THE DETOXICATION AND UTILIZATION OF CASTOR POMACE

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PREFACE

One of the serious economic hindrances to the large-scale production of castor seeds in Oklahoma is the fact that the toxicity of the residual meal after extraction of oil prevents its use as a high-protein supplement for livestock feeding. This toxicity is such that no use can be made of the material except as a fertilizer, thus materially reducing the value of the crop. Since the toxic constituents are known to be albuminous proteins, it was thought that a thorough investigation of methods known to denature and coagulate such proteins might disclose a means of detoxication. A project to investigate such detoxication procedures was therefore instituted and the preliminary results of these studies are presented in this report.

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REVIEW OF LITERATURE

The castor plant, Ricinus communis, was well known to the ancients and is said to have originated in India. It is now cultivated widely in nearly all of the temperate and tropical parts of the world. It is raised almost exclusively for its oil, which is extensively used for medicinal and industrial purposes.

The high protein content of the castor bean and the presence of an extremely toxic substance, long believed to be intimately associated with the protein, has stimulated an unusual interest in the proteins of this seed. That this interest still exists is indicated by a recent review of technical papers and information on the proteins of the castor bean by D. Breese Jones (1).

Among the early workers on castor bean proteins, Ritthausen in 1882 (2), (3) was the first to obtain the castor bean globulin in crystalline form. Both he and Vines (4) independently studied other protein fractions of the castor bean, but, because of the state of knowledge of protein chemistry at that time, many of their findings are contradictory.

The name, ricin, was given in 1889 by Stillman (5) to the highly toxic substance which he extracted from castor beans under Kobert's guidance. He believed the material to be a globulin. Among subsequent investigators, Jacoby (6) in particular has questioned the protein nature of the poison. Jacoby's experimental failure to destroy the toxic properties of his ricin solutions by digestion with trypsin gave rise to the view that the toxin of the castor bean was

a highly complex compound, but not a true protein.

Dixson (7) found that a more active preparation of ricin could be obtained by precipitation with alcohol than by neutralizing a hydrochloric acid extract with sodium carbonate. Stillmarck (8) approached the problem by extracting castor seeds with a 10 per cent sodium chloride solution and salting out with both magnesium and sodium sulfates. The precipitate was separated and the salts then removed by dialysis.

Cushny (9) next investigated this problem, directing his attention particularly to the isolation of the ricin and the determination of its chemical constitution.

Great interest in ricin was aroused by the studies of Ehrlich in 1891 (10), who succeeded in establishing a high degree of immunity to the castor seed toxin and in demonstrating the production of an antitoxic substance, antiricin, in the blood of immunized animals. Subsequently ricin has been extensively employed in studies of immunity. Cornevin (11) obtained a vaccine by heating an aqueous solution of ricin at 100° for two hours. When this vaccine was injected under the skin, various domestic animals were immunized against the subsequent administration of ricin. Two doses of vaccine administered at eight-hour intervals were sufficient to immunize pigs.

Baquet (12) attenuated ricin by hydrogen peroxide in the presence of traces of copper and thus produced a suitable vaccine.

Ricin is one of the most toxic substances known. For

example, Ehrlich (10) produced death (usually 24 to 36 hours) with 0.03 mg. per kilo of body weight. Cushny (9) obtained speedy lethal effects in rabbits with 0.04 mg. Muller (13) and Jacoby (6) produced death in rabbits within 30 hours with 0.5 to 0.6 mg. of Merck's ricin or their own purified ricin. Brieger (14) produced death in rabbits with 0.01 mg. per kilo with a partly purified preparation. Of the known compounds approaching ricin in toxicity, the snake venoms and botulins are conspicious.

The extensive investigations of Osborne and associates (15), conducted over a period of ten years or more, yielded most of the available information on the chemical properties, quantities, and composition of the different proteins of the castor bean. Castor beans contain a considerable quantity of crystallizable globulin, a much smaller amount of coagulable albumin, and proteoses. The physiological properties ascribed to the substance known as ricin are associated with the coagulable albumin of the castor seed.

The appearance of symptoms accompanying ricin intoxication is comparatively slow, even when large doses are administered. The animals refuse to eat after a few hours. The onset of severe symptoms, though delayed, is always sudden. Convulsions and opisthotonus are followed by extreme relaxation and again by a return of the convulsive effects. There are no truly specific symptoms to be noted, and the influence of variation in dosage is exhibited primarily in the shortening or prolongation of the latent period before the appearance of appreciable symptoms. In no instance does

death ensue sooner than fifteen to eighteen hours after the subcutaneous administration of the poison.

Among the gross pathological changes noted, punctiform hemorrhages in the peritoneal cavity scattered over the omentum and along the serous covering of the intestine are most frequently observed. Other similar ecchymoses are often noted. Peyer's patches and the retroperitoneal lymph glands are usually found somewhat swollen and markedly congested or hemorrhagic. Not infrequently the peritoneal cavity contains a large excess of somewhat opaque fluid. Acute ulcerations of the intestine are not found. Flexner (16) has pointed out that the toxin of the castor bean exerts its deleterious action upon cells and tissue, much like the bacterial poisons. The destructive effects are evident both at the point of entrance of the toxin in the subcutaneous tissue and at the place of exit, as along the stomach and intestinal wall. The elimination of the poison by the gut has been observed by several investigators. The passage of the poison through the wall of the intestinal tract undoubtedly accounts for the local histological lesions observed there. At the site of injections, a reddening of the subcutaneous tissue, which not infrequently presents a distinct hemorrhagic appearance, is found. A noticeable necrosis is not obtained. These appearances are all missing when non-toxic proteins are injected subcutaneously.

The characteristic influence of ricin preparations on extra-vascular blood or suspensions of corpuscles is exhibited in the agglutination of the erythrocytes followed

by the sedimentation of flocky masses upon the bottom of the retaining vessel. A clear, practically colorless serum is thus obtained. The bloods of various species show different degrees of sensitivity similar to that observed with bacterial lysins. The activity of ricin in agglutinating mammalian blood corpuscles is very great, 0.001 per cent sufficing to sediment dilute blood suspensions. In contrast, Kobert (17) was unable to effect an agglutination of the corpuscles of Sipunculus, a genus of elongate cylindrical marine worms, with ricin and concluded that their stroma must vary in composition from that of vertebrates.

