in

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[2] INCAP Publication I-420.

[3] Hale, F., and C. M. Lyman 1962 Effective utilization of cottonseed meal in swine rations. J. Animal Sci., *21:* 998 (abstract).

table 1. A ration containing 50% of soybean meal was used as a control and all the experimental rations contained 42% of a high quality cottonseed flour. This material was produced by the pre-press solvent extraction process and contained: (in %) protein, 50; fat, 3.2; crude fiber, 5.1; free gossypol, 0.058; total gossypol, 0.943; available lysine, 3.10 g/16 g N. Ferrous sulfate ($7H_2O$) and calcium hydroxide were added, alone and in combination. The residue from the crude oil purification step contained: (in %) protein, 52.1; fat, 39.7; crude fiber, 2.9; free gossypol, 0.332; total gossypol, 1.228; available lysine, 2.36 g/16 g N, and was added to rations 6 and 7 to increase the gossypol level both in the presence and absence of the combination of ferrous sulfate and calcium hydroxide. All rations were analyzed for free and total gossypol by the methods of the AOCS (7).

Care and feeding of animals. Throughout the experimental period, which lasted 15 weeks, each group of 8 pigs was confined in a pen which measured 5 × 2 m. The pens had concrete floors and were washed daily. Free access to feed and water was provided at all times. All animals received 1 cm^3 of a 20% zinc sulfate solution every other day throughout the experimental period. Weight and feed consumption data were recorded at weekly intervals.

Blood chemistry. Blood samples from the jugular vein were obtained from each pig at the beginning and every 15 days thereafter until the fifteenth week, for the determination of hemoglobin (8), hematocrit (9), serum glutamic-oxaloacetic transaminase[4] and serum total iron-binding capacity and serum iron (10). From the latter values, the unsaturated iron-binding capacity and the amount of circulating transferrin were calculated.

RESULTS

The growth performance, mortality and observations on the discoloration of the hair of the pigs are presented in table 2; also shown is the free gossypol concentration of the ration fed. The animals fed the control ration gained significantly more than those fed the ration containing cottonseed flour with or without additional $Ca(OH)_2$ and $FeSO_4 \cdot 7H_2O$, alone or combined. The addition of ferrous sulfate and calcium hydroxide combined (ration 5) resulted in no greater response, with respect to growth and feed efficiency, than the addition of iron alone (ration 3). There was, however, a marked difference in the appearance of the animals. Those receiving the ration with the combined chemicals showed no discoloration of hair and 5 out of 8 pigs showed discoloration of hair in the group fed the ration supplemented with iron alone.

The results on the blood constituents determined are presented for groups 1 through 5 only, because results for groups 6 and 7 are similar to those for groups 2

[4] Sigma Chemical Company 1964 Tech. Bull. no. 505 (May), St. Louis.

TABLE 1

Composition of the experimental rations

	Ration no.						
	1	2	3	4	5	6	7
Soybean meal	50.0	—	—	—	—	—	—
Cottonseed flour [1]	—	42.0	42.0	42.0	42.0	42.0	42.0
Mineral supplement [2]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Torula yeast	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cod liver oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5
$FeSO_4 \cdot 7H_2O$	—	—	0.1	—	0.1	0.1	—
$Ca(OH)_2$	—	—	—	1.0	1.0	1.0	—
Ground yellow corn	45.3	53.3	53.2	52.3	52.2	46.2	47.3
Cottonseed residue [3]	—	—	—	—	—	6.0	6.0
Aurofac [4]	0.2	0.2	0.2	0.2	0.2	0.2	0.2

[1] Processed for human consumption.
[2] Salmina, Riverside Company, Guatemala, C. A.: contains (in %) calcium carbonate, 33; bone meal, 33; iodized sodium chloride, 33; and minor elements, 1.
[3] A cottonseed filter press cake containing 332 mg/100 g of free gossypol.
[4] 1.8 g chlortetracycline per gram. American Cyanamid Company, Wayne, New Jersey.

