

STUDIES IN COTTON SEED MEAL INTOXICATION. I.¹
PYROPHOSPHORIC ACID.

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The injurious effect of the continuous feeding of cotton seed meal to calves and swine has been known for years. The cause has been ascribed by various investigators² to lint, oil, high protein content, a toxalbumin, choline, betaine, resin and decomposition products. Crawford³ in a preliminary paper published in March 1910 states that "The chief poisonous principle in certain cotton seed meals is a salt of pyrophosphoric acid." Crawford's conclusion is based upon the study of an extract obtained by digesting the meal at body temperature one day with pepsin and one day with pancreatin. He fed with a catheter extracts of cotton seed meal, these extracts corresponding to amounts of meal very much in excess of those which would be fed ordinarily. He made no study of the undissolved residues.

We began the study of the subject in 1908 and, since that time, have used many solvents in our efforts to extract the toxic substance. The undissolved residue having proved toxic in every case, we decided to investigate the residue undissolved by the pepsin-pancreatin treatment. We also tried other feeds which bear upon the question.

The animals selected were rabbits. Our normal daily feed for each animal was 15 grams of cotton seed meal or an amount of some fraction equivalent to 15 grams of meal. As the rabbits used averaged about 1.5 kilos the daily feed corresponds to 10

¹ This paper was read before the Biological Chemical Section of the American Chemical Society at the Washington meeting, December, 1911 (except the results of the last experiment on sodium pyrophosphate).

² *Exp. Sta. Record*, xxii, p. 502, 1910.

³ *Journ. of Pharmacol.*, i, p. 547, 1910.

grams per kilogram of initial live weight. The unconsumed feed was estimated and correction made. Each animal was allowed pea vines, cabbage leaves or other green feed each morning. We mixed molasses with the cotton seed meal and, when some fraction of the meal was being fed, the portion of meal removed was replaced by an equivalent weight of wheat bran. Six controls were run during the entire time of the experiment and all the control animals lived. The feeds, daily and total, are all calculated to the equivalent in grams of cotton seed meal per kilo of initial weight of animal.

Pepsin and pancreatin extract.

Raw cotton seed meal was digested one day each with pepsin and pancreatin. The mass was filtered, and the filtrate concentrated to small volume. The solution was fed through a catheter in amounts corresponding to 200 grams of meal.

Nine rabbits were taken, varying in weight from 1461 to 2240 grams, average 1752. All lost in weight an average of 123 grams. Two were made very sick but survived, two showed no ill effect except loss in weight and five died. The amount fed corresponded to ten times our normal feed of cotton seed meal. The P_2O_5 in the feed was 2.22 grams.

Sodium pyrophosphate.

Sodium pyrophosphate was prepared in the laboratory by igniting disodium phosphate. The product responded to the tests for pyrophosphate and for freedom from orthophosphates. 4.157 grams corresponded to 2.22 grams of P_2O_5 , the amount contained in the pepsin-pancreatin extract of 200 grams of meal. The pyrophosphate for each animal was dissolved in 65 cc. of water and fed through a catheter.

One rabbit weighing 2070 grams died during the night following feeding. Another rabbit weighing 1456 grams, similarly fed, died thirty-one minutes afterwards.

The results of the preceding experiments indicate that the pepsin-pancreatin extract of 200 grams of meal is generally toxic when given at one feeding and that the $Na_4P_2O_7$ corresponding to the amount of P_2O_5 in that amount of meal is toxic if administered at a single feeding.

Cotton seed meal.

Twelve rabbits were taken whose initial weights ranged from 970 to 2560 grams, the average being 1559 grams. All the animals died within from 8 to 21 days, average, 13 days. The average loss in weight was 379 grams. The total amount of meal consumed by each animal ranged from 105 to 225 grams, average, 157 grams. The total amount eaten was practically 100 grams per kilo of initial weight of the animal, making an average daily consumption of meal equivalent to 7.7 grams per kilo of animal. These figures indicate the degree of toxicity of the meal towards the animals under the conditions of the experiment. There were 2.76 grams of P_2O_5 in the average feed or 0.21 gram in the daily feed.

Sodium pyrophosphate corresponding to whole meal.

Our cotton seed meal contained 1.76 per cent of P_2O_5 . If it were all in the form of pyrophosphate, 0.4157 gram of sodium pyrophosphate containing 0.222 gram of P_2O_5 would contain an amount equivalent to 12.5 grams of meal.

Four rabbits varying from 790 to 1550 grams, averaging 1117 grams, were fed daily 0.4157 gram of $Na_4P_2O_7$. Each animal gained in weight, the average gain being 243 grams. At the end of fifty-two days the feed was discontinued, all the animals being in good condition. For each kilo of animal the equivalent of the feed in cotton seed meal was a total of 582 grams; daily, 11.2 grams.

This feed furnished each animal daily with more pyrophosphoric acid than the amount received by each animal eating the raw meal, yet this feed was non-toxic and the meal toxic. This indicates that pyrophosphoric acid is not the cause of toxicity in cotton seed meal.

Our next step was to ascertain which was the more toxic portion of the meal, the aqueous extract, the pepsin-pancreatin extract of the residue undissolved by water or the residue undissolved after both of these treatments.

Aqueous extract of cotton seed meal.

