FURTHER STUDIES ON THE DETOXIFICATION AND UTILIZATION OF CASTOR POMACE

By

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PREFACE

Domestic consumption of castor oil during recent years has increased sharply and its usefulness in the preparation of a drying oil of exceptional qualities promises even greater consumption in the future. The residue remaining from the extraction of the oil has a high protein content and would be suitable as a livestock feed were it not for the presence of a highly toxic substance, ricin. A vigorous attempt is being made to encourage the domestic production of castor seed; approximately 25,000 to 27,000 acres will be produced in Oklahoma during the current season. If methods for the successful detoxification of the pomace could be developed, a new source of income would be created for the castor-seed producer and a new source of high-protein supplement for the livestock raiser.

In previous work at this Station it has been shown that it is possible to detoxify castor pomace with respect to the acute oral toxicity of ricin by autoclaving. In preliminary feeding tests with rats and chicks no symptoms of chronic toxicity were observed. Workers at the Nebraska Station, however, subsequently presented evidence indicating that for chickens there was a second toxic factor which could be removed by exhaustive extraction with ethyl alcohol. In view of these later findings, a further investigation appeared warranted. The results of these studies are presented in this report.

CONTENTS

LITERATURE REVIEW

The castor bean plant has been known since ancient times and is said to have originated in India. The plant is grown in nearly all temperate and tropical climates. It is raised almost exclusively for its oil, which is used for both medicinal and industrial uses. The other portions of the plant have been of little economic value. Castor oil is used in the preparation of a synthetic drying oil and, because of its physical properties, has superior properties as a lubricant.

The castor bean consists of about 25% husk and 75% kernel. The kernel contains about 60-65% oil while the husk contains 45-55% oil. (1) The oil is extracted from the seed by means of hydraulic presses. The seed is first cleaned, then pressed while it is cold. This extraction produces the highest grade of oil which is used for medicinal purposes. The residue is then heated and pressed while it is warm. The remaining residue is then extracted with solvents. The oil resulting from the warm pressing and the solvent extraction is utilized in industry and as a lubrication agent. The cake that is left after the solvent extraction, usually referred to as pomace, is dried and sold as fertilizer. Because of the presence in the castor cake of a highly toxic element, the cake has had no other major economic use.

The high protein content of the castor seed and the presence of a highly toxic element in the seed which has been associated with the protein of the seed has stimulated an unusual interest in the proteins of castor seed. Some work has been done on the problem of detoxifying the

the castor seed meal, thereby enabling the cake to be utilized as a protein feed supplement for livestock.

Among the early workers on castor bean proteins were Rittenhausen (2), who in 1882 isolated the first crystalline globulin from the seed. Vines (3) working independently, also studied other protein fractions. Because of the state of knowledge of proteins existent at that time, many of the conclusions of the two men are contradictory. Stillmark (4) in 1889, working under the guidance of Kobert, isolated from a 10% saline solution of castor bean extract a highly toxic substance which he called ricin. He believed the material to be a globulin.

Many investigators did not believe that the material was a protein. Jacoby (5), in attempting to prove the material not to be a protein, failed in an experiment to digest the material with trypsin. Later Muller (6) also failed to digest the material or reduce its toxicity by digestion with pepsin. From the results of the experiments, it was concluded that the material, ricin, was a highly complex material but not a true protein.

Dixson (7) found that he could prepare a more potent ricin extract by precipitation with alcohol than by neutralization of a hydrochloric acid extract with sodium carbonate. Stillmarck had extracted the seeds with a 10% sodium chloride solution and then salted out the globulins and albumins with both magnesium and sodium sulfates. He then separated the precipitates and removed the salts by dialysis in cold running water.

Cushny (8) next investigated the problem. He was concerned mainly with the problem of the separation of the ricin. He extracted the seeds with a solvent that he does not name. He then saturated the solution with magnesium sulfate, thereby precipitating both the globulins and the

ricin. The precipitate is then dissolved by dialyzing into water. The solution was then filtered yielding a yellowish solution that contained ricin salts and a small amount of organic matter.

Osborne et al. (9) after working on the problem for ten years, concluded that ricin and the albumins are one and the same substance. He found that the seeds contain a considerable quantity of globulin which crystallizes in octhedra. He also found that they contain a smaller quantity of a proteose that belonged to several previously characterized groups. There was also found a small quantity of albumin which coagulates at 60-70° C. It was found that the toxic portion was always associated with the coagulable fraction and never with any other fraction of the proteins. He draws the conclusion that ricin is in the albuminous part of the protein and that it is an impurity on the albumin fraction. This may be the case as it is well known that colloids will carry other substances with them when they are thrown out of suspension.

The symptoms of acute oral ricin intoxication appear fairly slowly, usually after several hours. Upon the ingestion of ricin, the following symptoms appear: The general symptoms are refusal of the animals to eat. There is a paralysis of the respiratory and vasomotor systems. They then go into convulsions and exhibit opisthotonus. This is followed by extreme relaxation and then by a recurrence of the convulsions. The pathological symptoms are diarrhea, general prostration, hemorrhagic condition of the intestines, renal congestion, and hypermia of the spinal medulla and brain. The red blood cells are coagulated. The eyes are affected causing an inflammation and a severe panopthalmitis. The ingestion of ricin never causes instantaneous death but usually death occurs about fifteen to eighteen days after the administration of the ricin. Ricin

is toxic to all mammals, being most toxic to rabbits and guinea pigs. Osborne et al. found that 0.0005 mg. ricin/kg. body weight was fatal to rabbits when the ricin was administered subcutaneously.

Castor seeds also contain a most potent allergen. This allergen was first described by Alilaire (10), who first described human hypersensitiveness to this compound. He believed that the toxin and the allergen were the same compound. Ratner and Gruhel (11) subsequently showed that castor seeds contained both an anaphylactogenic agent in addition to the toxin, ricin. Barnard (12) prepared a non-toxic allergenic extract from castor seeds. He described his product as being water soluble, heat stable and non-dialyzable. Later Grabar and Koutseff (13) separated a non-toxic allergenic fraction from castor seeds that was water soluble, heat stable and dialyzable.

