Studies on the Detoxication of Castor Seed Pomace[1]

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ONE of the serious economic hindrances to the large-scale domestic production of castor seeds is the toxicity of the residual meal after oil extraction, which prevents its use as a protein supplement for livestock. This toxicity is of such a nature that, at the present time, no use is made of the pomace except as a fertilizer, thus materially reducing the value of the crop. Recent developments in the production of synthetic drying oils from castor oil have created an augmented demand for castor seed. In response to this need the domestic production of castor seed is being extensively encouraged at the present time. If these efforts are successful, an additional potential source of protein supplementation will become available.

The recent extensive review of Jones (1) on the protein constituents of the castor seed summarizes the pertinent data on this subject. Most of the investigations concerned with the possible use of the pomace as a feeding-stuff were conducted in the early part of this century, and most of the recent reports are confined to the patent literature. In contrast to this a thorough re-investigation of the nature and properties of the toxic protein, ricin, has been reported by Kabat, *et al.* (2) in 1947. These workers have prepared ricin in a very high state of purity and have studied its chemical and immunological properties.

In addition to this toxic protein, ricin, the castor seed contains a very powerful allergenic material. This has been isolated in highly purified form from both domestic castor seeds and from Brazilian castor-seed pomace by Spies and co-workers (3, 4). They have shown the allergen to be a protein-polysaccharide of relatively low molecular weight. The purified material was extremely active, provoking allergenic response in dilutions of $1:10^6$. It was both chemically and immuniologically distinct from ricin. Castor plants also contain a mildly toxic alkaloid, ricinine, some of which is present in the seed (5).

According to Jones (1), there are only a few recorded instances in which castor pomace has been fed to livestock, and most reports suggest that the high toxicity of the residual meal precludes such use. Numerous methods have been employed in an attempt to detoxify the meal by extraction of ricin or to effect its destruction. Thus Petrosyan and and Ponomarev (6) reported that the pomace was detoxified by boiling for one to two hours. Tangl (7) used heating at 140° C. for 60 to 90 minutes. Jaki (8) treated castor pomace with steam and then removed the excess moisture under vacuum. Massart and Massart (9) extracted the cake with alkali metal halides and hydroxides and subsequently autoclaved the filtered residue. By these techniques they reported successful detoxication. Rudolph (10, 11) has patented a process for detoxifying the meal by heating and subsequent extraction with various solvents.

In view of the known protein nature of ricin it appeared that a thorough re-investigation of conditions known to denature and coagulate proteins might reveal a more commercially feasible procedure for the destruction of ricin than any of those reported. A project directed toward this end was therefore instituted at this station; this communication reports the results of the first of these investigaions.

Experimental

Oral Toxicity Studies. The first objective of this study was to determine the oral toxicity for experimental animals of castor pomace produced by various commercial and laboratory processes. With a high order of toxicity established, the effects of various treatments on the oral toxicity of the pomace were then determined. Treatments were employed which might be expected to alter the physical and chemical state of the toxic protein and thus affect the toxicity of the pomace.

Rats and chicks were the principal experimental animals employed in these studies. Rats were fed an adequate stock ration, and the chicks received standard chick starter ration. Additions of castor pomace for toxicity tests were made at the expense of the entire ration. The results from a series of tests are summarized in Table I. It will be seen that without exception the solvent extracted meals exhibited a high order of toxicity. The symptoms observed following oral administration of castor pomace were not essentially different from those recorded by previous workers upon the injection of ricin preparations. In general, however, the initial symptoms of toxicity were delayed in their appearance by oral administration as compared with intraperitoneal injection of an equivalent amount of crude ricin.

