Some Factors Involved in the Detoxication of Cottonseed[1]

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IT has recently been shown that autoclaving has a deleterious effect on the *proteins* of cottonseed (9) and that commercial cottonseed meals differ in the amount of cooking to which they have been subjected (10). Inasmuch as these factors were not considered in previous interpretations of cottonseed meal nutrition studies, it was believed desirable to investigate the rôle of the factor in uncooked cottonseed responsible for the deleterious effects in stock feeding noted by many previous investigators.[3]

Experimental

Male rats (Sprague-Dawley) were kept in individual screen-bottom cages. Food consumption data were obtained in all experiments and water-consumption data in those concerned with iron administration. Hull-free cottonseed kernels were ground to pass a 20-mesh screen and extracted with ethyl ether (by percolation) to obtain an oil-free non-toxic meal or with petroleum ether to obtain an oil-free toxic meal. The meals were then reground to pass a 60-mesh screen. The diets (Table I) were similar to those previously described (9).

TABLE I

Constitution of Rations Used [a]

	Ration					
	A	B	C	D	E	F
	%	%	%	%	%	%
Ether-extracted cottonseed	24.8	49.6	.....	.....	37.2	22.9
Hexane-extracted cottonseed	.....	.....	24.2	48.8	12.1	.....
Ether-extract of cottonseed	.....	.....	.....	.....	.....	10
Hydrogenated cottonseed oil	19.5	19.0	19.5	19.0	19.0	10
Sucrose	51.7	27.4	52.8	28.2	27.6	53.1
Liver extract	2	2	2	2	2	2
Salts	2	2	2	2	2	2

[a] The amount of cottonseed meal was adjusted to give 12 or 24% protein (N×6.18) in the diets. The diets were also isocaloric with respect to oil content. One drop of haliver oil fortified with vitamin D was added for each 100 g. of ration. Thiamin hydrochloride (50 γ) was administered by mouth thrice weekly.

Osborne and Mendel (12) first reported that rats refused to eat raw cottonseed. In the present experiments an abrupt fall in food consumption occurred when diets containing ground raw cottonseed or petroleum ether-extracted meats were substituted for non-toxic diets. When the toxic meals were used at sufficiently high levels, the animals ate so little that their subsequent deaths could be attributed to starvation. In order to demonstrate toxicity in paired feeding experiments it was necessary to dilute the toxic diet with additional parts of the control diet so that sufficient food would be consumed to obtain usable data (Table II).

Detoxication by Air Oxidation. A diet containing the toxic principle in the form of an ether extract of ground cottonseed (Diet F) was fed to three rats. They lost weight for five days and then began to gain. At the end of 30 days they were growing at a rate comparable to that obtained with the non-toxic diets. In order to determine whether these results were due to air oxidation, a portion of the diet was permitted to stand uncovered at room temperature for three months. At the end of the period a new ration was compounded with the same ingredients, the oil components of which, however, had been kept cold to minimize oxidation. The bulk of this fresh ration was kept at 4° during the feeding trials. The results, presented in Fig. 1, indicate that the toxic factor had disappeared during storage. The possibility of oxidative destruction of gossypol has been discussed by Gallup and Reder (6).

The disappearance of toxicity from a ration containing the toxic factor dissolved in oil (Diet F)

TABLE II

Comparison of Ether-Extracted (Diets A and B) With Petroleum Ether-Extracted (Diets C, D, E) Cottonseed Meals by Paired Feeding Experiments [a]

Pair No.	Diet	Toxic Factor	Duration	Total Food Consumption	Original Weight	Change in Weight
			days	*gm.*	*gm.*	*gm.*
1	D	+	5	5.4	38	−10 died
	B	−	5	5.4	38	−7
2	D	+	5	3.8	39	−11 died
	B	−	5	3.8	39	−9
3	D	+	5	4.3	37	−7
	B	−	5	4.3	37	−13 died
4	C	+	14	34.6	69	−19 died
	A	−	14	34.6	74	−16
5	C	+	13	21.1	106	−43 died
	A	−	13	21.1	101	−34
6	E	+	14	60.6	64	+8
	B	−	14	60.4	70	+12
7	E	+	14	71.7	75	+13
	B	−	14	71.7	78	+21

[a] If permitted to eat diets A and B *ad libitum*, comparable rats consumed 5-10 g. daily (9).

