

## II. THE OCCURENCE AND DESTRUCTION OF GOS- SYPOL IN COTTON SEED PRODUCTS

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A chemical and nutritional study of cotton seed products, chiefly cottonseed meal, has been going on in the Department of Agricultural Chemistry Research of the Oklahoma Experiment Station for a number of years, and we have become especially interested in the toxic substance gossypol, which has been isolated from the cotton seeds. This paper as indicated by the title will deal mainly with the occurrence and destruction of this substance as found in cotton seeds and its related products. Previous results obtained in this laboratory on the toxicity of cottonseed meal, and the physiological effects of gossypol, are reported by Dowell and Menaul (1923), Menaul (1923), and Gallup (1926).

As early as 1861 Kuhlmann found a yellow pigment in the cotton plant with properties which lead us to think it the same as the substance later isolated by Marchlewski (1899) and given the name gossypol. Kuhlmann attempted to recover this substance from the "foots" of cottonseed oil after removal of the fatty acids. He obtained an impure product having a greenish-blue color, characteristic of the oxidation products of pure gossypol.

Longmore (1866) in an attempt to isolate the coloring matter of crude cottonseed oil obtained a brown powder which he described as having a pungent powerful dyeing properties. This substance was also quite impure, and likely a mixture of the decomposition products of gossypol rather than a single compound. The work of Marchlewski (l.c.) who gave the compound its name by a combination of the two words gossypium and phenol, seems to have led the way for the more recent investigations although very little more was published on the subject until the notable work of Withers and Carruth (1915). Carruth's (1918) translation of Marchlewski's method of obtaining gossypol gives some indication of the difficulties involved in obtaining a pure product without oxidation and possibly the occurrence of molecular rearrangements. Carruth states that gossypol "appears to be a constituent of the cotton plant only." He goes on to say, "It occurs in peculiar glands called 'gland dots,' 'secretion glands,' or 'resin glands' which are present in all parts of the plant except the woody tissue. These are 100 to 400 m. in diameter and are readily visible to the eye. They appear to be formed by disintegration of adjacent cells."

All of these glands as well as those found in the seeds, the source of material which we have been using for the isolation of gossypol, give a characteristic red color with sulphuric acid which suggests the presence of gossypol. However, color reactions, especially those with concentrated sulphuric acid, are quite unreliable, and we are sceptical about drawing any conclusions from them. Doubtless gossypol occurs throughout the cotton plant but I know of no work that has been done on the form in which it occurs in the leaf and stem and other parts, as compared to its form in the cotton seeds where it probably accumulates. I believe there is a fine opportunity for a study of the physiological role of gossypol in the cotton plant, its form, and distribution. Before such a study could be made however, our present methods for the determination of gossypol would probably need to be modified and micro or possibly microscopic methods applied.

We are particularly interested in gossypol as found in cotton seeds and cottonseed meal because of the deleterious effect it produces in live stock when these products are fed in excess. In defense of those people who do not consider the toxicity of cottonseed meal an important problem, I will say that cottonseed meal may be fed in small amounts to sheep and cows for a considerable length of time, possibly throughout the lifetime of these animals, without producing any apparent injuries. This appears to be true (especially) if silage is used in the ration, yet there has been no work done to determine to what extent silage can overcome those toxic properties or what effect its separate constituents might have. For young animals, especially swine the meal is very toxic and cannot be fed but in very limited amounts and for short periods of time. The symptoms of overfeeding are easily recognized as bulging of the eyes, stiff gait; the animals so affected standing apart and indisposed to eat or move around. Complete blindness often results if the feeding is continued.

The average amount of gossypol occurring in cotton seeds which have had the lint removed and examined in this same laboratory lies between 0.2 and 0.4%. These seeds were grown in this locality and were of several varieties. Schwartze and Alseberg (1923) found a correlation between the gossypol and the locality in which they grew. Their results show that cotton seeds grown in the southeastern part of the United States contain a larger percentage of gossypol than those grown in the southwest. Furthermore they (Schwartze and Alseberg, 1924) established a direct relationship between the gossypol content of cotton seeds and their toxicity.