TABLE I
Oral Toxicity of Various Laboratory and Commercial Preparations of Castor Bean Pomace for the Rat and Chick

Description of material	Per cent administered	Animal	Number of animals	Per cent mortality in 10-day period
Solvent process commercial meal (Sherwin-Williams Company).....	5	Adult rat	29	96
Solvent process commercial meal (Sherwin-Williams Company).....	5	Weanling rat	10	100
Ether extracted laboratory meal.......	5	Adult rat	10	100
Commercial meal (Baker Castor Oil Company)...................	2 50	Weanling rat	25	4
Solvent process commercial meal (Sherwin-Williams Company).....	5	Chick	20	95

Detoxification Studies. Having demonstrated a high degree of oral toxicity in both commercial and laboratory solvent extracted castor pomace, studies were instituted to determine the effect of various treatments. A protocol of some of the results obtained with various treatments is presented in Table II. A description of the treatments reported is as follows: toasting, heating with dry heat at 140°C.

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TABLE II

Effects of Various Treatments on the Oral Toxicity of Solvent Extracted Castor Pomace

Trial No.	Treatment	Level in diet, per cent	Animal	Number of animals	Response
1	Steaming for 15 min.	5	Rat	4	All survived
2	"Dry" autoclaving, 5 min.	5	Rat	3	2 died
3	"Dry" autoclaving, 10 min.	5	Rat	3	1 died
4	"Dry" autoclaving, 15 min.	5	Rat	12	All survived
5	"Dry" autoclaving, 20 min.	5	Rat	3	All survived
6	"Dry" autoclaving, 30 min.	5	Rat	9	All survived
7	"Wet" autoclaving, 15 min.	5	Rat	10	All survived
8	"Wet" autoclaving, 60 min.	5	Rat	10	All survived
9	Toasting for 30 min.	10	Rat	4	4 died
10	Cooking at 80°C. for 30 min.	10	Rat	4	3 died
11	Cooking at 80°C. for 60 min.	10	Rat	4	All survived
12	Incubating with 0.2 N HCl	10	Rat	4	All survived
13	Incubating with 0.2 N NaOH	10	Rat	4	All survived
14	Incubating with 3% H_2O_2	10	Rat	4	All survived
15	"Dry" autoclaving for 10 min.	10	Chick	10	All survived
16	"Dry" autoclaving for 20 min.	10	Chick	10	All survived
17	"Dry" autoclaving for 60 min.	10	Chick	10	All survived

for one hour; steaming, heating in an autoclave with valves open at 105°C.; "dry" autoclaving, heating the pomace spread in layers one inch in depth at 125°C. in a steam autoclave; "wet" autoclaving, heating the pomace made into a slurry with an excess of water at 125°C.; heating the pomace made into a slurry with an excess of water at various temperatures; incubating the pomace in water suspensions at room temperature in the presence of 0.2 NaOH or HCl or 3% H_2O_2 for 24 hours. Following these treatments the castor pomace was dried, ground, if necessary, and fed to rats and chicks in oral toxicity trials.

Examination of the data shows that autoclaving highly toxic castor pomace for periods of 15 minutes or more resulted in essentially complete destruction of ricin. Steaming for 30 minutes was also effective. Incubation of the pomace in the presence of mild alkali or acid followed by neutralization or mild oxidation with hydrogen peroxide were equally effective. Dry heating or cooking at temperatures up to 80°C. were ineffective.

Since it has been repeatedly demonstrated that prolonged autoclaving of soybean oil meal and other similar products reduces their value as sources of protein, it was deemed desirable to ascertain the minimum time that would effect essentially complete destruction of ricin as measured by the response of healthy rats. Repeated trials of different lengths of time showed that, under the conditions employed, autoclaving the dry pomace at 125°C. for 15 minutes gave adequate detoxication. No deaths attributable to ricin toxicity were observed in several hundred animals fed varying levels of castor pomace treated in this fashion. The autoclaved pomace was friable and could be readily mixed with other ingredients of the ration. When water was mixed with pomace the resulting product after autoclaving was difficult to process for ready mixing.