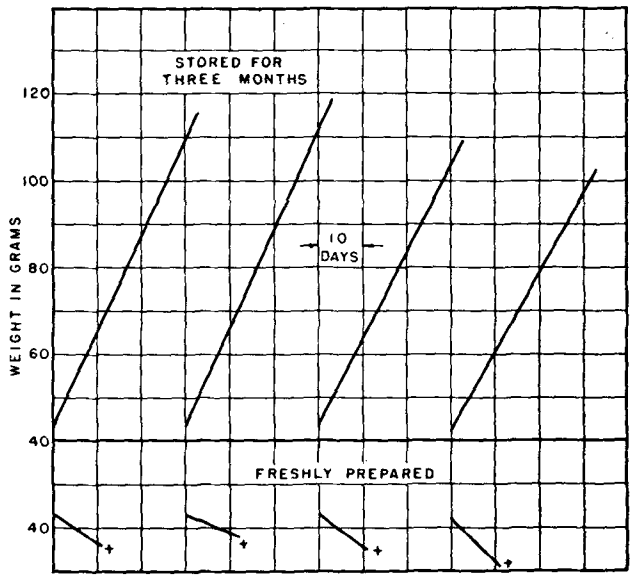

FIG. 1. Rat growth curves obtained with a diet containing an ether extract of cottonseed (Diet F).

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[3] At the time that these experiments were in progress, it was believed that the constituent of cottonseed responsible for its toxicity was gossypol (1, 2, 3, 4, 5, 13, 16). However, since no identification was made, the term "toxic factor" will be used to indicate the substance or substances responsible for the deleterious effects of feeding raw cottonseed.

was not observed to any similar degree in rations in which it was present in ground cottonseed meats or petroleum ether-extracted meal (Diets C and D) even though these components were intimately mixed with lard or cottonseed oil in the preparation of the rations. Diets of such character were found to be still toxic even after a year of standing at room temperature.

Gossypol is an effective antioxidant for fats and oils (cf. 11). If the toxic factor is or acts like gossypol, it would be expected, like other inhibitors, to disappear during the induction period. Apparently, in order to be susceptible to air oxidation in cottonseed rations, the toxic factor must be in solution in the oil which in turn must be in contact with air. In ground cottonseed many of the oil-containing glands undoubtedly escape contact with air while in petroleum ether-extracted meal much of the toxic factor apparently escapes contact with the added oil of the ration.

Detoxication by Soluble Iron Salts. The beneficial effect of feeding iron salts with toxic cottonseed rations was first noted by Withers and Brewster (16) and has since been investigated by Gallup (3) and others (13). Inasmuch as the toxic factor is readily destroyed by oxidation, as shown above, and iron is known to act catalytically as a pro-oxidant, it appeared of interest to study further the nature of the reaction of iron salts with the toxic factor. The following experiments indicate that induced oxidative destruction is of minor importance and that the detoxication is attributable to the formation of an unabsorbable complex, as was suggested by Gallup (3).

1. An otherwise toxic diet, but containing 1% ferric ammonium citrate, was compounded quickly and stored at 4°C. Only one day's ration was supplied at a time, and each day the feed cups were scrupulously cleaned to avoid the accumulation of oxidizing oil films. Rats fed under this regime grew as rapidly as did those given the same diet, with no care taken to avoid oxidation.

2. In confirmation of the results of Gallup (3) detoxication could not be demonstrated with insoluble iron salts. Ferric oxide (1%), added to toxic diets, caused no change in the toxicity.