These results suggest that the cotton seeds grown in the southeast and likewise the cottonseed meal produced in that locality are more toxic than those products found in this locality. The difference in toxicity however, are probably not great enough to be of practical importance other than a less restricted use of cottonseed meal as a feed in the southwest. The fact that there is this difference leads us to believe that the variable quantity of gossypol in cotton seeds may be dependent upon climatic conditions and if this is true, it would offer a possible clue as to the formation and perhaps translocation of gossypol in the cotton plant. As a preliminary step to such a study, the quantity of gossypol in cotton seeds at different stages of maturity is being investigated at the present time. The method being used for the determination of gossypol is as follows.

The seeds are ginned by hand and the lint removed by means of concentrated sulphuric acid. The excess acid is washed off and the seeds again washed in weak ammonia to neutralize any remaining acid and finally washed with clear water and dried at 60° C. The air dried seeds are ground to pass a 60 mesh sieve and 50 grams are weighed out in a Soxhlet thimble. The sample is extracted with ether for 24 hours after which time there is only a very faint yellow color imparted to the ether in the thimble and the extract thus obtained is evaporated below 100° C. The residue consisting of oil, gossypol and much coloring matter is taken up with petroleic ether and filtered after standing over night. Only a small amount of material usually settles out and the gossypol which is quite insoluble in petroleic ether, remains in solution in the oil-petroleic ether mixture. One cc. of aniline is added and the contents set aside for precipitation. After several days (usually about 14) the precipitate which is yellow-orange is filtered off and weighed using a tared Gooch crucible with an asbestos mat. Petroleic ether is used for washing the precipitate free of aniline. The formula for this precipitate has been calculated by Carruth as being  $C_{26}H_{42}O_9 \cdot C_6H_5NH_2$ .

The occurrence of gossypol in cottonseed meal as differentiated from its normal occurrence in the seeds has offered some interesting but very difficult problems in connection with its determination and destruction. If it were not for the fact that the meal is used as a food, the problem of gossypol and its toxicity would be less important one, but it was due to this fact that the substance was investigated.

It might be supposed that the removal of the majority of the oil from the cottonseeds at the oil mill would also affect the removal of the gossypol which is oil-soluble. Furthermore,

since the initial content of gossypol in the seeds is very small it might also be supposed that the resultant meal after removal of the oil would contain only exceedingly small traces of gossypol. Such suppositions are correct to a certain degree, yet they do not account for the proven toxicity of cottonseed meal. To account for this toxicity (which is relatively less than that of the seeds) the following hypothesis has been suggested.

At the oil mill in the preparation of "hot-pressed" meal which is in reality a by-product, obtained by the "hot-press" method of removing the oil from the seeds, the seeds are heated for several minutes in steam-jacketed drums. This is done to increase the quantity of oil which may be expressed in the large pressure presses. During this heating the gossypol undergoes some change whereby only a portion of it remains in its original form (ether and oil soluble) and the majority of it is either decomposed or converted into a compound which is not soluble in the usual fat solvents. This changed portion which is retained in the meal has been given the name D-Gossypol (Carruth, 1918) to differentiate it from the original gossypol which may be determined by the method stated above.

The toxicity of this changed form has not been determined and there are diversities of opinions. In the preparation of "cold-pressed" meal, the seeds are not subjected to such a rigorous heat treatment and for that reason it might be supposed that this meal would be less toxic due to the more complete removal of the gossypol. In the samples examined by biological methods we have found this to be true although the determinations of gossypol in some "hot-pressed" and some "cold-pressed" meals by Sherwood (1926) does not support such a supposition. We have also fed equal quantities of the ether extract of "cold" and "hot-pressed" meal and found the extract from the "hot-pressed" less toxic than that from the "cold-pressed" meal. This supports the above hypothesis.