TABLE 2

Performance of pigs fed cottonseed meal supplemented with iron and calcium

Ration no.[1]	Free gossypol	Initial wt	Wt gain [2]	kg feed/kg gain ratio	Mortality	No. of pigs showing hair depigmentation
	mg/100 g	*kg*	*kg*			
1	—	8.1	54.6	3.23	0/8	0/8
2	30	8.1	31.2	3.42	0/8	6/8
3	20	8.1	38.8	3.24	0/8	5/8
4	26	8.2	31.0	3.41	1/8 [3]	1/7
5	19	8.1	42.4	3.44	0/8	0/8
6	26	8.2	37.2	3.36	0/8	1/8
7	44	8.1	24.9	2.97	0/8	8/8

[1] See table 1 for treatments.
[2] Differences between control and experimental groups statistically significant ($P < 0.01$); least significant difference: 16.5 kg.
[3] Death of one animal due to diaphragmatic hernia.

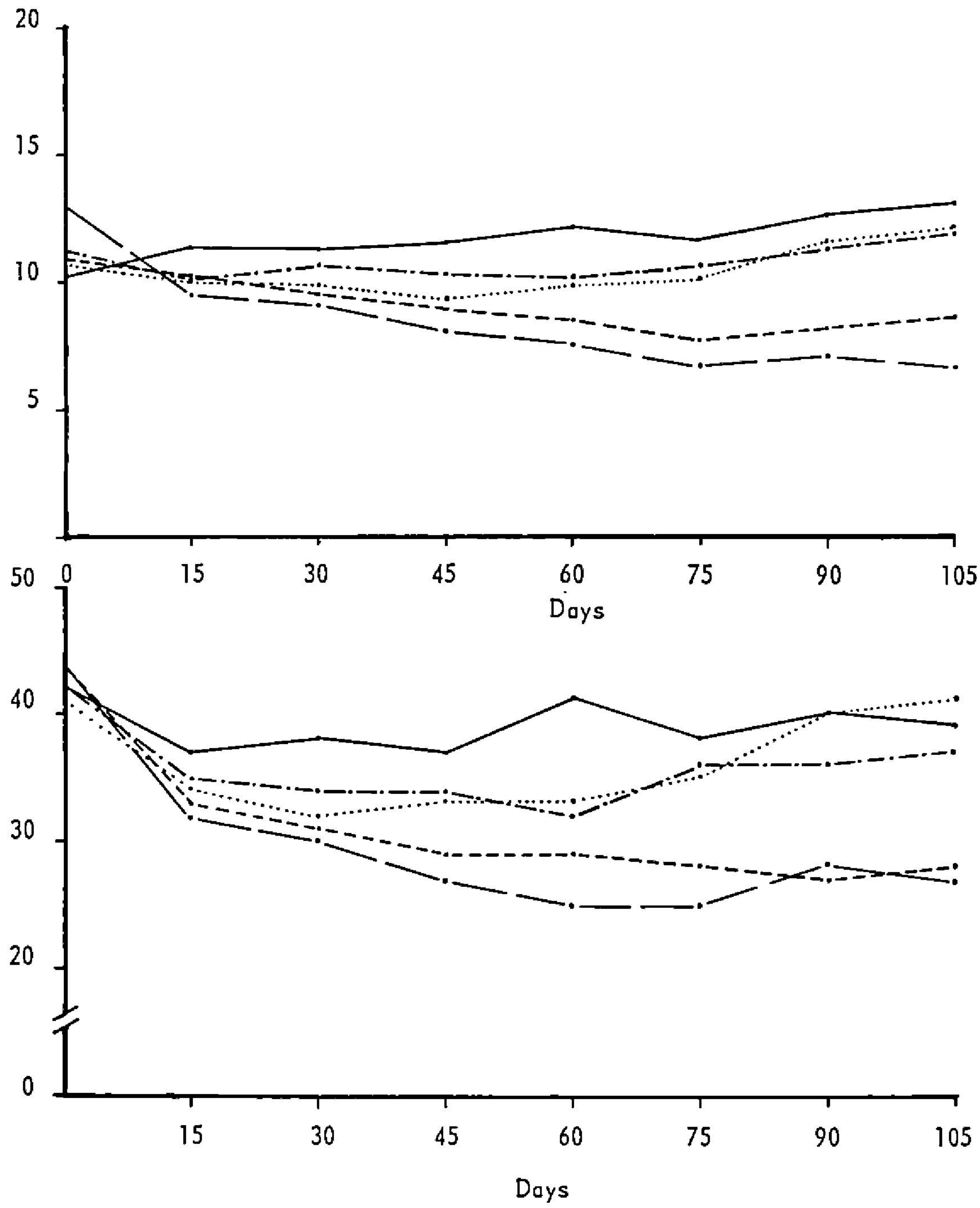

Fig. 1 Effect of the addition of calcium and iron on the levels of hemoglobin and hematocrit of pigs fed cottonseed meal; ——— control; – – – – – – cottonseed meal; —·—·— cottonseed meal + Fe; ·— —· cottonseed meal + Ca; ...... cottonseed meal + Fe + Ca.

and 3, respectively, except for higher levels of gossypol, and the blood values obtained were the same in trend and magnitude for each group and its counterpart.