Recently workers of the Bureau of Agricultural and Industrial Chemistry of the U. S. Department of Agriculture isolated from castor seeds a very powerful non-toxic protein polysaccharide fraction. Upon analysis this fraction proved to be almost identical in composition with that of the allergen found in the cottonseed. The compound is characterized by a very high content of arginine and cystine. It was found that the potency of the compound was not impaired by prolonged boiling in water. The allergen is reported to have a high sensitizing capacity.

Castor bean pomace, which is the residue left after solvent extraction, is high in protein, the decorticated seed having from 50-60 per cent crude protein. Studies by Kodras, Whitehair and MacVicar (14) showed that the pomace had a low concentration of methionine and lysine and possibly of tryptophan, but a rather high proportion of all the other essential amino acids. Thus, castor cake would make a very good protein

supplement for feeding livestock if some economic method of detoxifying the ricin could be found.

One of the first men to work on the problem of detoxifying ricin was Boquet (15). He treated ricin with a 1% solution of hydrogen peroxide containing 1 mg./ml. of copper sulfate. He found that the ricin was detoxified without the loss of the antigenic properties. The treated ricin immunized animals to the pure ricin toxic action. Another worker, Ruldolph (16), detoxified the ricin by boiling the powdered cake with water. The water was changed frequently. The powder was then washed with hot water, filtered and dried. This material was reported to be a good fodder for animals. Tangl (17) removed the ricin by heating the meal at 140° for 60-90 minutes. The meal was fed to sheep and was well utilized. Massart (18) removed the ricinine and ricin from the castor cake by treating the powder with a solution of alkali halides and hydroxides. The cake was then treated with steam in an autoclave. The material was found to be suitable as a feed.

Kodras (14) working on the problem found that treating the castor pomace with steam in an autoclave at 125° at 20 lb. pressure for fifteen minutes was effective in destroying the toxicity of ricin.

In 1949, Borchers (19) using castor bean meal experimented with various methods of destroying the toxin in the meal. In his first experiment he heated the crushed and defatted castor bean meal in flowing steam for fifteen minutes. This treatment destroyed about 95% of the ricin as measured by the hemagglutinating activity of the meal, and no symptoms of ricin toxicity were noted in the chick. The meal was fed to day-old chicks and their growth compared to that of chicks that were fed the untreated meal. There was little improvement of the treated meal

over the untreated meal. In a second experiment, Borchers (19) steamed the meal as above and then treated the meal with ten volumes of a 1% solution of sodium chloride by stirring for three hours then filtering and drying the meal residue. The meal showed no hemagglutinating activity. The meal when fed to chicks gave normal survival, but the growth was only one-half that of the basal group. In the third experiment, the meal was refluxed with ten volumes of 95% ethanol for three hours. The alcohol was then filtered and the meal dried. The alcoholic extract was mixed with a portion of the basal ration equivalent to 10% of the castor seed meal and dried. Neither the meal nor the alcoholic extract showed any ricin present as tested by the hemagglutinating activity. When a normal ration plus the ethanolic extract of the detoxified castor seed pomace was fed to chicks, the animals died in about 22 days. The extracted meal when fed to chicks resulted in normal livability, but the growth rate was definitely less than those fed the control ration. In a fourth experiment, he extracted the meal with six changes of boiling ethanol. Each change of alcohol was in contact with the meal for about thirty minutes. The six extracts were combined and evaporated to a small volume. The concentrated extract was then mixed with the basal ration in an amount equivalent to 10% of castor bean meal. The extract when fed to chicks resulted in normal survival, but reduced growth rate. The extracted meal when fed resulted in growth and survival similar to the controls.

From the above experiments Borchers concluded that there was present in the castor bean meal a heat stable toxic factor that was not identical with ricin. Since the material was heat stable, it may be the heat stable allergen of Ratner and Gruehl (11).

EXPERIMENTAL

All feeding and toxicity trials were made using rats of the Sprauge-Dawley strain. The animals in all of the experiments were kept in individual cages. Food and water were supplied ad libitum. A basal ration composed of sucrose, casein, cottonseed oil, and salts supplemented with known vitamins (Table 1) was fed to all the animals except when experimental rations were being fed.

TABLE 1

BASAL RATION[1]

Casein	22.0%
Cottonseed Oil	5.0
Salts[2]	4.0
Starch	69.0

[1] The following vitamins were added per kg. of ration: thiamine, 4 mg.; riboflavin, 6 mg.; calcium pantothenate, 20 mg.; nicotinic acid, 20 mg.; pteroylglutamic acid, 1 mg.; inositol, 20 mg.; p-amino benzoic acid, 20 mg.; choline chloride, 1 g.; pyridoxine, 6 mg. Vitamins A and D and alpha-tocopherol were administered by dropper twice each week.

[2] Hegsted et al., J. Biol. Chem. 138:460(1941).

DETOXIFICATION AND EXTRACTION OF CASTOR SEED POMACE

Experiment No. 1, Preparation of Extract

Finely ground solvent extracted castor pomace from Sherwin Williams Company was autoclaved for twenty minutes at a temperature of 125° C.

(fifteen pounds pressure). The material was spread in a thin layer in large shallow pans to permit rapid penetration of steam. At the completion of the autoclaving process, the material was dried and again reduced to a fine powder by grinding. That this process detoxified the ricin in the castor seed pomace was evidenced by the fact that no acute toxicity symptoms were observed when the product was fed to rats. The detoxified castor seed pomace was then refluxed for a period of three hours with five volumes of 95% ethanol. The extracted meal was filtered off and dried. The extracts of all the extractions were combined and evaporated to a volume of 1480 ml. The percentage of nitrogen in the detoxified castor seed pomace, the detoxified and extracted castor seed pomace and the extract was determined by the Keldjahl procedure.

Upon the basis of these analyses, rations containing an equivalent amount of nitrogen were prepared and fed to weanling rats.

Feeding Trials

Weanling rats weighing between 37-56 grams were used for tests of toxicity. Ten rats were placed in each lot having been allotted equally on the basis of sex and weight.

The four rations fed were as follows: Lot 1, a casein-containing basal ration; Lot 2, a ration containing detoxified castor seed pomace in an amount equivalent to the casein in the basal ration; Lot 3, a ration containing detoxified and extracted castor seed pomace in an amount equivalent to the casein in the basal ration; Lot 4, the basal ration upon which an amount of extract equivalent to the amount of detoxified castor seed pomace had been evaporated. All rations were supplemented with all known vitamins. The composition of these rations is shown in Table 2.