Several writers have assumed that the toxic, agglutinative, and hemorrhagic properties are distinct and separable. Fuchs and Falkensammer (18) and Karrer, et al. (19) have shown in their experiments that the agglutinating principle of ricin is inactivated fairly easily under conditions of proteolytic digestion, while the toxic principles are more resistant. Reid (20) found that when the cells of dog's brains were washed with physiological salt solution and agglutinated by adding a solution of ricin, the ricin was extracted from the precipitate by very dilute hydrochloric acid. The clear extract was found to promptly agglutinate a suspension of cat's red blood corpuscles; this showed that not only the toxin but also the agglutinin was united with the brain cells, thus indicating that the two effects were due to a single substance. A differentiation of ricin into a toxin and an agglutinin is not justified.

Within the last few years extensive studies on ricin

have been carried on by Kabat, et al. (21). Highly toxic hemoagglutinating preparations of ricin can be obtained by fractional precipitation with sodium sulfate. These products were electrophoretically, ultracentrifugally, and immunochemically homogeneous and identical in these respects with crystalline ricin, but were only two-thirds as toxic. The toxic and non-toxic forms can be separated by crystallization. Immunochemical and toxicity assays of crude extracts of castor beans indicate that these contained both forms of ricin. Ricin was found to have an isoelectric point of 5.2 to 5.5 and a molecular weight by sedimentation and diffusion of 77,000 to 85,000 for an assumed partial specific volume of 0.75.

Only a few cases are recorded of the use of castor cake or pomace as a protein concentrate for use in the feeding of farm animals. A preliminary heat treatment of the castor beans destroyed the toxicity according to early work by Stillmarck. Osborne, Mendel and Harris in 1905 (15) observed that heating at 60 to 80° coagulates ricin.

H. T. Atkinson (1918) (22) concludes his experience with castor seed meal as a poultry feed with the statement:

> In view of the danger that would ensue if castor meal was used as one of the constituents of a mixed meal, it would be advisable for all poultry-keepers to make sure that none was included in the meal purchased by them and corn merchants should rigorously exclude it from any mixture they might be selling, as there is no doubt its inclusion would be a source of great danger to the birds feeding on it and a consequent loss to the poultry-keeper.

Both White (23) and Wooldridge (24) reported cases of castor seed poisoning in horses. Two deaths were noted in

the first report and five in the latter, and the services of the other horses were lost for several weeks.

Moreschi (25) found the seeds poisonous for carnivores as well as herbivores.

Different methods to detoxify the ricin have been tried. Several investigators have attempted to remove the toxic material by extraction procedures employing water and various organic solvents. Rudolph (1942) (26), (27), (28) has patented a process for detoxicating castor pomace for animal or human consumption. The method consists of heating the pomace to over 80° C. to inactivate the ricin and the use of organic solvents such as ethyl alcohol, methyl alcohol, chloroform, acetone, etc. for extracting the ricinine, the immunologically active fraction. Petrosyan and Ponomarev (1934) (29) succeeded in removing the toxicity for pigs by cooking the pomace for one to two hours, while Tangl (30) used the method of heating for 60 to 90 minutes at 140°. Butz and Bottger (31) used a 25 per cent castor pomace mixture for dairy cows. No mention was made of the method used to detoxify the castor pomace.

Jaki (32) treated castor pomace with steam to destroy the toxic substances and removed the excess moisture by vacuum distillation. Massart (33) extracted the ricinine with alkali halides and hydroxides, and subsequently treated the cake with steam in an autoclave.

PART I

TOXICOLOGICAL AND DETOXICATION STUDIES ON CASTOR POMACE

The first objective of this problem was to determine the oral toxicity of castor pomaces produced by various commercial and laboratory processes. With a high order of toxicity established, the effects of various treatments of the pomace on its oral toxicity were then studied. Treatments were employed which might be expected to alter the physical and chemical state of the toxic albumin and thus destroy the toxicity.

Oral Toxicity Studies

Most of the trials to determine the toxicity of various pomaces were made using rats. A few trials were also made with chicks.

Rats of the Sprague-Dawley strain, varying in weight from 45 to 250 grams, were used. They were kept in individual cages and fed the following ration (Basal Ration #1):

Table 1. Basal Ration #1

Ground yellow corn	56.00%
Soybean oil meal	20.00
Whole-milk powder	20.00
Alfalfa meal	2.00
Dried brewer's yeast	1.00
Sodium chloride (iodized)	0.50
Calcium carbonate	0.50
Cod liver oil	0.50

Treated and untreated castor pomace were added at various levels at the expense of the whole ration. Feed and water were abailable ad libitum.

A summary of representative results on the oral toxicities of various samples of castor pomace is given in Table 2. It will be noted that the expeller-process pomace (Baker Castor Oil Company) was fed to rats at levels as high as 50 per cent without symptoms of toxicity. In contrast, solvent-extraction pomace, either of commercial (Sherwin-Williams Company) or laboratory origin, was extremely toxic. Death occurred in all animals consuming a ration containing 5 per cent of the material. Male rats varying in weight from 40 to 65 grams died within 4 days. It was observed, in general, that the larger, more mature animals were more resistant. As much time as ten days was occasionally required and in a few cases the animals apparently developed an immunity to the toxin.

A few hours after feeding rats the toxic ration, they appeared very nervous and did not eat. The rats assumed a crouching position and became very inactive. The animals lost weight principally because of refusal of food. Diarrhea was seldom noted and dehydration did not occur. Other symptoms were not noted until 36 to 72 hours in most cases. At this time the condition of the animals degenerated rapidly. Their breathing became rapid and shallow. Nervous symptoms, including occasional convulsions, were noted. Death ensued within a few hours after the onset of obvious nervous involvement, apparently from respiratory failure.