Hemoglobin levels. Figure 1 shows the hemoglobin levels of the animals in the various groups. The results indicated a significantly lower hemoglobin concentration ($P < 0.01$) in all groups fed cottonseed meal with and without supplementation when compared with the controls. Iron supplementation alone, or the combination of calcium and iron increased the levels of hemoglobin but not quite to normal values and calcium supplementation alone resulted in the lowest levels throughout the experimental period.

Hematocrit values. The same trends were observed in the hematocrit values (fig. 1) as those observed for hemoglobin. The lowest values were observed in the group fed the ration supplemented with calcium alone. The effects of treatment and time were highly significant ($P < 0.01$).

Serum glutamic-oxaloacetic transaminase (SGO-T). The results for this determination are shown in figure 2. The groups fed cottonseed meal, alone or supplemented with calcium, or with calcium and iron, showed values similar to those obtained for the control group. The values obtained for the group supplemented with iron alone, however, showed a significant increase ($P < 0.05$) in the levels of this enzyme.

Serum total iron-binding capacity (TIBC) and serum iron. Figure 3 shows the results for these 2 determinations. There was a significant difference ($P < 0.01$) due to treatments and to time for both determinations; the interaction between time and treatments was also significant. The addition of iron alone maintained the levels of TIBC and serum iron within normal limits; the combination of calcium and iron in the ration was less effective than iron alone, and the addition of calcium alone resulted in the highest levels of TIBC and lowest levels of serum iron.