TABLE 2

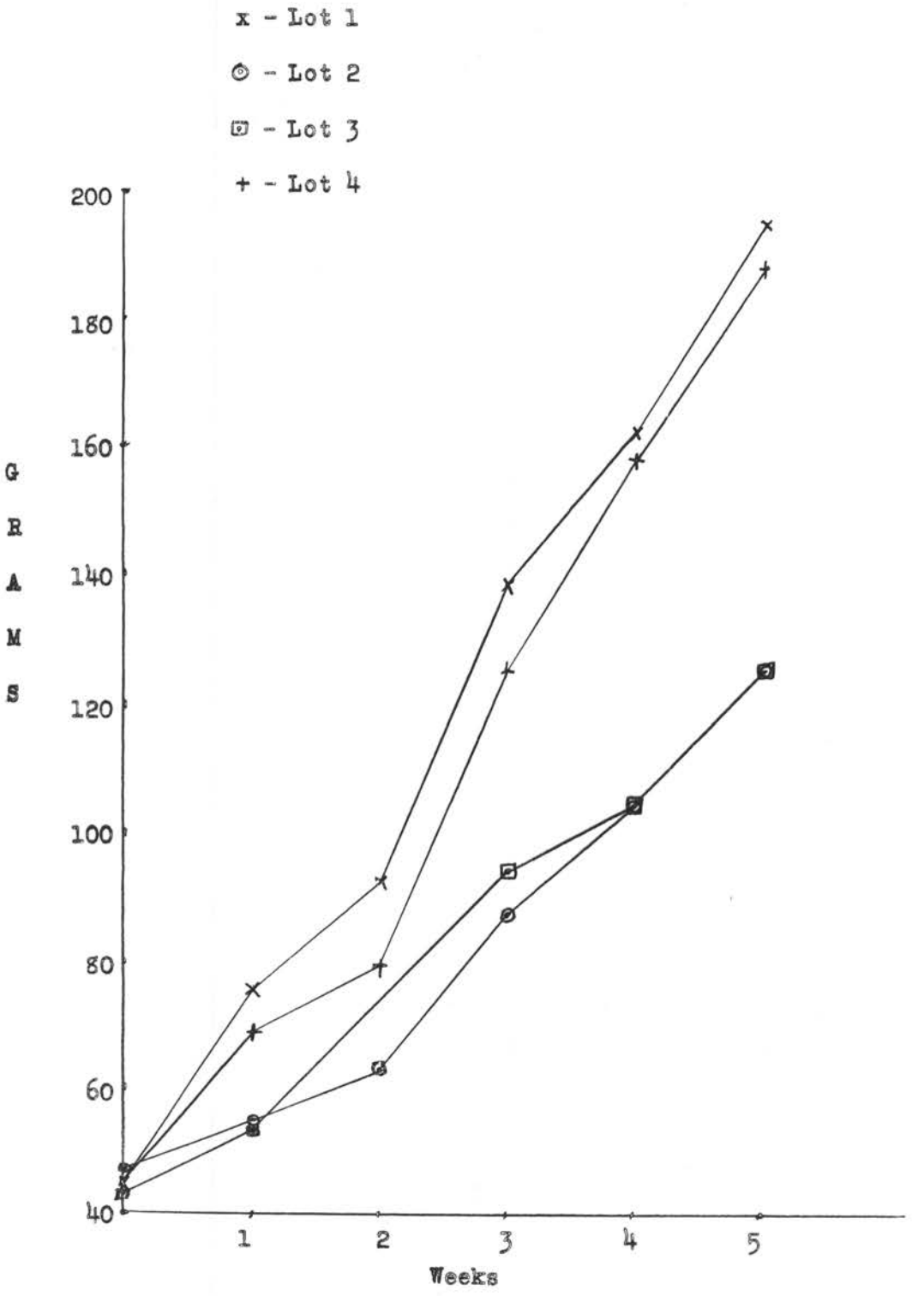
Component	Lot Number 1	2	3	4
Crude Casein	22.0			22.0
Detoxified Castor Pomace		34.8		
Detoxified and Extracted Castor Pomace			33.6	
Cottonseed Oil	5.0	5.0	5.0	5.0
Salts	4.0	4.0	4.0	4.0
Starch	69.0	56.2	58.4	69.0
Extract[1]				+
Number of animals	10	10	10	10

[1] Extract added at a level equivalent to 34.8% of detoxified castor seed pomace.

The animals were maintained on the experimental rations for a period of five weeks. The survival of the rats receiving the castor seed extract was normal. The growth rates are shown in Figure 1. It will be seen that the growth rate of those receiving the castor seed pomace, both the detoxified and the detoxified and extracted, was only half the growth rate of the controls. In the five-week growing period the control animals gained an average of 149 grams while the animals receiving the detoxified castor seed pomace gained an average of 77 grams. The animals receiving the detoxified and extracted castor seed pomace gained an average of 76 grams. This lack of difference in the average gains between the two lots indicated that the pomace was only slightly affected or changed by extraction with boiling 95% ethanol. The lot fed the basal ration which had been treated with the extract of the castor seed pomace gained an

EXPERIMENT 1

FIGURE 1



average of 143 grams over the five-week period. This result indicated that there were no toxins present in the alcoholic extract of the detoxified castor seed pomace.