3. Detoxication was demonstrable within 24 hours when the iron was administered as a solution in the drinking water. There was increased food consumption and resumption of growth (Fig. 2).

4. In order to determine whether the benefit of iron ingestion could be detected irrespective of actual contact with the food in the gastro-intestinal tract, four groups of rats were given the toxic diet and water containing 1% ferric ammonium citrate as follows:

a) Diet and iron solution during the day, 12-hour period (9 a.m. to 9 p.m.); distilled water at night.

b) Diet and distilled water during the day; iron solution at night.

c) Diet and iron solution at night; water by day.

d) Diet and water at night; iron solution by day.

Results are shown in Table III. The benefit of administering the toxic diet and the iron solution during the same 12-hour period is evident and suggests, in conformity with the observations described above and with those of Gallup (3), the formation of a non-toxic complex. Swensen, Fieger, and Upp (15) have shown

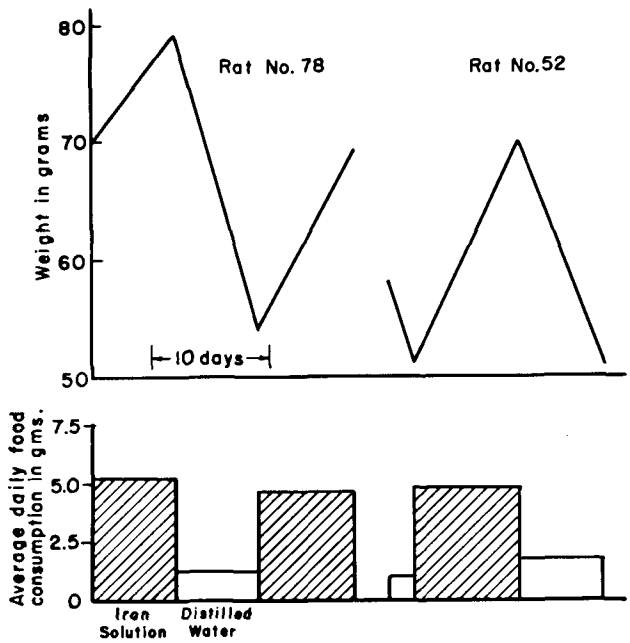

FIG. 2. Effect of the administration of iron in the drinking water (1% ferric ammonium citrate) to rats supplied with a diet containing petroleum ether-extracted cottonseed (Diet D).

that the excretion of gossypol in chicks fed cottonseed meal was increased when ferric chloride was added to the ration.

TABLE III

Effect of Administering Ferric Ammonium Citrate (1% in the Drinking Water) Together or Alternating With a Toxic Diet (Diet D) for 12-Hour Periods

Treatment	Number Rats	Days	Average Original Weight	Average Food Consumption	Average Iron Solution Consumption	Average Change in Weight
			gm.	*gm.*	*ml.*	*gm.*
Diet, P.M.; Iron, P.M.	3	7	81	28	98	+11
Diet, A.M.; Iron, A.M.	3	7	88	31	96	+6
Diet, A.M.; Iron, P.M.	3	7	91	24	97	−11
Diet, P.M.; Iron, A.M.	3	7	96	12	42	−19

In this laboratory iron salts were found useful for differentiating those cottonseed meals, the low nutritive value of which was due to excessive heat treatment of the protein, from those which contained residual amounts of the toxic factor. In the latter case only did the addition of iron salts improve the growth response. In the former, as for example with some samples of overcooked commercial meals, added iron salts did not affect the growth rate in either direction.