Many attempts have been made to render cotton seeds and cottonseed meal non-toxic and antidotes such as iron and copper salts have also been suggested. None of the earlier attempts proved to be satisfactory since the methods employed either impair the feeding value of the meal or else were of experimental interest only. Dowell and Menaul (l.c.) working in this laboratory were probably the first investigators to obtain success along this line of work in a practical way. They were able to feed swine autoclaved cottonseed meal in relatively large amounts without producing any toxic symptoms, whereas the untreated meal produced slow growth in the animals and in some cases

death resulted. Their method of destroying the gossypol was to autoclave the damp meal for about 20 minutes at 15 pounds pressure. This method proved so satisfactory that the work was continued in the following year with much the same results (Gallup); 1926. We found that by simply steaming the meal in well-insulated containers we were able to reduce its toxicity to about the same degree as was accomplished by autoclaving.

From these feeding experiments it appears that the destruction of gossypol was brought about by heat and moisture, and we reserved for later study the effectiveness of these two factors when allowed to act separately. Our methods of determining these effects have been to subject the seeds to dry heat (110°C) for varying lengths of time and by chemical means determine the decrease in gossypol. At the same time we have subjected the seeds to the action of steam (as a source of heat and moisture) for the same period of time, and finally allowed the seeds to stand in contact with water a little above room temperature until they had sprouted. The decrease in gossypol was determined chemically on all of these products. As a means of checking this work we have fed the products thus obtained to experimental animals and noted the effects in growth and reproduction. The results of this work, altho not complete are bringing out some interesting facts. We do not believe that heat alone can bring about the decomposition of gossypol altho it does have some action, and if prolonged may cause such changes that the gossypol can no longer be determined by the usual chemical methods.

The action of both heat and moisture in this respect is most favorable altho certain precautions are necessary to produce a meal free of toxic principle and yet retaining its high feeding value. The meal should be thoroughly dampened and autoclaved in thin layers not over four or five inches in thickness. Cooking is incomplete if the meal is too dry and prolonged autoclaving does not remedy the condition but rather tends to burn the meal at the exposed surfaces. Furthermore, the prolonged action of steam probably brings about the coagulation of protein with a decrease in digestibility. The most satisfactory product has been obtained by autoclaving the wet seeds at 20 pounds pressure for one hour. In this manner we have been able to destroy the toxicity in whole cotton seeds which had been previously soaked in water for several hours to soften the hulls. From this it appears that the process is not one of oxidation but possibly hydrolysis or molecular rearrangement.

In our nutritional studies we have been able to successfully rear rats thru three generations when they were receiving in their diet as high as forty-five per cent autoclaved cottonseed

meal. In no case have we been able to successfully rear rats thru more than one generation when using the untreated meal unless the amount in the diet was relatively small. Some of the other investigators have not had this trouble, probably due to the use of a non-toxic meal, and few have attempted to carry rats thru several generations on cottonseed meal diets. With but few exceptions we have always obtained better growth, better reproduction, and lower mortality among rats fed the treated meal than among those fed the untreated. The amount of cottonseed meal used in these rations varied over a wide range.

### Summary.

Gossypol probably occurs throughout the cotton plant and accumulates in the seeds. It occurs in greatest quantities in the southeast where cottonseed meal poisoning is quite prevalent. A study of gossypol contents of seeds at different stages of maturity is being made. During the expression of the oil from cotton seeds only a portion of the gossypol is removed, considerable of it being destroyed and some of probably undergoing a chemical change. In this latter form it appears in relatively large quantities in the meal and a study of its effects upon the feeding value of the meal is being made.

Gossypol is destroyed by heat and moisture under favorable conditions such as autoclaving and cottonseed meal so treated is not only non-toxic, but proves to be a feed of exceptionally high quality.

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