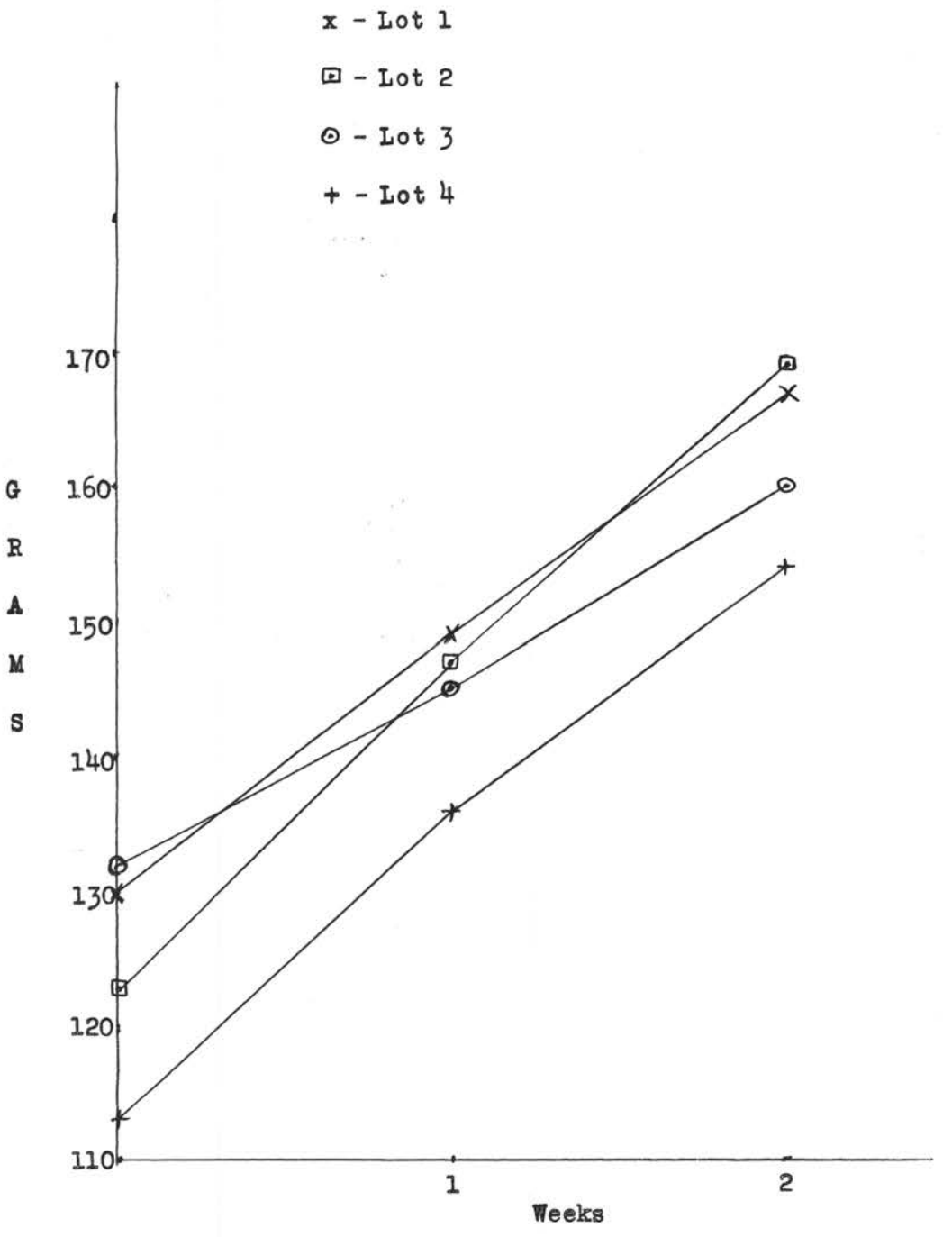
Experiment 1 a

Upon the completion of the above experiment, it was decided to investigate the effects of supplementation of the two castor seed pomaces, detoxified and the detoxified and extracted, with three of the amino acids that had been reported to be in low concentration in the pomace. The rations of Lots 2 and 3 of Experiment No. 1 were supplemented with 0.4% D-L methionine, 0.2 % D-L tryptophan, and 0.3% L lysine. The animals receiving the respective rations were divided into four groups on the basis of sex and weight. Two groups continued to receive the castor pomace rations (Rations 2 and 3, Table 2) while the other two received the same rations supplemented with the three amino acids. The animals were maintained on the experimental rations for an additional period of two weeks. The growth response is shown in Figure 2.

The results of the experiment indicated that supplementation with methionine, tryptophan and lysine resulted in faster growth. The animals being fed the detoxified castor seed pomace supplemented with the three amino acids grew 46 grams in the two weeks while the animals being fed the unsupplemented ration grew 37 grams in the same period. The rats receiving the detoxified and extracted material responded in a similar manner, those that received the supplement growing 41 grams in the experimental period while the animals being fed the same unsupplemented ration grew 28 grams. These results indicated that the concentration of one or more of the indicated amino acids were present in the ration in sub-optimal amounts.

FIGURE 2

EXPERIMENT 1a



Experiment 2

Since the results of the experiment just described did not confirm the results obtained by the Nebraska workers, it was decided to obtain a more complete extraction of castor seed pomace with ethanol. This was effected in the following manner. Castor seed pomace (2193 gm.) was detoxified by autoclaving as in Experiment 1. The detoxified material was then extracted by refluxing for 120 minutes with three volumes of ethanol. Each change of the extractor was extracted seven times. After each extraction the pomace was filtered and the alcoholic extract was evaporated to a small volume. All the extracts were then combined and the volume of the extract measured.

Toxicity Trials

Five lots of rats, weighing between 81-122 grams, and of the Sprauge-Dawley strain, were used for the feeding trials. The composition of the experimental rations is shown in Table 3.

TABLE 3

Component	Lot Number 1	2	3	4	5
Crude Casein	22.0				22.0
Detoxified Castor Pomace		34.8			
Detoxified and Extracted Castor Pomace			33.6	33.6	
Cottonseed Oil	5.0	5.0	5.0	5.0	5.0
Salts	4.0	4.0	4.0	4.0	4.0
Starch	69.0	56.0	58.4	58.4	69.0
Extract[1]					+
DL-Methionine				0.4	
L-Lysine				0.3	
Number of animals	10	10	10	10	10

[1] Extract added at a level equivalent to 34.8% of detoxified castor pomace.

