

## XXIX. THE POISONOUS SUBSTANCE IN COTTONSEED

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There have been various suggestions as to the cause of poisoning and death from the feeding of cotton seed meal. It has been variously ascribed to the lint, the oil, the high protein content, to a toxic protein or toxic alkaloid, to cholin and betain, to resin present in the meal, to decomposition products, and to a salt of pyrophosphoric acid. In 1915 Rammel & Veeder of the U. S. Bureau of Animal Husbandry suggested that poisoning by cotton seed is similar to beri-beri, and is caused by deficient diets. Richardson and Green of the University of Texas, concluded that cottonseed caused injury by being deficient in mineral salts and vitamins.

### The Occurrence and Properties of Gossypol

If a cross section of a cottonseed kernel is examined with a lens, many small yellowish brown spots may be seen; these are secretion cavities filled with a compound called gossypol, first isolated in 1899, from by products in the manufacture of cottonseed oil. Gossypol is a yellow colored substance having the chemical properties of phenol (carbolic acid) and of tannic acid. It is insoluble in water but soluble in alkalis, the solution at first being yellow, then

changing to a violet, then black, the changes being due to oxidation.

#### Experimental Work with Gossypol

Ether will extract the gossypol from cottonseed meal, the extracted meal is not toxic to rabbits; the ether extract after the removal of the ether is toxic and consists of crude gossypol. One-tenth of a gram of gossypol fed to rabbits each day with their ration, results in their death, in from eight to twenty-five days, even if the gossypol is put in their ration for only three or four days. In some cases a rabbit which had been given only one-tenth of a gram died fourteen days afterwards. One-tenth of a gram given in a single dose seems to be the smallest amount that will cause death in a rabbit. A peculiar feature about the effects of gossypol or cottonseed, is that the animals may eat these substances for several days without seeming affected, then they may suddenly cease eating, waste away and finally die.

Five-hundredths of a gram of gossypol injected into the blood vessel of a rabbit caused death in about four minutes. The animal acted as though suffocated, leaped high in the air and gasping during this interval. On post mortem examination, the blood was found to be black in color as though death was due to suffocation. One-half this amount was given in the same manner, the animal became apparently paralyzed, and lay on the floor, unable to move its limbs. The animal recovered the use of its limbs after an hour and sat up, but died sixteen hours later.

These experiments indicate that gossypol affects the oxygen carrying power of blood, so experiments to determine the oxygen carrying power of blood treated with gossypol were made. Sheep's blood saturated with oxygen, arterial was used, and the "oxygen capacity" i. e., the amount of oxygen that a definite volume of blood would liberate was determined. Two hundredths of a gram of gossypol in two c. c. of blood, i. e., with  $1\frac{1}{2}\%$  gossypol, the blood gave off only 45.6% of the oxygen that the same amount of blood without the gossypol, would liberate. Two thousandths of a gram of gossypol added to two c. c. of blood i. e., with  $1\frac{1}{2}\%$  gossypol, the blood gave off only 64% of the oxygen liberated by the arterial blood.

Venous blood will liberate 75% of the amount of oxygen that arterial blood will liberate. It is evident that gossypol prevents the liberation of oxygen from the blood, and this property is very marked even when very small quantities of gossypol are present.—one-tenth of one per cent of gossypol.

These results are in accordance with the symptoms observed after feeding cottonseed to animals, noticeably, a shortness of breath.

### Hemolytic Action

Gossypol dissolves in alkaline solutions, thereby neutralizing them. If such a solution of gossypol is shaken a thick foam is formed, resembling the property of saponins. Saponins are characterized by their power to hemolyse red blood cells.

The hemolytic power of gossypol was determined and found that one-hundredth of one per cent (.01%) gossypol causes hemolysis.

A solution of gossypol at a dilution of 1:100,000 will kill fish.

Our experiments on the toxicity of cottonseed products indicate that the toxicity varies with the conditions of cooking the raw seed, raw kernels being the most highly toxic,—the dark colored, cottonseed meal which had been cooked at a high temperature the least toxic. Gossypol is destroyed under favorable conditions at high temperatures due to oxidation. At Dr. Dowell's suggestion we carried out an experiment in which one pen of pigs (28 pounds each) was fed on a well balanced ration containing commercial cottonseed meal. Another pen was fed the same amount of cottonseed meal which had been moistened, cooked at 15 pounds pressure for 15 minutes and then dried.

At the end of three weeks no difference in the two lots was noticeable, neither in condition or gain in body weight, but from then on the pigs fed on commercial cottonseed meal were noticeably inferior to the pigs fed on the autoclaved product. At the end of 73 days the pigs fed the autoclaved cottonseed had gained 9.5 pounds more than those fed the commercial cottonseed meal. Gain of each pig fed cottonseed meal 23.5 pounds. Gain of each pig fed autoclaved cottonseed meal 33.0 pounds. The gain in weight of each pig fed commercial cottonseed meal was 71% of the gain of those fed autoclaved cottonseed meal.

All hogs fed the commercial cottonseed meal died the following week. All showed the same post mortem appearances. The post mortem examination showed serous fluid in the pericardial sac and in the thoracic and abdominal cavities. The heart was enlarged and the cardiac muscle congested. The lungs were decidedly edematous and mottled with a number of small subpleural hemorrhages. The liver and many glands, the stomach and small intestines were congested. The blood is very black and does not clot after death.

The pigs fed autoclaved cottonseed meal were then turned in with the college hogs and have shown normal growth.