GOSSYPOL AND COTTONSEED MEAL POISONING

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For a long time stock feeders have known that cottonseed meal may has also been known that the toxicity of meals is likely to vary with the locality and the year in which the cottonseed was grown. This indicates that during the growth of cottonseed seasonal and climatic conditions play a role which later affects the toxicity of the meal. The fact that the meal made from cottonseed grown in the southwestern part of the United States may usually be fed in moderate quantities without danger points to the conclusion that regional conditions of growth are responsible, in part at least, for the general differences in feeding results. In 1918 Dr. C. L. Alsberg, then Chief of the Bureau of Chemistry, initiated a study of this problem ([10], [11], [12]) in the hope of fixing definitely the causes for the toxicity of different lots of cottonseed meal.

Several theories had been advanced to account for the toxicity of cottonseed meal. Of these the one offered by Withers and Carruth (16, 17) seemed the most plausible. It was therefore selected as a basis for the investigation. Briefly, the hypothesis of these observers was that the toxicity of cottonseed meal is due to a phenolic substance which they called gossypol.

For the investigations here discussed the cottonseed kernel was chosen as the source of the necessary material because it lends itself more readily to extraction with ordinary solvents for the preparation of toxic and nontoxic fractions than does the cottonseed cake. An examination of thirty-nine authentic samples of cottonseed, representing eleven varieties grown in the United States in different years, showed that the gossypol content varies directly with the oil content.

The method of Garruth as modified by Schwartze and Alsberg ([10]) was used for the determination of gossypol. The kernels were extracted with ether, the ether was evaporated, and petroleum ether was added. Aniline was added to the petroleum ether solution. At the end of suitable intervals, the analine-gossypol compound was weighed on the Gooch crucible. Corrections were made for susequent precipitation in the mother liquor and for the nitrogen content in the aniline-gossypol compound.

The gossypol content of the hull-free kernels ranged from approximately 0.4 to 1.2 per cent and the oil content from approximately 25 to 40 per cent. As the gossypol content increase with the amount of oil in the seed, it seems proper to use the "rule of oil" as an index of the gossypol content of cottonseed. The protein content varied inversely with the gossypol content. We found no certain indication of a varietal factor influencing the amount of gossypol.

The oil and protein contents determined by analysis of the seeds obtained from various sources are in general agreement with the practical findings

of cottonseed millers. There is therefore a tendency to a regional variation in the gossypol content of cottonseed. In agreement with this conclusion are practical observations of stock feeders, namely, that in the Southwest cottonseed meal may be fed to farm animals with little or no precautions with respect to the presence of a toxic agent, whereas on the Atlantic coast caution is necessary in feeding because the seed grown there has a higher gossypol content.

Our results and also those of Stanford and Vichoever ([13]) indicate that cottonseed free from gossypol does not exist. The toxicity problem therefore can be solved only by working out an effective industrial procedure to eliminate this substance.

Four samples of kernels and the oils extracted from them were used for the pharmacological part of our investigation. The meals were prepared by removing the hull and lint from the seed and properly grinding them. The four lots of seed covered extreme variations in gossypol content. The prepared meals were incorporated into diets which contained all the essential ingredients for the maintenance of the experimental animals. The quantity of each fed was based upon the quantity of gossypol therein as determined by chemical analysis. The effect of the cottonseed kernel diet was compared with that of a peanut meal diet containing equivalent quantities of purified gossypol. With moderate variations the toxicity of each diet corresponded to the gossypol content. Samples of the four varieties of cottonseed meals used in the feeding experiments were extracted with ether, and the ether extract was made up to volume with purified peanut oil. Doses calculated on the basis of the chemical analyses of the meals were injected into rats. The acute toxicity of the seed corresponded, with moderate variations, to the gossypol content. In no instance was any toxicity greater than that indicated by chemical analysis. The conclusion drawn from these experiments was that the cottonseed kernels are practically as toxic as the results of chemical analyses indicated them to be.

As rats showed no symptoms of chronic poisoning from gossypol, except loss of appetite and failure to grow well, it was necessary to use other animals. The cat was chosen because it can be handled in relatively large numbers in a small laboratory, is a meat eater, and is less susceptible to acidosis and the ill effects of starvation than herbivorous and omnivorous animals. Cats fed on gossypl suffered paralysis of the voluntary muscles and lost control of the bladder sphincter. Hypertrophy and enlargement of the heart, edema of the lungs, and effusion into the serous cavities also resulted. In acute cases of intoxication an intense edema at the site of injection (or diarrhea if fed) and a severe edema of the lungs occurred. Gossypol is a cardiac poison, as shown by blood pressure experiments.