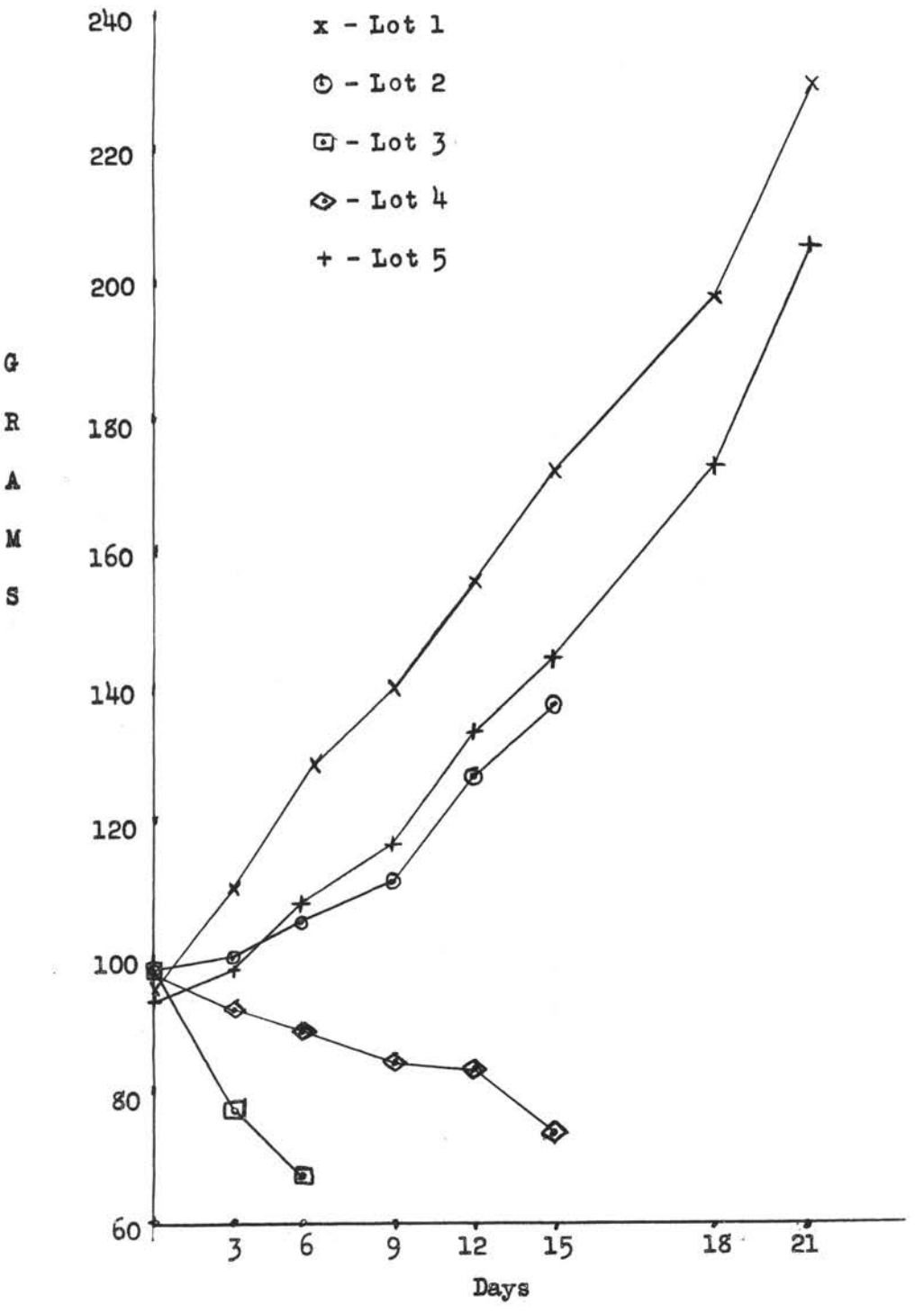
Rations for Lot 5 were prepared in the same manner as they had been prepared for Lot 4 in Experiment 1.

The rats were weighed every three days for a period of fifteen days. The weight gains of the animals are shown in Figure 3.

The results obtained in this experiment were quite different than those that had been obtained in Experiment 1. Survival of the rats being fed the detoxified and extracted material was much less than in the previous experiment. Of the ten animals used, only one survived nine days on the ration. Partial inanition was noted in Lot 3 and Lot 4. Those animals in Lot 4 survived longer than the animals of Lot 3. It was therefore decided to maintain them on the experimental ration for a few

FIGURE 3

EXPERIMENT 2



days longer. At the end of the 24th day only one animal survived. The animals in Lot 3 and Lot 4 did not grow well; in fact, they steadily lost weight. Those animals in Lot 4 lost an average of 24 grams in 15 days. The animals in Lot 5 which received the extract-treated basal ration grew well. They gained an average of 61 grams compared to a 76-gram gain by the controls. All of the animals in Lot 5 survived.

Experiment 2 a

To further check the effect of the ethanol extracted pomace ration, supplemented with the amino acids, it was decided to feed that ration to the animals of Lot 2. They were fed the ration for a period of 21 days. The growth response is indicated in Figure 4. At the end of this period three of the ten animals still survived. They all lost weight regularly during the experiment and lost an average of 46 grams in fifteen days. On the fifteenth day 10% casein was added to the rations. The average weight of the remaining animals began to increase slightly.

Experiment 3

Because of the severe death losses and poor performance produced by the second experiment in contrast to the first, it was decided to repeat the former experiment a third time. The castor seed pomace was autoclaved as before and 3500 grams of the detoxified material was extracted seven times with boiling 95% ethanol for 120 minutes. The extracts were combined and evaporated to a small volume and this volume measured. Composition of the experimental rations used in Experiment 3 are shown in Table 4.