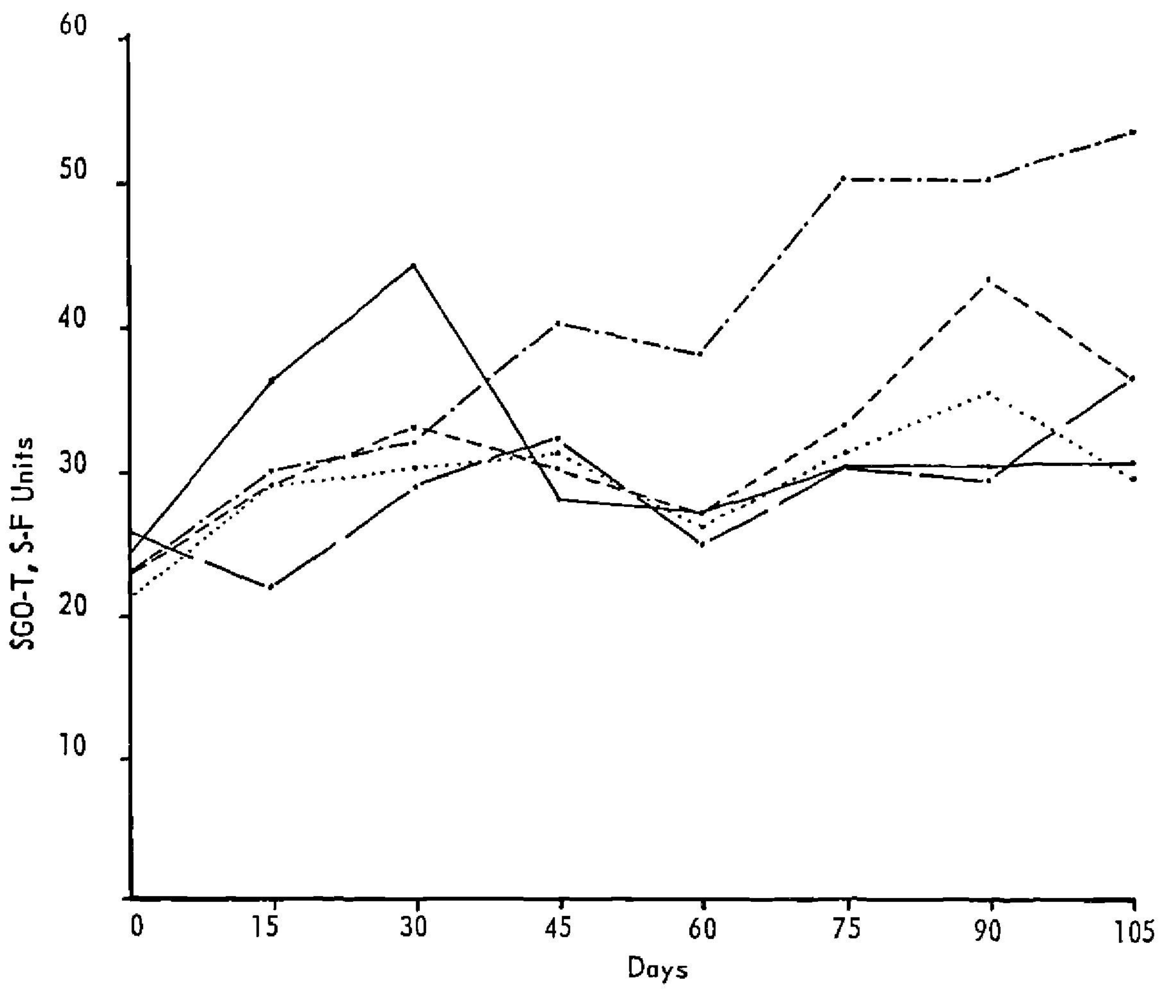

Fig. 2 Effect of the addition of calcium and iron on the levels of serum glutamic-oxaloacetic transaminase of pigs fed cottonseed meal; ——— control; – – – – – – cottonseed meal; —·—·— cottonseed meal + Fe; ·— —· cottonseed meal + Ca; ...... cottonseed meal + Fe + Ca.