The gross pathology was not very specific and varied

Table 2. Toxicity of Castor Pomace

Trial	Type of Material	Level Administered (% in feed)	Animal and Size	Number of Animals	Response
1	Expeller-extraction (Baker Castor Oil Co.)	2	Rat (60-75 g.)	6	One died, cause unknown; rest survived; no symptoms of toxicity.
2	" "	6	Rat (60-75 g.)	6	All survived; no symptoms of toxicity.
3	" "	15	Rat (60-75 g.)	6	All survived; no symptoms of toxicity.
4	" "	50	Rat (85-140 g.)	7	All survived; no symptoms of toxicity.
5	Solvent-extraction (Sherwin-Williams Co.)	5	Rat (55-65 g.)	4	Died in 4 days; typical symptoms: emaciation; general inflammation and edema of the gastro-intestinal tract with punctiform hemorrhage; liver hyperemic; kidney congested; contents of small intestine liquid.
6	" "	5	Rat (250 g.)		One survived. Three died in 3,7, and 8 days respectively typical symptoms.
7	" "	5	Rat (40-50 g.)		Died within 4 days; typical symptoms.

Table 2. Continued

Trial	Type of Material	Level Administered (% in feed)	Animal and Size	Number of Animals	Response
8	Solvent-extraction (Sherwin-Williams Co.	5	Rat (150-180 g.)	14	Died in 6 days; typical symptoms.
9	" "	5	Rat (170-240 g.)	11	4 died in 7 days, 5 in 8 days and 2 within 10 days; typical symptoms.
10	Solvent-extraction (Laboratory preparation)	5	Rat (130-170 g.)	3	Died within 5 days; typical symptoms.
11	" "	5	Rat (250 g.)	2	Died within 10 days; typical symptoms.
12	Solvent-extraction (Sherwin-Williams Co.)	5	Chick (40-60 g.)	10	2 died in 2 days, 8 died in 6 to 10 days; typical symptoms.
13	" "	5	Chick (40-60 g.)	10	Died in 6 to 10 days; typical symptoms.
14	" "	50	Chick (175 g.)	7	One survived; others died in 6 days; typical symptoms.

according to the level of castor pomace fed and the age of the animals. On autopsy, the following gross pathological changes were noted: emaciation of the body, punctiform hemorrhages of the intestinal tract, general inflammation and edema of the tissues and membranes, hyperemia of the liver, congestion of the kidneys, and sometimes a serious fluid in the body cavities. The contents of the small intestine were liquid in character. Blood was frequently found in the gastro-intestinal tract.

In order to prove the presence of ricin in these samples of castor pomace, it was extracted from two castor-bean meals of different origins in a form suitable for intraperitoneal injections. One method of extraction followed was the procedure of Osborne, et al. (15). Two hundred twenty-five grams of a commercial castor pomace was extracted with 500 cc. of 10 per cent sodium chloride solution, the extract filtered, and the filtrate dialyzed in running tap water for 48 hours. The considerable precipitate which formed was filtered from the dialysate. The dialysate was saturated with ammonium sulfate and the resulting precipitate removed by centrifugation. This was dissolved in 10 per cent sodium chloride solution, and again reprecipitated by saturation with ammonium sulfate. The precipitate was taken up in a small quantity of water. Ricin was also extracted from a laboratory-prepared castor-bean meal essentially as described by Corwin (21). Seventy grams of ether-extracted castor-bean meal prepared in the laboratory were extracted with water at pH 3.8; the ricin was precipitated by saturation with sodium sulfate. The crude

product was then dissolved in a small quantity of water.

The preparations were injected intraperitoneally in rats at the dosage given in Table 3. Each milliliter of ricin extract represents two grams of original material.

Table 3. Effect of Injecting Ricin Extracts in Rats

Preparation	Dose	No. of animals	Effects noted
No. 1	1.0 ml	2	Survived
No. 1	2.0 ml	2	Died in five days
No. 2	0.5 ml	4	Died within 20 hours
No. 2	1.0 ml	4	Died within 20 hours
No. 2 (1:10 dilution)	0.25 ml	1	Died in 48 hours
No. 2 (1:10 dilution)	0.5 ml	2	One died in 24 hours One survived

The characteristic pathological changes observed in animals receiving ricin preparations intraperitoneally were quite similar to those observed in animals poisoned by ingestion of pomace. The subcutaneous tissue and underlying muscles at the site of injection were hemorrhagic. Extensive ecchymoses on the omentum and outer layer of the caecum and hyperemia of the intestinal and mesenteric vessels were noted.

To observe the histological changes that were occurring, tissue sections of liver and intestine were made.[1] Microscopic examination of the sections of the jejunum portion of the small intestine showed hyperemia and edema of the submucosa with desquamation of the epithelium. A muco-fibrinous exudate containing some blood was observed in the lumen of the

[1]The author wishes to acknowledge with thanks the assistance of Miss Dorothy North in the preparation of the slides and Dr. H. W. Orr in their interpretation.

intestine owing to the villi being denuded of epithelium. The membrane propria was infiltrated with numerous leucocytes. Liver sections appeared hyperemic and edematous; some occlusion of the bile ducts was observed. The sections showed no marked degeneration of liver cells. It appears from the above observations that the toxicity of ricin is a direct chemical effect such as that produced by a local irritant. The irritation is severe enough to cause sloughing of the epithelium. It first sets up a hyperemia and edema symptoms of inflammation. Upon further action, an exudate appears as the epithelium is sloughed away.

A study of the toxic action in young chicks was also made. Day-old chicks receiving a highly toxic castor-bean pomace at the 5-per cent level died within 6 to 10 days. In a few instances the chicks developed an immunity and survived. In contrast to rats, the birds ate the ration. They steadily lost weight, however, and became progressively less active.

Before death they became severely emaciated and dehydrated. The face was pale; the plumage was ruffled and unthrifty in appearance. Gross pathology on autopsy was not essentially dissimilar from that observed in the rat. The liver was dark and congested; the kidneys were swollen and hemorrhagic. Hemorrhage and edema was observable in the entire gastro-intestinal tract including the gizzard, which showed occasional inflammatory patches.

Detoxication Studies

A high degree of toxicity in both solvent-extracted commercial and laboratory preparations having been demonstrated, studies were instituted to determine the effects of several treatments on the toxicity of the pomace.

The following treatments were effected: Heating in a dry state to 140° C. in a drying oven, to 125° C. (20 pounds pressure) in an autoclave, adding a quantity of water and autoclaving at 125° C, and boiling in an excess of water. These treatments, it was felt, offered the greatest chance of developing a profitable commercial process for detoxication with the minimum of time, equipment, and damage to the nutritive quality of the protein in the pomace. The treated pomace was dried, finely ground, and added to the rations.