Metabolism experiments with cats on a meat diet showed that gossypol causes loss of nitrogen both in the feces and the urine, regardless of the fact that the amount of food consumed was small and the animals were in nitrogenous equilibrium. Recovery followed when gossypol was withdrawn. The evidence, therefore, justifies the assumption ([14]) that cottonseed meal poisoning is a metabolic disturbance.

Rabbits fed with gossypol became paralyzed in the hind legs and lost control of the bladder. Recovery followed early when gossypol was withdrawn. There may appear to be some degree of convalescence with both cats and rabbits even if gossypol is not withdrawn, but eventually the animals relapse.

The diets fed to experimental animals of each type were the same from day to day. They were prepared in a uniform way and each animal received food containing a definite concentration of gossypol. The only variable was the amount of food eaten per day, which was controlled by establishing the concentration of gossypol on which a certain number of the animals would not go "off the diet" appreciably. Two hundred and twenty-five parts of gossypol, obtained from purified gossypol-acetate, per million parts of dry oats was the minimum effective concentration for rabbits and the same concentration of gossypol in fresh meat-milk powder diet for cats. These concentrations produced a toxic effect other than any that could possibly be ascribed to loss of appetite and consequent inanition. Rats would not grow well on 1350 parts of gossypol per million of standard dry mixed rat diet. It should also be noted that the dosages specified apply to animals which were exceptionally well fed. The state of nutrition and other factors undoubtedly will modify these figures.

A brief resumé of the more recent work on cottonseed meal poisoning and its relation to our work is desirable. Rommel and Veddar ([9]) reasoned that cottonseed poisoning and beriberi were "analogous if not identical" because the symptoms are alike. They believed that both are deficiency diseases. The iron deficiency theory advanced by McGowan and Crichton ([3], [4]) was reached by reasoning by analogy. Osborne and Mendel ([7]) and Macy and Mendel ([5]), however, have shown that cottonseed protein is nutritionally adequate and that cottonseed meal contains some water-soluble vitamin.

As a matter of fact, gossypol alone in other than a cottonseed diet produces all the symptoms of cottonseed poisoning. Theoretically, cottonseed poisoning may be a deficiency disease. After the gossypol or decomposition products of gossypol reach the intestinal tract, or even after they have been absorbed, they might affect the vitamin utilization, the protein digestion or metabolism, or the iron economy of the animal in question. Previous investigators have shown that gossypol reacts with iron in vitro ([1]) and also that experimental animals being fed cottonseed

meal are benefited when iron is added to their diet ([15]). It has also been shown that gossypol inhibits protein digestion in vitro ([2]), probably in the animal intestine ([6,8], [12]) as well, and causes metabolic disturbances ([12], [14]). There is some evidence, however, against the hypothesis that gossypol owes its effect upon the system to an attack upon the vitamins, the protein utilization, or the iron within the body. The presence in the diet of more than enough of any one of these ingredients has not been found completely effective in warding off gossypol or cottonseed poisoning. Certain clinical features, such as remissions and relapses, also mitigate against this hypothesis. It is important, therefore, in the present state of our knowledge to distinguish between what causes cottonseed poisoning and the effect and manner in which it acts.

It seems evident that cottonseed meal poisoning is due to gossypol *per se*, or to one of its decomposition products which has a similar action, or perhaps to both. The question then arises, how can cottonseed meal be detoxified. Before this question can be answered it seems necessary to establish the structure of the gossypol molecule and its chemical reactions. In addition, some of its physico chemical properties, such as its tendency to become bound or adherent to the meal, must be studied. Benzol extraction of raw cottonseed will not settle the entire question, because the complete extraction of the toxic material would require much more labor than is needed for a good recovery of oil. Moreover, such a treatment might not be successful with some lots of seed, particularly those that had become heated. Future progress of the cottonseed meal problem, therefore, depends upon the successful solution of the gossypol problem.

Discussion

The reality of cottonseed meal poisoning is not debatable since it has already received too much practical and academic verification.