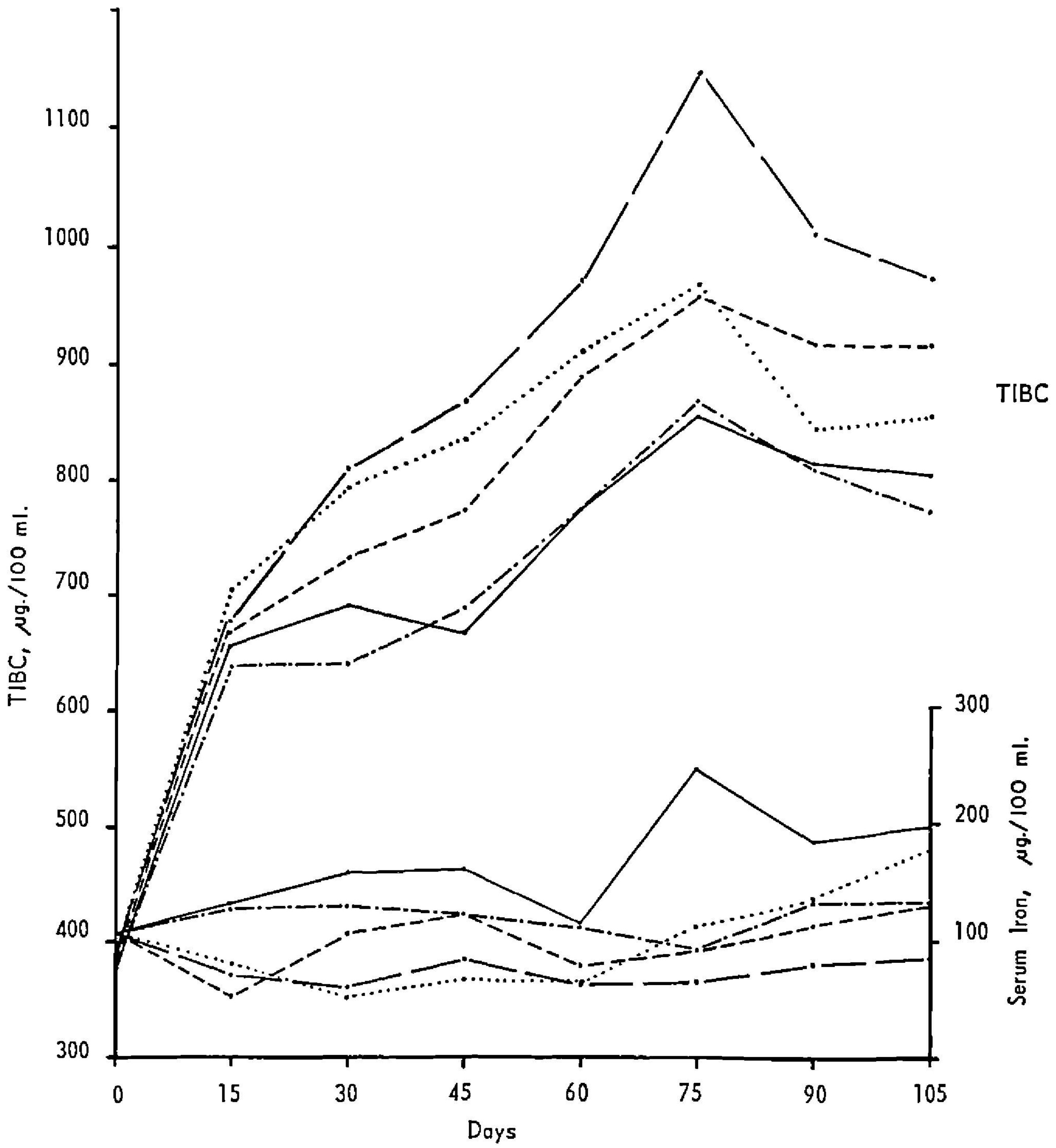

Fig. 3 Effect of the addition of calcium and iron on serum total iron-binding capacity and serum iron of pigs fed cottonseed meal; ——— control; – – – – – – cottonseed meal; —·—·— cottonseed meal + Fe; ·— —· cottonseed meal + Ca; ...... cottonseed meal + Fe + Ca.

The percentage of saturation of transferrin is shown in figure 4. As expected, the lowest values correspond with the group fed the ration supplemented with calcium alone, and iron alone was more efficient than the combination of both chemicals.

When iron alone was fed, the unsaturated iron-binding capacity values (UIBC) of the serum were almost as low as those obtained in the control group. Its combination with calcium was less effective in this respect, and the supplementation of calcium alone resulted in the highest UIBC. The amount of circulating transferrin was not actually determined. It was calculated from the molecular weight of transferrin (89,000), number of iron atoms bound by a molecule of this serum protein (2 atoms) and determined values of TIBC. The average figures obtained were 60.2, 65.8, 58.7, 74.1 and 67.8 g % of transferrin for groups 1, 2, 3, 4 and 5, respectively. Iron alone maintained normal levels, and its combination with calcium increased the levels of transferrin. Calcium alone increased the levels of the iron-binding protein to the highest values obtained within the experimental conditions used.