The effect of keeping the castor pomace at room temperature in the presence of excess free acid or base was studied. The dry pomace was thoroughly mixed in a 0.2 N solution of acid or base and allowed to stand overnight. The slurry was then neutralized and mixed with the ration.

Mild oxidation with hydrogen peroxide was tried and its effect on ricin toxicity was determined. The dry pomace was mixed with a 3 per cent solution of hydrogen peroxide and allowed to stand for 24 hours. By this time the peroxide was spent and the treated pomace was mixed with the ration.

The effects of various treatments upon the toxicity of castor pomace are summarized in Table 4. Examination of the data shows that autoclaving castor pomace at 125° C. (20 pounds

pressure) for 10 minutes or more resulted in essentially complete detoxication of the pomace. In only one instance (Trial 2) did death occur in rats or chicks fed such treated pomace. Even steaming (4 pounds pressure) for 30 minutes was effective in destroying the toxicity. Dry heating (Trial 16) at 140° C. for one-half-hour and one-hour periods did not destroy the toxicity and all animals receiving pomace so treated died in 10 days. Heating a suspension of the pomace at 80° C. (Trial 17) was also ineffective. Treating the castor pomace with 0.2 N solutions of either NaOH or HCl and later neutralizing the solution removed the toxic effects. Mild oxidation (Trial 15) with 3 per cent hydrogen peroxide was also satisfactory for detoxication.

Table 4. The Effects of Various Treatments upon the Toxicity of Castor Pomace

Trial	Type of Material	Treatment	Level Administered (% in feed)	Animal	Number of Animals	Response
1	Expeller-extraction (Baker Castor Oil Co.)	4 lbs. steam for 30 minutes	5	Rat	4	All survived.
2	" "	20 lbs. steam for 15 minutes	5	Rat	2	" "
3	" "	20 lbs. steam for 1 hour	5	Rat	3	" "
4	Solvent-extraction (Laboratory preparation)	2 lbs. steam for 15 minutes	5	Rat	4	" "
5	" "	20 lbs. steam for 5 minutes	5	Rat	3	Two died in 10 days; 1 survived.
6	" "	20 lbs. steam for 10 minutes	5	Rat	3	1 died in 18 days; rest survived.
7	" "	20 lbs. steam for 20 minutes	5	Rat	3	All survived.
8	" "	20 lbs. steam for 30 minutes	5	Rat	9	" "
9	" "	20 lbs. steam for 1 hour	5	Rat	3	" "

Table 4. Continued

Trial	Type of Material	Treatment	Level Administered (% in feed)	Animal	Number of Animals	Response
10	Solvent-extraction (Sherwin-Williams Co.)	12.5 g. pomace plus 7 ml H_2O and 20 lbs. steam for 15 minutes	5	Rat	14	All survived.
11	" "	12.5 g. pomace plus 20 ml H_2O and 20 lbs. steam for 1 hour	5	Rat	3	" "
12	" "	12.5 g. pomace plus 30 ml H_2O and 20 lbs. steam for 1 hour	5	Rat	3	" "
13	" "	0.2 N HCl overnight, then neutralized	10	Rat	2	" "
14	" "	0.2 N NaOH overnight, then neutralized	10	Rat	2	" "
15	" "	3% H_2O_2 overnight	10	Rat	3	" "
16	" "	Dry heat at 140° C. for 30 minutes	10	Rat	4	All died in 10 days.
17	" "	Excess H_2O at 80° C. for 30 minutes	10	Rat	4	2 died in 8 days others in 11 days
18	" "	Excess H_2O at 80° C. for 1 hour	10	Rat	4	All survived.
19	" "	20 lbs. steam for 10 minutes	10	Chick	10	" "
20	" "	20 lbs. steam for 30 minutes	10	Chick	10	" "
21	" "	20 lbs. steam for 1 hr	10	Chick	10	" "

PART II

BIOLOGICAL VALUE OF CASTOR BEAN PROTEIN

Since nearly complete detoxication of castor pomace had been accomplished by autoclaving, determination of biological value of the proteins of the seed became desirable. Biological tests were therefore made using both the rat and the chick as experimental animals. Since the biological value of a protein is almost wholly dependent upon its amino-acid composition, determination of the essential-amino-acid content of the total protein of the bean was also needed.

Biological Value of Castor Seed Protein by Rat Growth Method

Of the several methods of determining the biological value of a protein, those based upon the ability of the protein to support growth of young animals are most widely used. In these studies, the ability of graded amounts of castor pomace to support growth of rats was determined when this material was substituted on the basis of nitrogen equivalence for casein, a protein of high quality.

Experiments 1 and 2. In these experiments two different commercial castor pomace preparations were compared for growth-promoting ability. One (Experiment 1) was an expeller-type meal (Baker Castor Oil Company) containing 37.8 per cent protein; this pomace contained the entire cortex of the seed and, hence, was high in crude fiber. The other (Experiment 2) was a partially decorticated, solvent-extracted meal (Sherwin-Williams Company) with a protein content of 57.5 per cent. The approximate analysis of these two preparations is given

in Table 5.

Table 5. Approximate Analysis of Castor Pomace

	Decorticated (Solvent-extracted)	Undecorticated (Expeller-type)
Moisture	6.18 %	4.52 %
Protein	57.50	37.80
Fat	2.95	1.03
N.F.E.	13.12	19.12
Crude Fiber	9.68	29.23
Ash	10.57	8.30

Since tests had shown the expeller-process meal to be relatively non-toxic, it was used without further treatment to replace casein at the 25 per cent level in a purified ration. Since castor pomace was substituted on the basis of nitrogen equivalence, those diets low in castor pomace were higher in energy content owing to the fiber added by the pomace. The composition of the various rations is shown in Table 6 together with the gain made by the various lots during a six-weeks period.

Table 6. Composition of Diets Used and Results Obtained in Experiment 1.[1]

	Lot I	Lot II	Lot III	Lot IV	Lot V
Castor Pomace	0.00%	16.50%	33.10%	49.60%	66.10%
Casein	26.30	19.70	13.10	6.60	0.00
Cornstarch	64.70	54.80	44.80	34.80	24.90
Fat	5.00	5.00	5.00	5.00	5.00
Salts[2]	4.00	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00	100.00
Per cent Protein supplied by Castor Pomace	0.00%	6.25%	12.50%	18.75%	25.00%

Total gain in weight for Six Weeks	177 gm.	148 gm.	136 gm.	110 gm.	62 gm.