The proof that gossypol or a decomposition product of it, or both is the cause of this poisoning is indirect owing to the necessity of employing quantitative chemical methods of analysis which are unfortunately uniformly applicable only to cottonseed kernels. Such problems as "How much gossypol in cottonseed meal may be fed to farm animals with safety" and "Is gossypol or its decomposition products as toxic in the bound state in which they exist in the meal as when not so bound" are awaiting the discovery of satisfactory methods of chemical analysis of the meal so that chemical experiments can parallel the feeding experiments. No amount of simple negative evidence obtained in feeding tests will alone suffice to further this problem since we already know that some lots of toxic cottonseed meal can be fed in small amounts without apparent injury.

The gossypol issue should not be confused with other issues. Experi-

ments with gossypol alone cannot be expected to throw light on the salt, protein, and vitamine adequacy of diets. Neither can a gossypol study inform one concerning moldy, heated or otherwise decomposed meal. Gossypol intoxication is due to the addition of something to the diet, which experimentally is quite the opposite to a deficiency phenomenon.

It may be that all of these factors just enumerated are encountered at times in agricultural practices and experiments. To what degree they have supplemented or have been confused with gossypol intoxication remains to be established.

Bibliography

1. Carruth, F. E.
   1918. Contribution to the Chemistry of Gossypol, The Toxic Principle of Cottonseed. Jour Amer. Chem. Soc. **40**: 647-663.
2. Jones, D. B., and Watrman, H. C.
   1923. Studies on the Digestibility of Proteins in Vitro. IV. On the Digestibility of the Cottonseed Globulin and the Effect of Gossypol upon the Peptic-Tryptic Digestion of Proteins. Jour. Biol. Chem. **56**: 501-511, illus.
3. McGowan, J. P. and Crichton, A.
   1924. Cottonseed Meal Poisoning, Bioch. Jour. **18**: 265-273.
4. McGowan, J. P.
   1924. On the Pathology of Iron Deficiency and Cottonseed Poisoning in Pigs. Jour. Pathol. and Bact. 27: illus.
5. Macy, I. G., and Mendel, L. B.
   1920. Comparative Studies on the Physiological Value and Toxicity of Cottonseed and Some of Its Products. Jour. Pharmacol. **16**: 345-390, illus.
6. Mendel, L. B., and Fine, M. S.
   1912. Studies in Nutrition. V. The Utilization of the Proteins of Cottonseed. Jour. Biol. Chem. **11**: 1-3.
7. Osborne, T. B., and Mendel, L. B.
   1917. The Use of Cottonseed as Food. Jour. Biol. Chem. 29: 289-317, illus.
8. Rather, J. B.
   1913. Digestion Experiments on Men with Cottonseed Meal. Tex. Agr. Exp. Sta. Bul. 163, 26 p.
9. Rommel, G. M., and Vedder, E. B.
   1915. Beriberi and Cottonseed Poisoning in Pigs. Jour. Agr. Research **5**: 489-493.
10. Schwartze, E. W., and Alsberg, C. L.
   1923. Quantitative Variation of Gossypol and Its Relation to the Oil Content of Cottonseed. Jour. Agr. Research **25**: 285-295, illus.

11.

1924. Pharmacology of Gossypol. Jour. Agr. Research, 28: 191-196, illus.

12.

1924. Relation Between Toxicity of Cottonseed and Its Gossypol Content. Jour. Agr. Research 28: 173-189, illus.

13. Stanford, E. E., and Viehoever, A.

1918. Chemistry and Histology of the Glands of the Cotton Plant, with Notes on the Occurrence of Similar Glands in Related Plants. Jour. Agr. Research **18**: 419-436, illus.

14. Wells, C. A., and Ewing, P. V.

1916. Cottonseed Meal as an Incomplete Food. Jour. Biol. Chem. **27**: 15-25.

15. Withers, W. A., and Brewster, J. F.

1913. Studies on Cottonseed Meal Toxicity. II. Iron as an Antidote. Jour. Biol. Chem. **15**: p. 161-166.

16. Withers, W. A., and Carruth, F. E.

1915. Gossypol, The Toxic Substance of Cottonseed Meal. Jour. Agr. Res. **5**: 216-288, illus.

17.

1918. Gossypol, the Toxic Substance of Cottonseed. Jour. Agr. Research, **15**: pp. 83-102, illus.

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