In none of these series of investigations using autoclaved castor pomace was there any gross evidence of toxicity attributable to the alkaloid ricinine. Since any appreciable effect on the alkaoidal content due to autoclaving seems unlikely, it may be that rats and chicks have a relatively high tolerance. None of the experimental animals showed symptoms which might have been caused by the protein-polysaccharide allergen. Several laboratory workers, however, did become sensitized, necessitating special precautions in the handling of the pomace. Our experience would suggest that the autoclaved pomace under normal laboratory conditions produced less response in the sensitized individuals. This may well be due to the change in physical state, the autoclaved material being relatively free of dust.

Biological Value of Castor Pomace. A procedure for essentially complete destruction of ricin toxicity of castor pomace having been developed, it became possible to institute studies on the biological value of castor pomace. Therefore biological tests were made using both the rat and the chick. Since the biological value of a protein supplement is dependent upon its amino acid composition, the pomace was also analyzed for the principal essential amino acids.

Initial studies measured the ability of graded amounts of detoxified, partially decorticated castor-seed pomace to support growth of weanling rats when this substance replaced casein on the basis of nitrogen equivalence. The proximate analysis of the castor pomace used was as follows: protein, 57.5; fat, 2.9; nitrogen free extract, 13.1; crude fiber, 9.7; ash, 10.6; moisture, 6.2. Weanling rats of the Sprague-Dawley strain were employed. The animals were fed *ad libitum* and were weighed at intervals of one week. The composition of the rations used and the results obtained are presented in Table III.

It was observed that the feed consumption of rats fed rations containing castor pomace was appreciably less than that of those consuming the casein basal. A part of the inferior growth observed with increased amounts of castor pomace in the ration might be attributable to a decreased food intake. Paired feeding experiments were therefore conducted, five to six-week-old male littermates being used. The rations were the same as Rations I and IV in the previous trial. The average feed consumption per rat for 16 days in four sets of experiments was as follows: Casein-containing ration, 142 gm.; castor-pomace containing ration, 143 gm. The respective average gains in weight were 48.5 gm. and 26.5 gm. for the 16-day period.

Studies were also made with chicks to determine the nutritive value of detoxified castor pomace for this species. The partially decorticated product, detoxified by autoclaving at 125°C. for 15 minutes, was also employed in these tests. Day-old chicks of mixed New Hampshire and Brown Leghorn strains were used; they were kept in thermostatically controlled electrically heated battery brooders and fed *ad libitum*. The composition of the rations fed and total growth for a three-week period are also shown in Table III.

Amino Acid Composition of Castor Pomace. The foregoing results indicated that the protein quality of

the castor seed was probably inferior. In order to permit a more accurate evaluation of the pomace as a protein supplement, the material was analyzed for the principal essential amino acids. The microbiological methods of Henderson and Snell (12) modified slightly to conform to our conditions were employed. The castor pomace was hydrolyzed for the determination of amino acid content by refluxing with 6 N HCl for 24 hours; alkaline hydrolysis was employed for determination of tryptophane using the method of Kuiken, Lyman, and Hale (13). Several hydrolysates were prepared and several assay levels used on each hydrolysate.

The following amino acid content was found: arginine, 11.0% calculated to 16% nitrogen on an ash-and moisture-free basis; aspartic acid, 4.6; glutamic acid, 18.0; histidine, 2.5; isoleucine, 5.3; leucine, 7.2; lysine, 3.1; methionine, 1.5; phenylalanine, 4.2; proline, 3.9; threonine, 3.6; tyrosine, 3.2; valine, 6.6; tryptophane, 0.8. A total of 75.3% of the total nitrogen was accounted for by these amino acids.

Discussion

It will be seen from the data presented in Tables I and II that the oral administration of solvent-extracted castor seed meals results in severe toxicity with high mortality in rats and chicks. The only sample of a commercial screw-pressed-type meal which was tested did not have a high order of acute toxicity, which suggests that the higher temperatures involved in processing may have denatured the toxic protein. The detoxicating effect of heat, particularly moist heat, is clearly demonstrated by these studies. Autoclaving without the addition of water for periods of 15 minutes or more resulted in essentially complete detoxication as evidenced by the administration of this material to several hundred animals under varying conditions without acute toxic symptoms. The resulting meal was readily mixed with other feed ingredients and proved palatable for both rats and chicks when included in the ration at levels as high as 35%. Various chemical treatments which might be expected to denature the protein were also effective in destroying the oral toxicity of the meals. From a practical point of view however the autoclaving process or some modification appears to be a more suitable treatment for further investigation.