[1] Each diet was supplemented with the following vitamins per kilogram of feed: thiamine chloride, 3 mg.; riboflavin, 6 mg.; nicotinic acid, 20 mg.; pyridoxine hydrochloride, 3 mg.; calcium pantothenate, 30 mg.; choline chloride, 2 g.; para-aminobenzoic acid, 50 mg.; inositol, 1 g.; folic acid, 2.5 mg. In addition, each rat received weekly two drops of cod-liver oil containing 1 mg. of alpha-tocopherol.

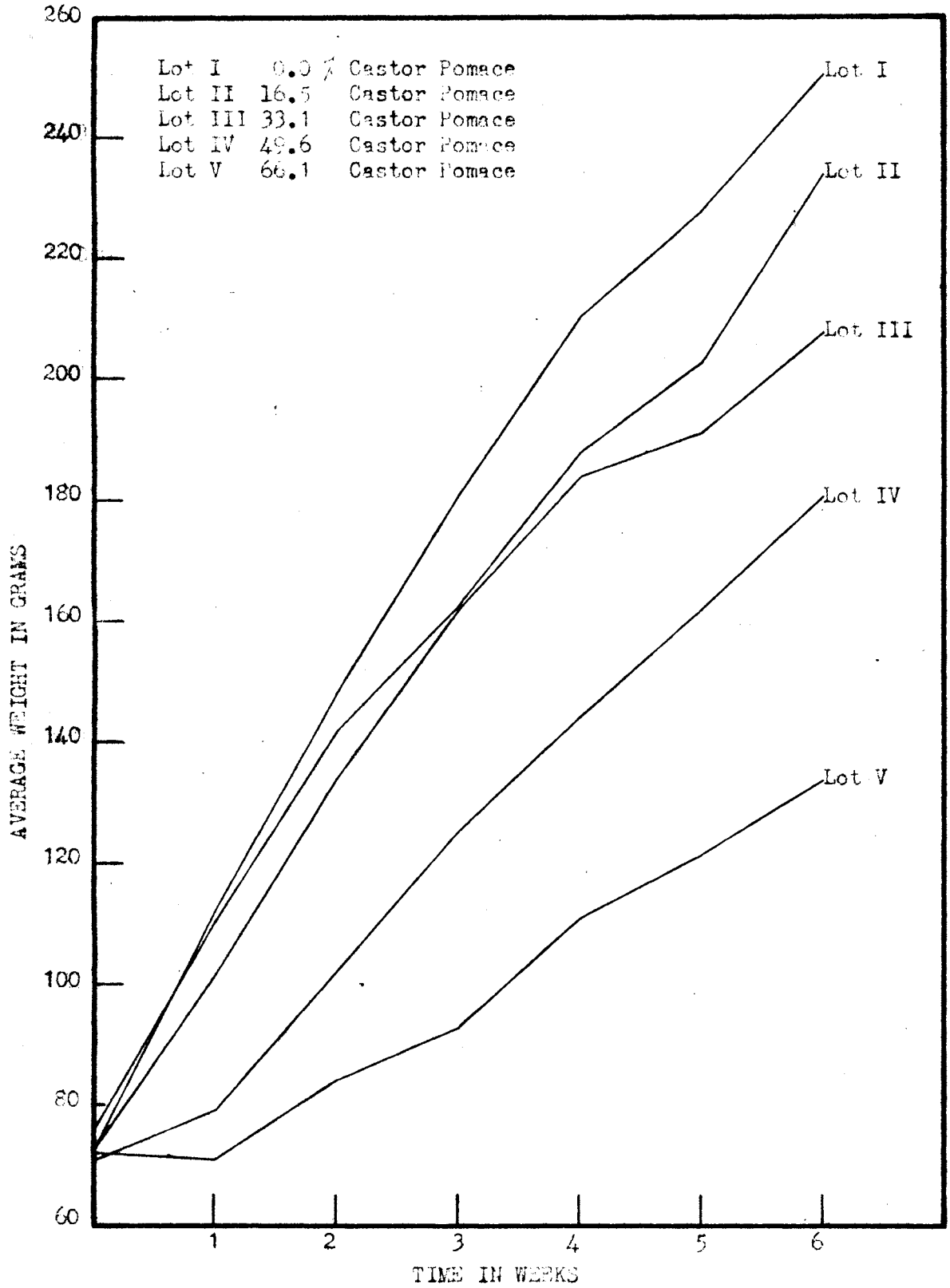
[2] Salt mixture was compounded according to Hegsted et al. (34).

Weanling rats of the Sprague-Dawley strain were equally allotted according to weight and sex and placed in individual cages, five rats per lot. Feed and water were supplied ad libitum. The rats were weighed weekly under as nearly the same conditions as possible.

The results of Experiment 1 are presented graphically in Figure 1. Examination of the data indicates that the protein in casein was definitely superior in growth-promoting value to the protein in castor seeds. The rats receiving casein as the sole protein source grew at a normal rate (30 grams per week). As castor pomace replaced casein, they grew at a slower rate. On the ration containing castor pomace as the sole source of protein, the rats grew at a comparatively slow rate (10 grams per week).

The growth experiment was repeated using a solvent-extraction product (Experiment 2). Since this product had been shown to be highly toxic, it was detoxified by autoclaving for twenty minutes at 125° C (20 pounds pressure) as previously described. Weanling male rats of the Sprague-Dawley strain were divided into four lots of eight rats each and treated as in Experiment 1. The composition of the rations is shown in Table 7. Each diet

Figure I
EFFECT ON GROWTH OF REPLACING CASEIN
WITH CASTOR POMACE IN THE DIET OF RATS



contained a 20 per cent level of protein and was fed for five weeks.

Table 7. Composition of Diets Used and Results Obtained in Experiment 2.