1260 grams of cotton seed meal were stirred at room temperature with 1050 cc. of water and 10 cc. of chloroform for twenty-

four hours. The solution was filtered, the liquid evaporated over a water bath to a syrup, poured over 240 grams of bran, mixed thoroughly, dried and fed to each animal in amounts corresponding to 30 grams of cotton seed meal daily—twice our normal feed. Six animals were taken. One animal died after 16 days, having lost 369 grams. The death being from causes other than the feed the observations are not included. The other five animals ranged in weight from 1137 to 1947 grams, average, 1400. The feed was discontinued after thirty-eight days, the animals having gained an average of 203 grams. There were 2.26 grams of P_2O_5 in the total average feed or 0.06 gram in the daily feed.

Pepsin and pancreatin extract.

The residue left upon the filter in preparing the preceding feed was washed thoroughly with hot water and then digested at 40° for one day each with pepsin and pancreatin. The mass was filtered, the filtrate evaporated over a water bath to a syrup, poured over bran and dried. Each animal was fed daily the amount corresponding to 30 grams of cotton seed meal—double our normal.

Six rabbits were taken ranging in weight from 1516 to 1864 grams, average, 1683. At the end of thirty-eight days the feed was discontinued, the animals having gained an average of 73 grams each.

There were 5.22 grams of P_2O_5 in the total feed or 0.14 gram in the daily feed.

Residue undissolved by treatment with water, pepsin and pancreatin.

The mass left after filtering off the solution from which the preceding pepsin and pancreatin extract feed was prepared was washed with hot water and dried. It was then finely ground in a mill. 8.8 grams, corresponding to 15 grams of meal, were mixed with bran and molasses and fed to each of six rabbits. The rabbits ranged in weight from 1374 to 1773 grams, average, 1592 grams. Five died in 14 to 16 days and the remaining one died at the end of 27 days—the average of all being 18 days. The average loss in weight was 325 grams. The total feed consumed corresponded to cotton seed meal equivalent to 116 grams per kilo initial average weight of animal, the average for a day being 6.4

grams per kilo. The total P_2O_5 consumed by the average was 1.96 grams, the average for a day being 0.11 gram of P_2O_5 .

It is thus seen that the residue undissolved by the treatment of the meal with water, pepsin and pancreatin solutions is toxic, but both of the extracts, although they corresponded to almost three or four times as much meal, were non-toxic.

The total P_2O_5 in the toxic portion (1.96 grams) was less than in either of the non-toxic fractions.

Residue undissolved by pepsin and pancreatin.

Cotton seed meal was digested as in our first experiment with pepsin and pancreatin. The mass was filtered and the residue was washed with water, dried and ground. 10.7 grams corresponded to 15 grams of meal. Three rabbits were taken, weighing 1860 and 2289 grams, average, 2124 grams. All lost in weight, the average being 552 grams, and all died in an average of 21 days—the range being from 15 to 27 days. The total feed per kilo of animal corresponded to an equivalent of 128 grams of cotton seed meal, the average daily feed being 6.1 grams per kilo. There were in the total average feed 1.40 grams of P_2O_5 , making a daily average of 0.07 gram.

This feed resembled the preceding feed closely, both in composition and results, and confirms our conclusions that the most toxic part of the meal is in the residue undissolved by pepsin and pancreatin.

We next prepared a feed which had an inappreciable amount of P_2O_5 and yet which was toxic. Our solvent was ammonium citrate solution.

Residue after citrate extraction.

450 grams of cotton seed meal were extracted with 1500 cc. of a solution of ammonium citrate following the A. O. A. C. method for determining insoluble phosphoric acid. The mass was filtered, the residue washed and dried. 7.8 grams were the equivalent of 15 grams of meal.

Five rabbits were fed on this mixture. The weights ranged from 1350 to 2380 grams, average, 1602 grams. All lost in weight, the average loss being 438 grams. All died in from 17 to 29 days, the average being 21 days. The total feed equivalent in cotton

seed meal was 168 grams and the daily feed 8.0 grams per kilo of initial live weight.

The total P_2O_5 consumed by each was 0.60 gram, a daily average of 0.03 gram. This feed was almost as toxic as the whole meal or the pepsin-pancreatin residue, although it contained but an insignificant amount of P_2O_5 .

Sodium pyrophosphate.

The residue after ammonium citrate extraction contained a total of 0.60 gram of P_2O_5 in the amount consumed in twenty-one days. If all the P_2O_5 were in the form of pyrophosphate it would be the equivalent of 1.11 grams of $Na_4P_2O_7$. This amount dissolved in 60 cc. of distilled water was fed on January 17, 1912, at one feeding, through a catheter, to each of six rabbits, whose average weight was 1827 grams. The weights ranged from 1732 to 2037 grams. The average gain for each animal was three grams. The animals did not seem to experience any ill effects from the feed.

As the citrate residue, which proved toxic in 21 days, contained a total of 0.60 gram of P_2O_5 in the form of $Na_4P_2O_7$, and as this amount of P_2O_5 given at a single feeding to each of six rabbits proved harmless, the cause of the toxicity of the citrate residue is not pyrophosphoric acid.

SUMMARY.

Experiments show that the pepsin-pancreatin extract of cotton seed meal is toxic generally to rabbits, when fed in amounts corresponding to from fifteen to twenty times the amount of meal normally fed, and that the pyrophosphoric acid corresponding to this extract is toxic if fed similarly.

The pepsin-pancreatin residue is more toxic than the aqueous or pepsin-pancreatin extracts of the meal—in fact it is the only one of the three fractions which is toxic under the conditions of the feeding.

A fraction of cotton seed meal containing a non-toxic amount of pyrophosphoric acid may be toxic.

The results indicate that pyrophosphoric acid is not the cause of toxicity of cotton seed meal.