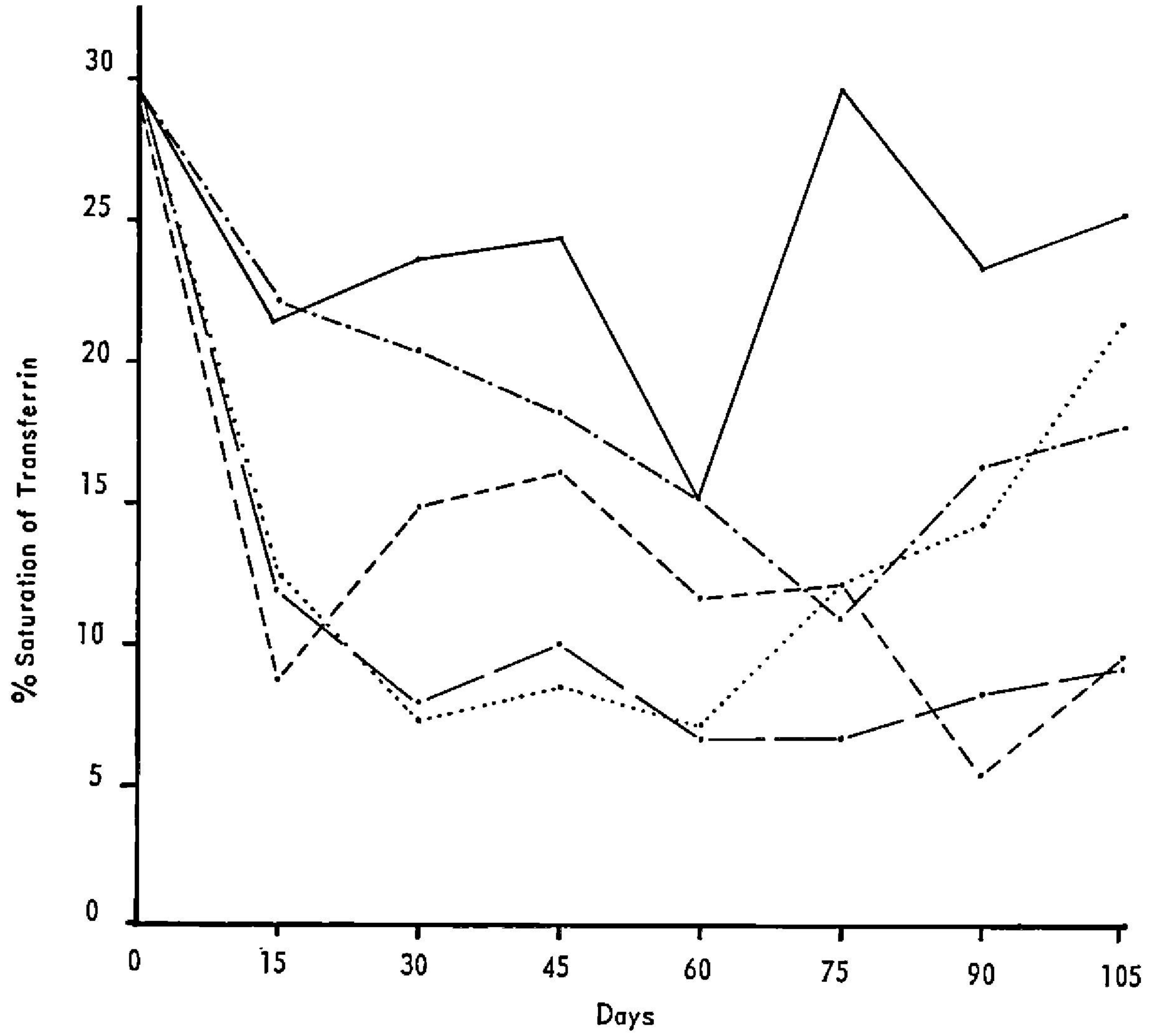

Fig. 4 Effect of the addition of calcium and iron on the saturation of serum transferrin of pigs fed cottonseed meal; ——— control; – – – – – – cottonseed meal; ·—·—·— cottonseed meal + Fe; ·—— ——· cottonseed meal + Ca; . . . . . . cottonseed meal + Fe + Ca.

DISCUSSION

The data on the performance of the pigs show the same trend as those reported previously from this laboratory (4). Neither iron nor the combination of iron and calcium resulted in weights comparable to those obtained with the control group, which may likely be due to some interference, by gossypol, with the utilization of calories. However, as the control group was fed a soybean meal ration, the soybean protein may have supported better growth than the cottonseed protein. When the cottonseed flour control ration (ration 2) is used as a basis of comparison, there is a definite effect of iron and of its combination with calcium; but not of calcium alone with respect to weight gain. This again corroborates the reports on the effect of iron and the detoxification of gossypol (1, 2).

As in previous studies (5), it was observed that some animals showed discoloration of the hair. The usual shiny, red hair coat of the animals turned a dull yellow and the smooth texture of the hair became coarse and kinky. As shown in table 2, gossypol in the ration appears to cause the hair changes. Iron alone in the ration did not have much protective effect, whereas calcium did. The combination of both protected the animals completely.

The role of gossypol in relation to color and structure of the hair is still obscure. Some minerals, such as zinc and copper, might be involved. Such possibilities are being studied in this laboratory.

The hematological findings characterized by low hemoglobin and hematocrit values, high iron-binding capacity, unsaturated iron-binding capacity and transferrin concentration, and low serum iron and percentage of saturation of transferrin, indicate a typical picture of iron-deficiency anemia (11). The results show that when calcium alone is added to the ration, the symptoms of anemia are made more acute than when iron was added alone or combined with calcium.

When 0.1% ferrous sulfate was added alone, there was a hematological response toward normal values which, however, never reached the values obtained with the group fed the soybean meal ration. The combination of iron and calcium was better than iron alone, and calcium alone resulted in the lowest hematological values obtained in this study.