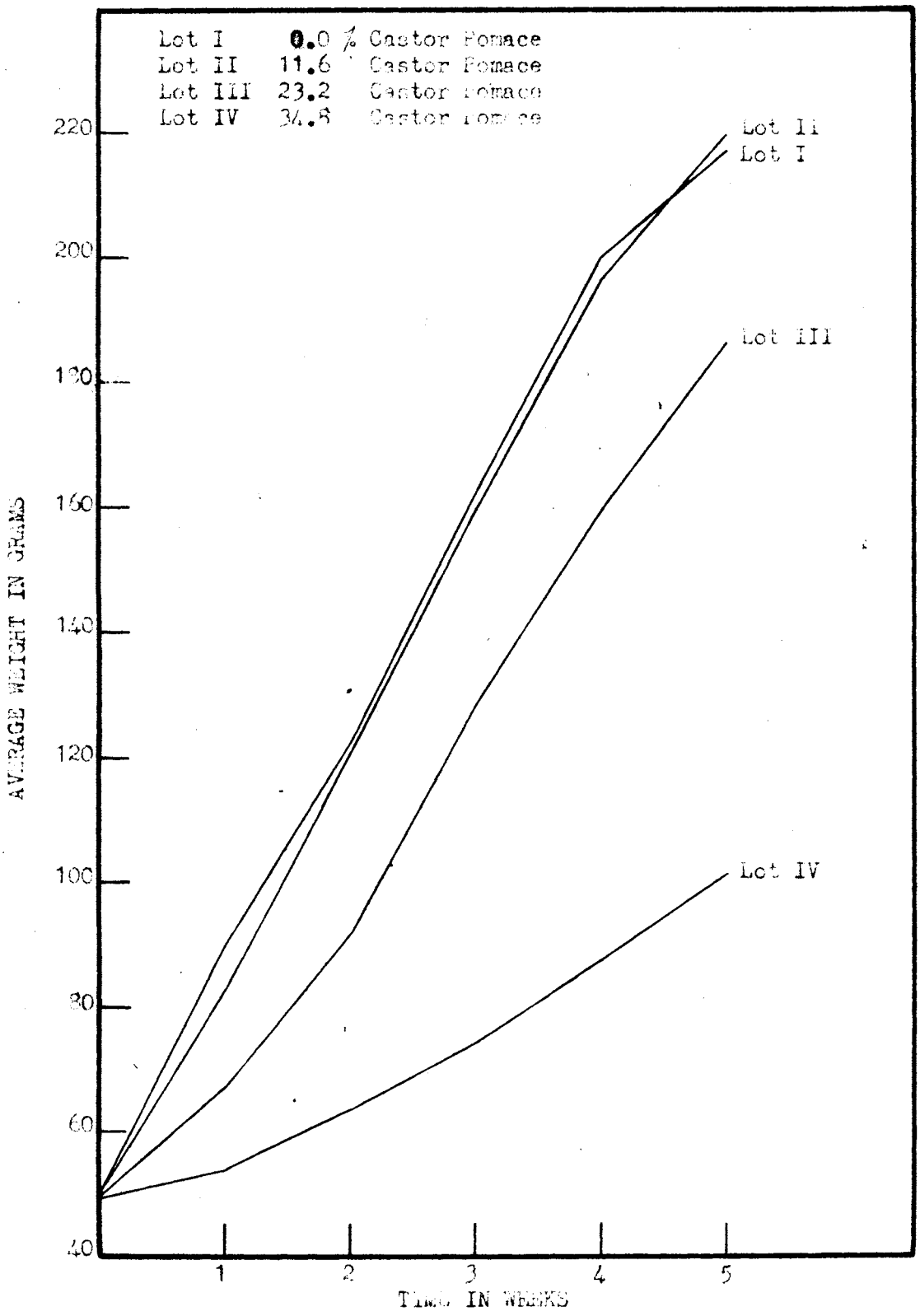
	Lot I	Lot II	Lot III	Lot IV
Castor Pomace	0.00%	11.60%	23.20%	34.80%
Casein	20.00	13.33	6.67	0.00
Cornstarch	71.00	66.07	61.13	52.20
Fat	5.00	5.00	5.00	5.00
Salts	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00
Per cent Protein Supplied by Castor Pomace	0.00%	6.67%	13.33%	20.00%
Total Gain in Weight for Five-Week Period	167 g.	169 g.	137 g.	5? g.

The effect on growth of replacing casein with castor pomace in this experiment is shown graphically in Figure 2. Exam- of the data shows that in this experiment, substitution of one-third of the casein by castor pomace did not materially reduce the rate of growth. Reduction of 28 per cent at two-thirds substitution, and 69 per cent at complete replacement was found. The differences in response in this experiment may be due to the lower fiber content of these diets as compared with those in Experiment 1.

Experiment 3. In the growth experiments in which food was available ad libitum, the rats with access to rations containing only casein as protein consumed more ration during the growth period than those on diets having the casein partially or completely replaced by castor pomace. Consequently, paired feeding