FIGURE 4

EXPERIMENT 2a

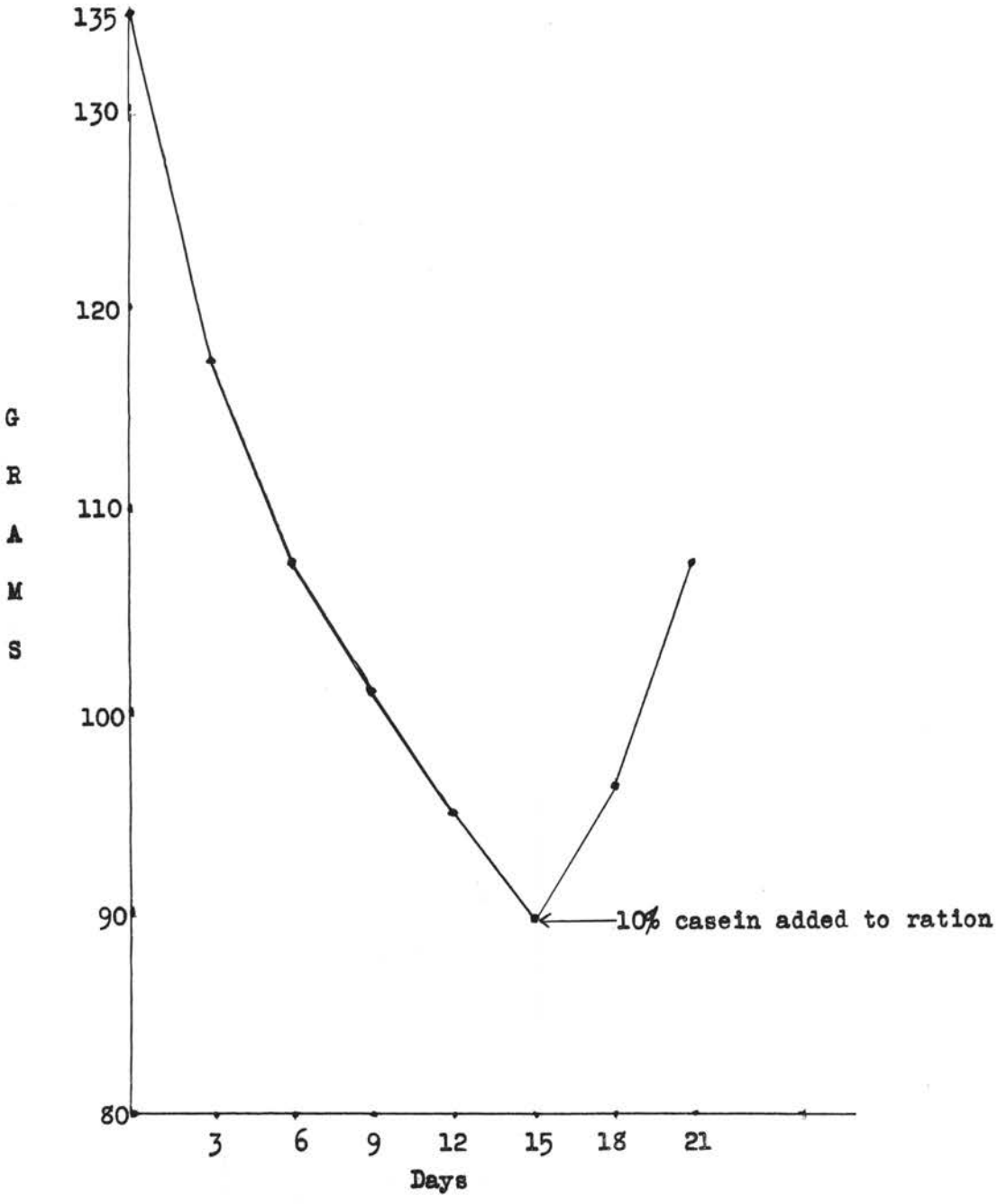


TABLE 4

Component	Lot Number 1	2	3	4	5
Crude Casein	22.0			22.0	
Detoxified Castor Pomace		31.3			31.3
Detoxified and Extracted Castor Pomace			26.8		
Cottonseed Oil	5.0	5.0	5.0	5.0	5.0
Salts	4.0	4.0	4.0	4.0	4.0
Starch	69.0	59.7	64.2	69.0	59.7
Extract[1]				+	
DL-Methionine					0.4
L-Lysine					0.3
Number of animals	10	10	10	10	10

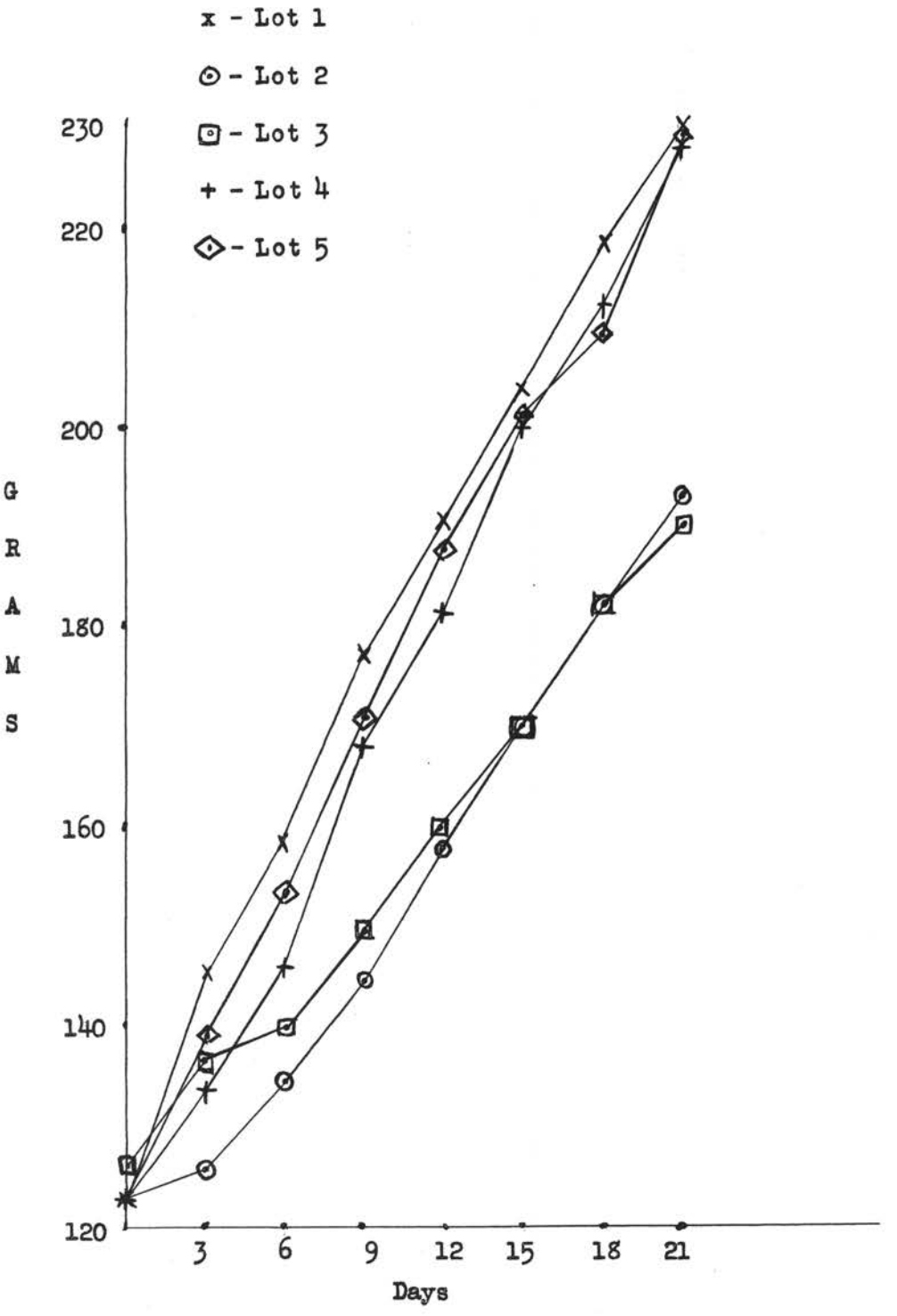
[1] Extract added at a level equivalent to 31.3% of detoxified castor seed pomace.

