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Inhibitory Effect of Gossypol on Microorganisms

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Cottonseed meal is known to contain a considerable amount of gossypol [1,1'-6,6'-7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl-(2,2'-binaphtalene)-8,8'-dialdehyde]. This pigment is produced mainly in the glands of the cotton seed, where its concentration may reach up to 1.7% and occasionally more than 6% of the seed's dry weight. After extraction of the oil, the majority of the pigment, which is liberated from the glands during processing, is retained in the meal partly in a protein-bound form (R. Adams, T. A. Geissman, and J. D. Edwards, Chem. Rev. **60**:555, 1960). Since cotton seeds are commercially processed in the oil industry, gossypol and its derivatives may be obtained in large quantities. The extraction procedure and the preparation of pure gossypol have been described (F. E. Carruth, J. Biol. Chem., **32**:87, 1917).

After the introduction of cottonseed meal as a feedstuff during the first half of the 19th century, it was found that substances toxic to pigs, chickens, and other nonruminant animals were contained in the seedmeal; this has limited its use to ruminants. With the isolation of gossypol and its chemical characterization, this toxicity was shown to be caused by this pigment or by related pigments (J. R. Couch, W. Y. Chang, and C. M. Lyman, Poultry Sci. **34**:178, 1955). The physiological effects of gossypol have been described in the literature. Erythrocyte hemolysis, pulmonary edema, and cardiac irregularities have been the most common effects of gossypol poisoning (P. Menaul, J. Agr. Res. **26**:233, 1923). Very low concentrations of gossypol, 40 ppm, in the diet of laying hens have resulted in discoloration of the egg yolks and whites of stored eggs (B. W. Heywang and M. G. Vavich, Poultry Sci. **44**: 84-89, 1965). Gossypol is a powerful poison when introduced into the blood stream, with 50 mg causing almost instant death in the rabbit, although per os an LD_{50} of only 2,400 to 2,801 mg/kg has been reported (E. Eagle et al., J. Am. Oil Chemist's Soc. **33**:15-21, 1956). The effect of gossypol on some of the respiratory enzymes has recently been described. Succinic dehydrogenase and cytochrome oxidase from sweet potato were completely inhibited by gossypol at 75×10^{-3} and 2×10^{-3} M, respectively (B. D. Meyers and G. O. Throneberry, Plant Physiol. **41**:787-791, 1966).

The fact that free gossypol is poisonous to nonruminants, whereas large amounts can be fed to ruminants with no sign of toxicity (D. S. Ramsey and J. T. Miles, J. Dairy Sci. **36**:1308-1312, 1953) drew out attention to the possibility that microbes could be involved in a detoxification process of this pigment. A literature survey revealed that one group of workers (R. Reiser and H. F. Fu, J. Nutr. **76**:215-218, 1962) had studied the effect of rumen liquor on gossypol, and had shown that detoxification was due to the binding of gossypol to rumen protein with a

TABLE 1. *Inhibitory effect of gossypol on microorganisms*

Organism	Minimal inhibitory concn
	µg/ml
Bacteria	
Staphylococcus aureus	10
Sarcina lutea	25
Bacillus polymyxa	50
B. megaterium	50
B. licheniformis	25
B. cereus	50
B. thermoacidurans	50
Leuconostoc mesenteroides	10
Lactobacillus delbrückii	20
Escherichia coli	>200
Proteus mirabilis	>200
Pseudomonas aeruginosa	>200
Yeasts	
Saccharomyces cerevisiae	>200
S. carlsbergensis	>200
Zygosaccharomyces mellis	>200
Hansenula anomala	200[a]
Hanseniaspora sp.	200[a]
Candida utilis	>200
Debaryomyces nicotianae	100
Pichia membranefaciens	25[b]
Cryptococcus neoformans	25
Rhodotorula mucilaginosa	>200

[a] Caused slight inhibition.
[b] Caused complete suppression of film growth.

simultaneous disappearance of 2 moles of lysine E-amine groups per mole of toxin. It was suggested that the gossypol-protein complex was highly resistant to the action of proteolytic enzymes and was, therefore, excluded from metabolic processes. Since the recovery of gossypol from the feces of gossypol-fed animals has not, however, been reported, the possibility of microbial intervention in vivo is not eliminated. There are also no reports on the direct effect of gossypol on microorganisms in pure cultures.

Aqueous solutions of gossypol acetate, obtained from the Southern Utilization Research Branch, U.S. Department of Agriculture (purity over 95%) were prepared with a few drops of 2 N NaOH. Antibiotic activity was tested by the addition of a Seitz-filtered gossypol solution to sterile tubes containing 5 ml of nutrient broth (0.5% yeast extract, Difco; 0.5% peptone, Difco; 2% glucose). The *p*H of the broth was adjusted to 6.4 before autoclaving, since gossypol oxidizes at neutral and alkaline *p*H levels, as indicated by brownish discoloration. The tubes were inoculated with one drop of a slightly turbid suspension of the test organism and incubated at 30 or 37 C, for 24 or 48 hr.

Table 1 gives the minimal inhibitory concentration of gossypol for the complete inhibition of growth of the respective test organism; concentrations over 200 μg/ml could not be examined because of limited solubility. It is evident that gossypol displays pronounced antibiotic activity towards aerobic sporeformers and lactobacilli, and is also antagonistic to some of the more oxidative yeasts. These data indicate that the activity of gossypol in cottonseed meal-fed animals might lead to a substantial change in the equilibrium of the microflora of the gastrointestinal tract; thus, this activity should be taken into consideration in studies on the effect of gossypol in the nutrition and metabolism of nonruminants, as well as in the microbial population of the rumen.