Figure II

EFFECT ON GROWTH OF REPLACING CASEIN WITH CASTOR POMACE IN THE DIET OF RATS



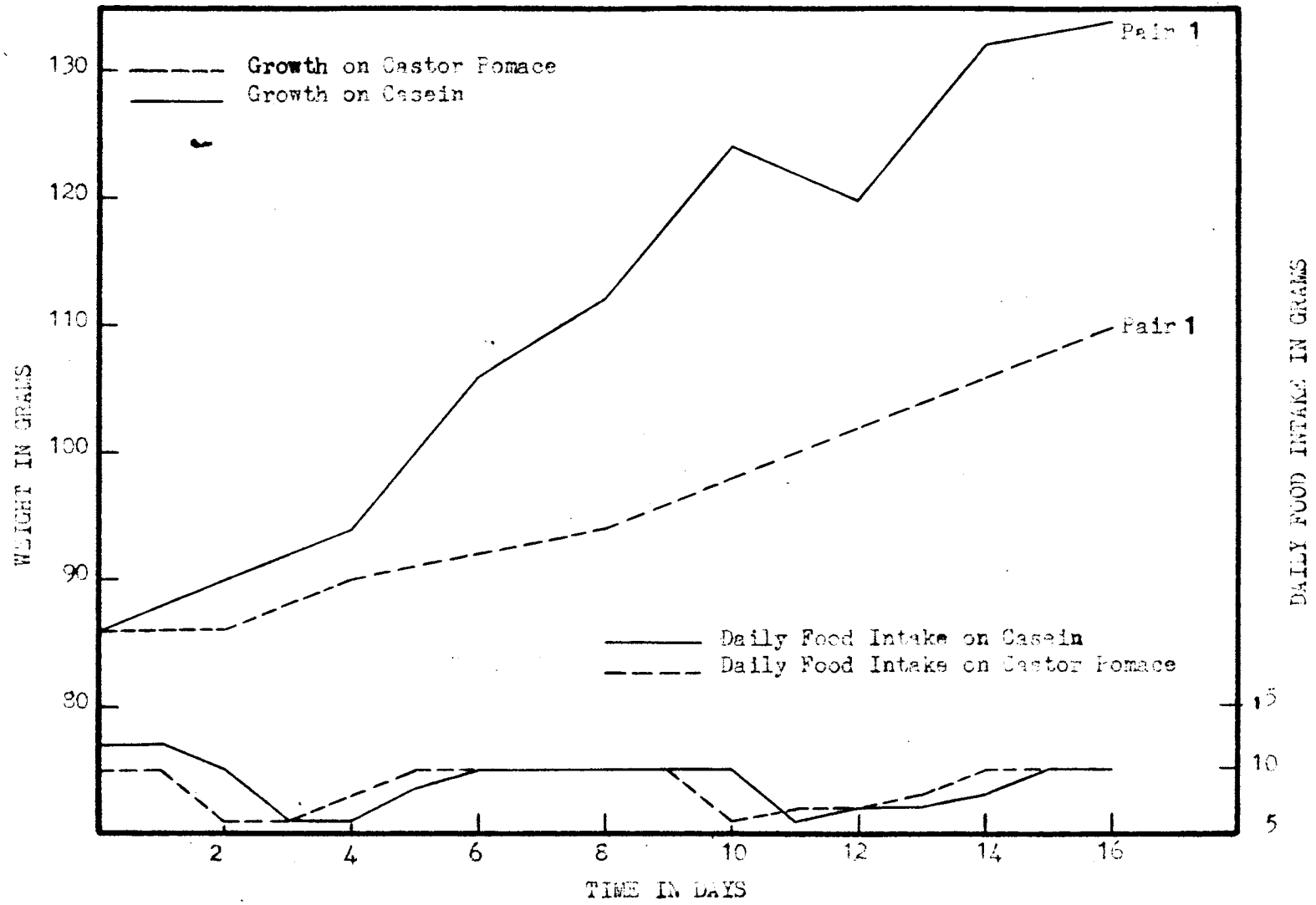
experiments were carried out in order to compare the growth rates when equal quantities of ration were consumed. These were carried out as follows: four pairs of male rats were used in each experiment. Litter mates of initial weight within 2 grams were used; initial weights varied between 86 and 108 grams. One animal received a purified diet containing casein as the protein source (Lot I, Table 6). The other was fed an identical ration except that partially decorticated castor pomace (57.5% crude protein) had replaced, on the basis of nitrogen equivalence, the casein and a portion of the cornstarch (Lot IV, Table 6). The food consumption of the casein-fed animals was restricted to that of the pomace-fed. The animals were weighed every other day. The feed consumed and total growth for the four pairs are given in Table 8. The data for a representative pair are presented graphically in Figure 3.

Table 8. Results Obtained in Paired Feeding Trials Composing Casein and Castor Pomace Protein.

	Diet I (Casein-fed)		Diet IV (Pomace-fed)	
	Food intake g.	Gain in 2 days g.	Food intake g.	Gain in 2 days g.
Days				
1	13.5		8.0	
2	5.0	1.0	8.0	-2.0
3	6.0		6.5	
4	5.5	6.5	8.0	5.5
5	7.0		10.5	
6	10.5	9.0	10.5	3.0
7	10.5		11.5	
8	11.5	11.5	9.0	3.5
9	9.0		10.0	
10	10.5	8.0	7.5	3.0
11	7.5		7.5	
12	7.5	-5.0	7.5	3.5
13	7.5		11.0	
14	11.0	15.5	8.5	6.0
15	8.5		9.5	
16	9.5	2.0	9.5	4.0
	141.5 g.	48.5 g.	143.0 g.	26.5 g.

Figure III

COMPARISON BY THE PAIRED-FEEDING TECHNIQUE OF GROWTH OF RATS ON CASEIN AND CASTOR POMACE AS A PROTEIN SOURCE



The average total food intake of rats on Diet I and IV was 141.5 and 143.0 grams respectively. The average gain of rats on Diet I was about twice that of Diet IV, being 48.5 and 26.5 grams.

The two-week assay period is relatively short for an animal assay. This assay is useful in a preliminary rating of proteins. It is interesting to note that whereas casein-fed rats on the same ration in Experiment 1 and 2 with ad libitum feeding tripled their weight when compared to castor-pomace-fed rats, they only doubled in weight in this paired-feeding experiment. Thus it would appear that the casein ration was more palatable than castor pomace-containing ration. The higher energy content of the casein ration might also partially account for these differences.

Biological Value of Castor Seed Protein by Chick-Growth Method

In these studies the ability of castor pomace when substituted on a nitrogen equivalence basis for casein to support growth of chicks was determined.

A partially decorticated, solvent-extracted meal with a protein content of 57.5 per cent was detoxified by autoclaving for twenty minutes at 125° C (20 pounds pressure). The treated pomace was fed to chicks at various levels as shown in Table 9.

Table 9. Composition of Diets Fed to Chicks[1]

	Lot I	Lot II	Lot III	Lot IV
Castor pomace	0.00	8.70	17.40	34.80
Casein	20.00	15.00	10.00	0.00
Cornstarch	63.50	59.80	56.10	48.70
Gelatin	5.00	5.00	5.00	5.00
Cottonseed oil	5.00	5.00	5.00	5.00
Salts[2]	5.00	5.00	5.00	5.00
Fish solubles	1.50	1.50	1.50	1.50
Total	100.00	100.00	100.00	100.00
Per cent of protein supplied by castor pomace	0.00	5.00	10.00	20.00
Total gain in weight for three weeks	79 gm.	103 gm.	75 gm.	45 gm.