Rats of the Sprauge-Dawley strain, weighing between 93-145 grams, were used in this experiment. They were allotted so that each lot had about an equal average weight. The animals were weighed every three days.

Survival of the animals in all lots was normal. Graphic results are shown in Figure 5. No deaths occurred in any of the lots during the course of the trial. The animals receiving the casein ration grew 106.4 grams in 21 days as compared to a gain of 69.7 grams for Lot 2 and 63.9 grams for Lot 3. The animals fed the detoxified castor seed pomace that had been supplemented with methionine and lysine made markedly greater gains, 103.8 grams. This compared favorably with the casein control

FIGURE 5

EXPERIMENT 3



which gained 106.4 grams. Those animals fed the ethanol extract on the basal ration showed an average gain of 105.0 grams in the feeding trial. This gain equals that of the control lot. The results obtained in this experiment do not support the presence of a heat stable second toxin extractable from castor seed pomace by ethanol. The data do indicate, however, supplementation of the detoxified castor seed pomace with lysine and methionine very markedly enhanced its protein quality. It would seem from these experiments that the concentration of menthionine or lysine or both is deficient in castor seed pomace for maximum growth in the rat when protein is supplied at the 20% level.

Experiment 4

As it had not been possible to obtain the same results with rats as had been observed by Borchers in the chick, it was decided to feed some of the detoxified and the detoxified and extracted meal that had been prepared at the Nebraska station.[1] This material was incorporated into 3 rations and fed to rats. The composition of the experimental rations is shown in Table 5.

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[1] The supplying of the castor meal by the Nebraska Experiment Station is gratefully appreciated.

TABLE 5

Component	Lot Number 1	2	3
Detoxified Castor Pomace		33.3	
Detoxified and Extracted Castor Pomace			33.3
Untreated Castor Pomace	33.3		
Starch	65.8	65.8	65.8
Cottonseed Oil	5.0	5.0	5.0
Salts	4.0	4.0	4.0
Number of animals	10	10	10

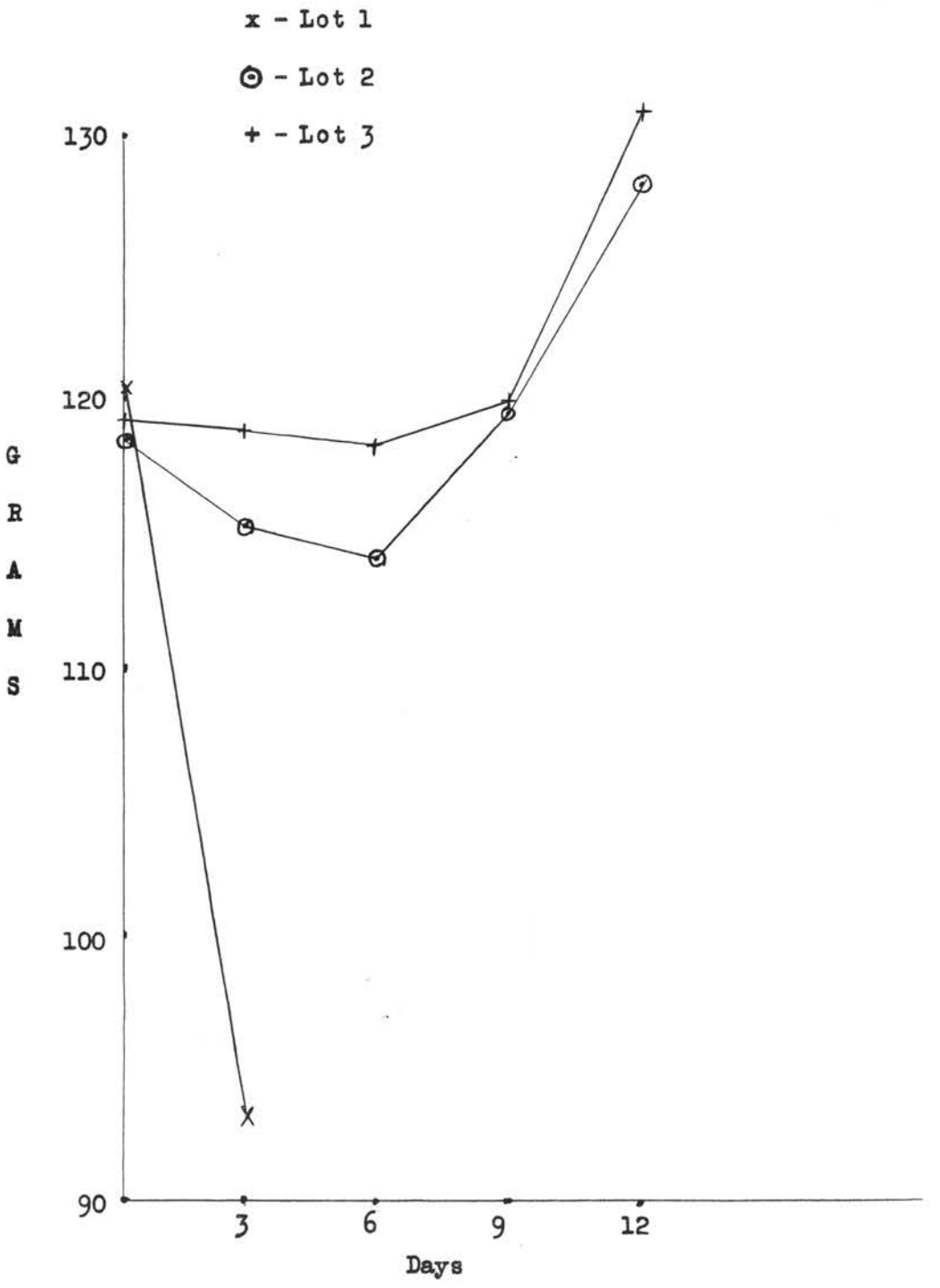
Rats of the Sprauge-Dawley strain were used. These animals weighed between 95-134 grams. They were allotted in such a manner that all lots had an almost equal average weight. The animals were kept in separate cages and food and water were always available ad libitum.