Detoxication by Moist Heat. In the commercial cooking of cottonseed meal the rolled meats are subjected to steam heat varying from 104° to 111°C. in stack cookers. The meats are introduced into a top compartment, from which, after being stirred for a short time, they are dropped into a second compartment. There are usually five compartments, and the total cooking time varies from 40 to 90 minutes. In an attempt to duplicate these conditions as closely as possible, ground cottonseed were autoclaved at 109° for periods ranging from 30 to 90 minutes. The cooked meats were then incorporated into diets and fed to weanling rats. The diets were still toxic although the meals had undergone as

much heat treatment, as measured by the extent of protein denaturation (10), as had commercial cottonseed meals. Similar results were obtained with petroleum ether-extracted cottonseed meats.

Two conditions present in commercial cooking practice had not been controlled, i.e., the moisture content and the stirring. Preliminary attempts to control the moisture content during autoclaving were not successful, but increasing the surface of the meal particles, as by ballmilling, gave products which were readily detoxified by autoclaving (8). Typical results are shown in Table IV. Discontinuance of the research program prevented a more detailed study of this phenomenon. Others (7, 14) have since described the importance of the amount of moisture present in cottonseed flakes for their detoxication by steam heat.

TABLE IV

Effect of Ball Milling (24 Hours) on the Susceptibility of the Toxic Factor in Hexane-Extracted Cottonseed to Destruction by Wet Heat (Autoclaving for 50 Minutes at 109°C.)[a]

Treatment	Number Rats	Average Original Weight	Average Change in Weight	Remarks
		gm.	*gm.*	
None	3	47	−14	Av. survival 7 days
Ball-milled	3	47	+99	24 days

[a] The diets contained 48% cottonseed meal (see Diet D). The autoclaved ball-milled meal was considerably darker than the autoclaved 60-mesh-size meal.

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Summary

The toxic factor in cottonseed can be nullified by three apparently unrelated mechanisms: oxidation, combination with soluble iron salts, and destruction by steam autoclaving. Oxidation is of minor importance except where the toxic factor has first been extracted with ethyl ether and is present in the diet in solution in oil. Detoxication with soluble iron salts is demonstrable even when the iron is administered in the drinking water. Ball-milled petroleum ether-extracted cottonseed meal can be detoxified by autoclaving under conditions which are not effective for the original meal.

These results were obtained from feeding tests with rats.

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Germination and Free Fatty Acid in Individual Cotton Seeds[1]

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SEEDS from cotton that has been exposed to wet weather in the field are likely to be lower in viability and to contain higher percentages of free fatty acids than those from seed cotton harvested without unfavorable exposure (3, 4). Similar observations have been made of cottonseed stored under conditions of high moisture or temperature (4). Conventional methods of approach to the relationship of free fatty acid content to germination would require that a sample of several hundred grams of cottonseed for the free fatty acid determination and another sample of several hundred seed for germination tests be drawn from each lot tested. When sufficient data were obtained, statistical methods could be used to study the relationship between the two variables. A second approach to the problem consists of the application of microchemical methods to the analysis for the free fatty acid content (2) of part of the nongerm portion of a single seed and the germination of the remainder of the seed.

In order to establish whether the free fatty acid content of the nongerm end of a hulled cotton seed was correlated with that of the germ end, 50 seeds were carefully peeled and cut approximately in half; each half was weighed and placed in a numbered, small, glass-stoppered Erlenmeyer flask. To each flask 5 ml. of petroleum ether (American Oil Chemists' Society, Specification H 2-41) was added and allowed to stand for about 30 minutes to soften the seeds. The seeds were then ground by means of a glass rod with a flattened end. Any material adhering to the rod was washed into the flask by means of an additional 5 ml. of the petroleum ether. The flasks were then stoppered and allowed to stand for about 16 hours with occasional shaking. After the extraction was completed, 10 ml. of neutralized alcohol containing m-cresol purple indicator was added and the mixture immediately titrated with 0.005 N alcoholic KOH. During the titration the effect of atmospheric carbon dioxide was eliminated by bubbling a stream of carbon dioxide-free air through the titration flask. The free fatty acid content is calculated as per cent oleic acid by multiplying the milliequivalents of alkali used by 28.2 and dividing the product by the weight

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