Preliminary studies to determine the biological value of the protein show that, in common with the proteins of many plant seeds, those of the castor seed are not of high quality. Thus for both the rat and the chick detoxified castor pomace did not provide for normal growth when it was the principal protein-carrier of the ration. Paired feeding experiments with the rat indicated that the impaired growth was due only in part to a failure of the rats to consume the rations containing castor pomace as readily as rations containing other sources of protein. For the chick the inclusion of castor pomace improved the physical state of the ration, and this is believed to account for the superior growth observed when one-fourth of the casein was replaced by castor pomace on the basis of nitrogen equivalence. In this species as well however the complete replacement of casein by castor pomace produced inferior growth in *ad libitum* feeding experiments. From these data it is inferred that the proteins of the castor seed are inferior to casein in growth promoting quality for both the rat and the chick. It is recognized however that the data presented here is not conclusive in that the inferior growth could be due to either incomplete destruction of ricin or to the presence of other toxins, either ricinine or the allergenic protein-polysaccharide. Further studies aimed at elucidating this are being conducted. It is also proposed to study the response of other mono- and polygastric animals to autoclaved castor pomace.

The distribution of the essential amino acids in castor-seed pomace however does suggest that a principal reason for the low biological value as revealed by feeding tests is poor protein quality. Examination of the amino acid composition shows that the proteins of the whole castor seed are comparatively deficient in tryptophane, this amino acid being found only in amounts of 0.8%. Methionine was not present in adequate amounts for rats and chicks, and the lysine content was minimal. More extensive studies of the amino acids of the various protein fractions are in progress.

Summary

Solvent extracted castor seed pomace has been shown to have a high acute oral toxicity for the rat and chick. This acute toxicity has been destroyed by various physical and chemical treatments designed to denature the toxic protein constituent, ricin. Of these, autoclaving for 15 minutes at 125°C. produced essentially complete destruction of ricin with minimal changes in the physical character of the pomace. Biological tests of the feeding value of the detoxified

TABLE III

Composition of Rations Used and Results Obtained in *Ad Libitum* Feeding Studies With Rats and Chicks Using Detoxified, Partially Decorticated Castor Seed Pomace

Ration component	Lot I		Lot II		Lot III		Lot IV	
	Rats[1]	Chicks[2]	Rats[1]	Chicks[2]	Rats[1]	Chicks[2]	Rats[1]	Chicks[2]
Castor pomace	0.00	0.00	11.60	8.70	23.20	17.40	34.80	34.80
Casein, crude	20.00	20.0	13.33	15.00	6.67	10.00	0.00	0.00
Cornstarch	71.00	63.50	66.07	59.80	61.13	56.10	56.20	48.70
Cottonseed oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salts[3]	4.00	5.00	4.00	5.00	4.00	5.00	4.00	5.00
Gelatin		5.00		5.00		5.00		5.00
Fish solubles		1.50		1.50		1.50		1.50
Percentage of protein supplied by castor pomace	0.0	0.0	6.67	5.00	13.33	10.0	20.0	20.0
Total gain in weight of rats for five weeks and chicks for three weeks	167 gm.	79 gm.	169 gm.	103 gm.	137 gm.	75 gm.	52 gm.	45 gm.

[1] These rations were supplemented with the following vitamins: thiamine, 3 mg. per kilogram of feed; riboflavin, 6 mg.; nicotinic acid, 20 mg.; pyridoxine, 3 mg.; calcium pantothenate, 30 mg.; para-aminobenzoic acid, 50 mg.; folic acid, 1.25 mg.; inositol, 1 gm.; choline chloride, 2 gm. Vitamins A and D as cod-liver oil and alpha tocopherol were administered by dropper once each week.