Survivals of the animals in Lots 2 and 3 was normal, but in Lot 1 all ten of the animals were dead by the sixth day and only five survived by the third day of the trial. These results were expected as the ration fed to them contained untreated castor seed pomace.

The growth response is shown in Figure 6. The animals in Lot 2 gained an average of 10.3 grams over a period of twelve days while those animals in Lot 3 gained an average of 12.6 grams in the same period of time. This short period of time was necessitated by meager supplies of the various castor seed pomace preparations. The results indicate that the pomace received from the Nebraska station had the same characteristic as that prepared locally which had been used in the previous feeding trials.

FIGURE 6

EXPERIMENT 4



General Discussion

The detoxified and the detoxified and extracted castor seed pomace rations used in Experiment 1 gave a poorer growth response than did the casein-containing basal ration. The growth of the animals receiving the castor seed pomace rations was only about half that of the animals receiving a ration of adequate protein quality. The animals that received the casein basal ration upon which the ethanolic extract of the detoxified castor seed pomace had been evaporated, gained as much as did the control group being fed the casein basal ration. This indicated that if there were any toxic substances in the ethanolic extract, the rat was relatively unaffected by them at the level fed, which was equal to 34.8% detoxified castor seed pomace. The fact that the growth response of the animals fed the detoxified and extracted castor seed pomace was not improved compared to the autoclaved meal would also indicate that no toxic material was removed by boiling 95% ethanol.

Upon supplementation of the castor seed pomace rations with the amino acids methionine, tryptophan and lysine, the animals grew at a faster rate. They gained on the average 9 grams more than the animals that were fed the same unsupplemented ration. These results suggest that castor seed pomace is deficient in one or more of the indicated amino acids methionine, tryptophan or lysine.

Exhaustively extracted detoxified castor seed pomace which was used in Experiment 2 caused the death of most of the animals to which it was fed. The detoxified and extracted pomace when incorporated into rations fed to rats resulted in a high death rate (Lots 3 and 4). The animals did not like the ration and partial inanition was noted. This may have been part of the reason for the high death rate.

Supplementation of the detoxified castor seed pomace with methionine and lysine did not bring about a diminution of the death rate. The animals continued to lose weight, but at a slower rate than was true when the ration was not supplemented with the aforementioned amino acids. The improvement in the response of the animals when casein was added to the ration suggests that the prolonged heat treatment of the pomace resulted in a reduced availability of the protein.

When the basal ration upon which the ethanolic extract of the detoxified castor seed pomace had been evaporated, was fed to rats, no decrease in growth was noted. The animals fed the ration grew at essentially the same rate as the control animals. This would indicate that there were not toxic substances present in the ethanolic extract for the rat at the level fed.

Because of the unexpected results obtained in the preceding experiment, the experiment was again tried using the same pomace and extracting it as had been done in Experiment 2. The growth response of the animals in this experiment was essentially the same as that found in Experiment 1. The animals that were fed the detoxified castor seed pomace ration that had been supplemented with methionine and lysine grew at a rate equal to that of the casein controls. The animals fed the casein basal ration supplemented with the ethanolic extract of the detoxified castor seed pomace grew as well as the controls. This fact again indicates that there are not toxic substances for the rat in an ethanolic extract of the detoxified castor seed pomace.

No explanation can be advanced for the high death rate in Experiment 2. The conditions under which the detoxified castor seed pomace was extracted were the same as in Experiment 3. The castor seed pomace which

was detoxified came from the same source and was detoxified in the same manner as that used in Experiment 3. The extraction procedure was essentially identical. The failure of animals to eat the ration containing the material in Experiment 2 was in distinct contrast to previous and subsequent experience.

In order to check the results that had been obtained with the locally detoxified and extracted castor seed pomace, an experiment was conducted using castor seed pomace obtained from the Nebraska Experiment Station. Upon feeding a detoxified, a detoxified and extracted, and an untreated meal to rats essentially the same results were obtained as had been obtained from the meal that had been prepared locally. The growth response of the animals being fed the detoxified and the detoxified and extracted castor seed pomace was very similar. The animals being fed the untreated castor seed pomace died within six days.

From all these studies it seems obvious that if there is a toxic substance in detoxified castor seed pomace, it can not be extracted from the meal with boiling ethanol. The value of castor seed pomace for growth of rats is not improved by the extraction with boiling ethanol.

It may be that the sub-optimal amounts of methionine and lysine are responsible for the failure of the rations containing castor seed pomace to give optimum growth in the rat. This fact is indicated by the results which were obtained when the rations were supplemented with the indicated amino acids. In every case the animals grew at a rate comparable to the growth of the control animals.

SUMMARY

Detoxification of the castor seed pomace was accomplished by autoclaving at 125° C. at a pressure of 15 lbs. This was confirmed by feeding the resulting meal to rats and noting any signs of toxicity. The detoxified meal was then refluxed with boiling ethanol. The resultant filtrate was evaporated to a small volume and used in the preparation of rations. The residue from the extractions was also used in the preparation of rations.

It was found that the detoxified and extracted castor seed pomace and the meal which was only autoclaved produced essentially equal growth response in the rat. The detoxified meal did not seem to be improved by extraction with boiling ethanol. No heat-stable toxic substances for rats were found in the alcoholic extracts. The results which Borchers (19) had observed at Nebraska with the chick were not confirmed with the rat. No toxic substance was indicated in the ethanolic extract of the detoxified castor seed pomace in any of the experiments conducted here. Also no essential difference was noted between the meal prepared locally and that which had been prepared at the Nebraska station.

When the detoxified and the detoxified and extracted castor seed pomace rations were supplemented with methionine and lysine, a significant increase in growth response was noted. The supplementation of the castor seed pomace rations with these amino acids brings the protein quality up to that of casein. This is indicated by the fact that the animals fed the amino acid supplemented castor seed rations grew as well as the animals receiving the casein basal ration.

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