The mechanisms through which iron-deficiency anemia is being produced may be as follows: when iron is added alone, in the absence of added calcium, part of it would be used to bind the gossypol present to the extent that the calcium present from the mineral mixture added and that from other components of the ration would allow. That this amount of calcium is not enough to result in significant binding of iron to gossypol is shown by the fact that enough iron is available to maintain a hematological picture approching normality. On the contrary, when calcium hydroxide alone was added, it exerted its stimulatory effect on the trapping of gossypol by the iron present from the other components of the ration so efficiently that very little iron was absorbed, as shown by the low levels of serum iron and the other hematological measurements. When the 2 compounds were added, the results were very similar to those obtained when iron alone was added. Evidently iron is being bound by gossypol in the presence of calcium, but the level of 0.1% ferrous sulfate used in this study is still not enough both to bind all the gossypol present and maintain a normal blood picture.

The results suggest that the toxic effect of gossypol is not the result of a systemic or specific action of the pigment on the hematopoietic mechanism. The binding of gossypol by iron probably takes place at the intestinal level, although it is clear from the results of Bressani et al.[5] that this binding is effective in vitro, and therefore already effective in the ration itself before it is consumed. Both mechanisms may be taking place, but whether in vitro or in vivo, the process appears to be very inefficient. This view is supported by the fact that, although chemically the ratio might be 1:1, biologically it is not. In this respect, Jarquin et al. (4), in experiments with swine, calculated that 5 moles of iron were needed to bind 1 mole of gossypol.

When a measure of the specific effect of gossypol at the organ level, such as the determination of serum glutamic-oxaloacetic transaminase as an indication of liver necrosis, was used, the results corroborated those discussed previously. This enzyme was significantly increased only when iron alone was supplemented, which indicates again that the level of iron used was not capable, in the absence of added calcium, of binding all the gossypol present. Enough iron was thus available for absorption, as judged by the hematological picture of these animals, and enough gossypol was also free for absorption, as indicated by the serum transaminase levels. Once calcium and iron were added, the levels of the enzyme dropped to normal. Calcium, when supplemented alone, maintained normal levels of transaminase, since all of the gossypol, or as much as the iron from the other components of the ration will allow, is being bound. We have no explanation for the low levels of transaminase obtained when cottonseed meal alone was fed.

In conclusion, the 2 effects of gossypol are unrelated. Animals whose rations are supplemented with iron alone will probably have normal hematology but enough gossypol may be absorbed, producing toxic effects, and calcium alone will result in an abnormal blood picture, but otherwise no toxic symptoms will be detected. The combination of the two will result in normal animals, as long as the amount of iron added can successfully bind all the gossypol present and still support normal hemopoiesis.

LITERATURE CITED

1. Withers, W. A., and F. E. Carruth 1917 Iron as an antidote to cottonseed meal injury. J. Biol. Chem., 32: 245.
2. Gallup, W. D. 1928 The value of iron salts in counteracting the toxic effects of gossypol. J. Biol. Chem., 77: 437.
3. Bressani, R., L. G. Elías, R. Jarquín and J. E. Braham 1964 All-vegetable protein mixtures for human feeding. XIII. Effect of cooking mixtures containing cottonseed flour on free gossypol content. Food Technol., 18: 1599.

[5] Bressani, R., L. G. Elías and J. E. Braham 1965 Effect of pH on the free gossypol level and nutritive value of cottonseed protein concentrates. Federation Proc., 24: 626 (abstract).

4. Jarquín, R., R. Bressani, L. G. Elías, C. Tejada, M. González and J. E. Braham 1966 Effect of cooking and iron supplementation on gossypol toxicity in swine. J. Agr. Food Chem., *14:* 275.
5. Braham, J. E., R. Jarquín, L. G. Elías, M. González and R. Bressani 1967 Effect of calcium and gossypol on the performance of swine and on certain enzymes and other blood constituents. J. Nutr., *91:* 47.
6. Danke, R. J., and A. D. Tillman 1965 Effect of free gossypol and supplemental dietary iron on blood constituents of rats. J. Nutr., *87:* 493.
7. American Oil Chemists' Society 1945–1950 Official and Tentative Methods of the American Oil Chemists' Society, ed. 2. Chicago.
8. Cannan, R. K. 1958 Proposal for a certified standard for use in hemoglobinometry. Clin. Chem., *4:* 246.
9. Wintrobe, M. M. 1951 Clinical Hematology, ed. 3. Lea and Febiger, Philadelphia.
10. Ramsay, W. N. M. 1958 Plasma iron. Advance. Clin. Chem., *1:* 1.
11. Lahey, M. E., M. Béhar, F. Viteri and N. S. Scrimshaw 1958 Values for copper, iron and iron-binding capacity in the serum in kwashiorkor. Pediatrics, *22:* 72.