[2] These rations were supplemented with the following vitamins: Vitamin A and D feeding oil, 1.15 gm. per pound of feed; thiamine, 0.9 mg.; inositol, 100 mg.; para-aminobenzoic acid, 5.0; calcium pantothenate, 5.0 mg.; pyridoxine, 1.6 mg.; riboflavin, 14 mg.; nicotinic acid, 8.0 mg.; folic acid, 0.45 mg.; choline, 700 mg.

[3] Hegsted, *et al.*, J. Biol. Chem., *138*, 459 (1941).

pomace as a protein source indicated that the material was not of high biological value. Microbiological analysis of the total seed protein for the principal amino acids showed that the protein contained relatively large amounts of glutamic acid and was seriously deficient in tryptophan. The methionine content was also inadequate and the lysine was marginal.

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Some Additional Notes on the Kinetics and Theory of Fatty Oil Hydrogenation*

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SOME time ago Bailey and Fisher (2) examined a series of hydrogenated linseed, soybean, and cottonseed oils, and from the composition of samples taken at different iodine values were able, by methods of approximation, to calculate roughly the reaction rates of the different unsaturated fatty acids in the oils, under conditions leading to "selective" and to "non-selective" hydrogenation. Somewhat surprisingly, it appeared that the reactivity of the different acids was by no means determined by the total degree of unsaturation but depended in any case upon the position as well as the number of double bonds. Furthermore by operating under conditions conducive to increased "selectivity" it was not possible materially to increase the differences in reactivities amongst all the unsaturated acids but merely between two groups of acids, comprising linolenic and linoleic on one hand, and isolinoleic (9:10, 15:16 octadecadienoic) and oleic on the other. Although the saturation of linoleic or isolinoleic acid proceeded in a step-wise manner, *e.g.,* linoleic → oleic → stearic, it was noted that, of the linolenic acid reacting, a portion apparently was hydrogenated directly to oleic acid without intermediate desorption of linoleic (or isolinoleic) acid from the catalyst. However no attempt was made to evaluate this effect quantitatively.

Later it was pointed out by Hilditch (4, 5) that these results indicated a fundamental difference in the properties and reactivities of unsaturated aliphatic compounds containing isolated double bonds and those with double bonds separated by a single CH_2 group and that the behavior of the different fatty acids upon hydrogenation was closely paralleled by their behavior upon reaction with atmospheric oxygen.

In view of the considerable theoretical and practical interest attached to the hydrogenation reaction it was considered worthwhile to go back to the original data of Bailey and Fisher and attempt a more careful analysis of the reaction kinetics through a somewhat different mathematical approach. The present communication will set forth the results of this analysis and will also discuss the theory of hydrogenation and catalyst action in view of the present as well as previous data.

Equations for the Reactions

The different reactions which occur simultaneously and consecutively in the hydrogenation of an oil containing linolenic, linoleic, isolinoleic, and oleic acids are shown diagrammatically in Figure 1.

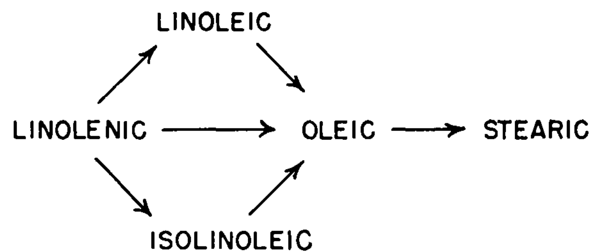

FIG. 1. Schematic representation of reactions occurring in the hydrogenation of a fatty oil containing oleic, linoleic, and linolenic acids.

In setting up expressions relating the composition of the oil to the reaction rates of the different fatty acids it is assumed here, as assumed previously by Bailey and Fisher, that, of an increment of hydrogen taken up by the oil in an infinitesimal period, the fraction going to each acid is proportional to the concentration of the acid times a figure determined by the reactivity of the acid. It is to be noted that this is not quite equivalent to assuming that the reaction rate at any instant is equal to the concentration times

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