[1] The ration was supplemented with the following vitamins per pound of feed: vitamin A and D feed oil, 1.15 g.; thiamine, 0.9 mg.; inositol, 10 mg.; para-aminobenzoic acid, 5.0 mg.; pantothenic acid, 5.0 mg.; pyridoxine, 1.6 mg.; choline, 700 mg.; riboflavin, 30.0 mg.; nicotinic acid, 8.0 mg.; and folic acid 0.45 mg.

[2] Salts were compounded according to Hegsted, et al. (35).

Day-old chicks of crossed New Hampshire and Brown Leghorn strains selected at random without regard to sex were used. They were kept in thermostatically-controlled electrically-heated battery brooders. The chicks were wing-banded and weighed at weekly intervals. Food and water were provided ad libitum. Average weekly weights for a three-week period are presented graphically in Figure IV and the total gain in weight for the three-week period presented in Table 9. Rather heavy losses from Newcastle virus occurred during the fourth week. Although the pattern of results did not change, it was

Figure IV

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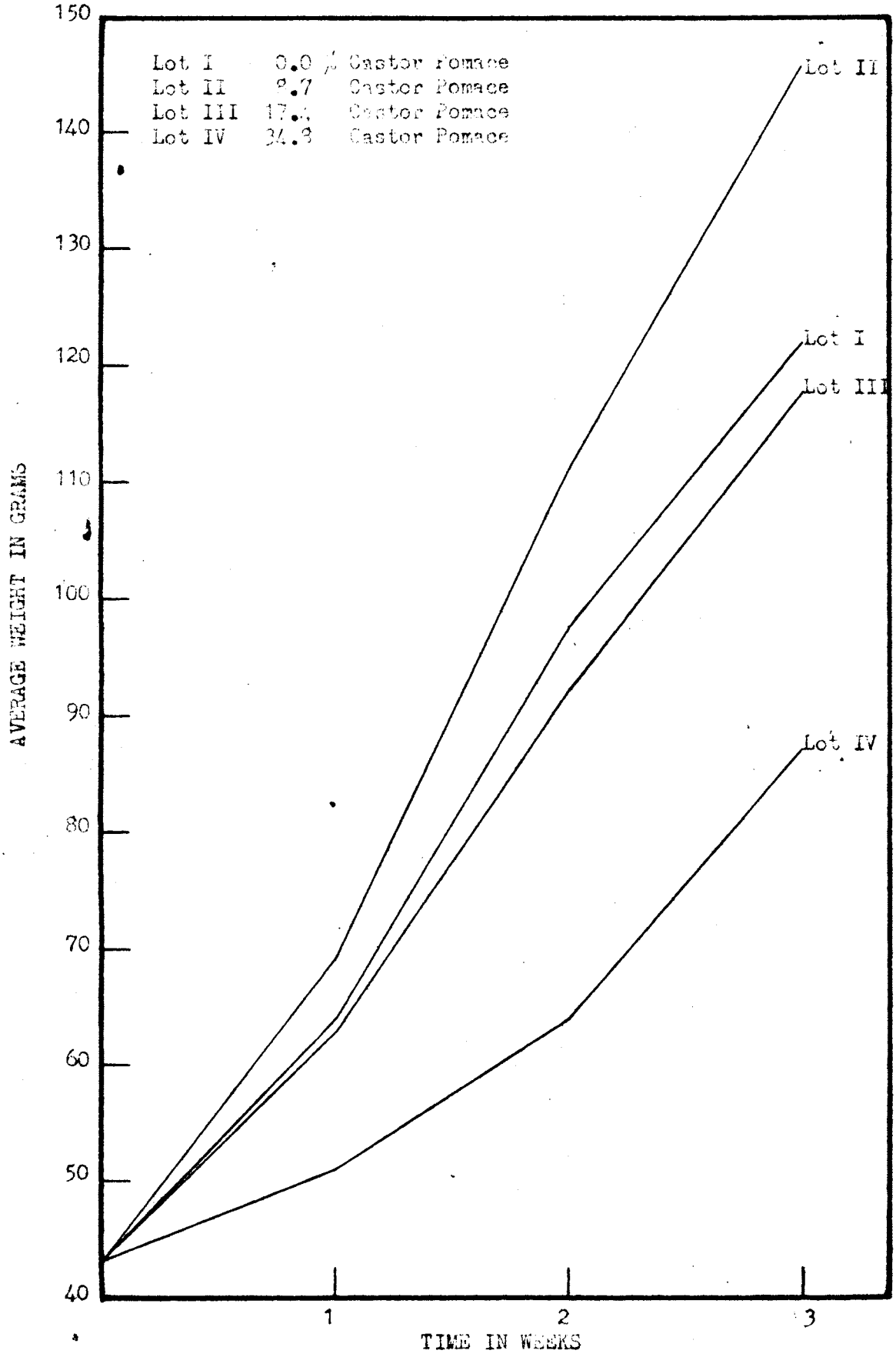

considered expedient to discard the growth data for the fourth week.

It will be seen from the data that replacing 25 per cent of the casein by castor pomace increased the rate of growth; there was a gain of 79 g. for the three-week period with casein and 103 g. with a 75 per cent casein-25 per cent castor pomace ration. Essentially the same rate of growth occurred on a ration containing casein as the principal protein source and a ration containing 50 per cent casein and 50 per cent castor pomace. Additional pomace in the ration in place of casein resulted in diminished rates of gain. Compared with the rat, the chick appears to be better able to utilize castor pomace. This may be in part due to the ability of the chick to better utilize a ration containing greater amounts of crude fiber. As has already been shown, this animal is also more resistant to the toxic principle of castor pomace. Species differences in amino-acid requirements may also play some part in determining the apparently more efficient utilization of castor pomace by the chick. Another factor, unrelated to nutritive value of protein, is the palatability of the ration.

THE AMINO ACID COMPOSITION OF CASTOR POMACE

The results of the paired-feeding and ad libitum-feeding growth experiments indicated that the protein of castor pomace was incomplete. It was deemed desirable, therefore, to determine the composition of the castor bean protein in terms of the essential amino acids.

Chemical methods for the quantitative determination of amino acids are time-consuming and subject to many procedural errors. During recent years the use of micro-organisms to determine various materials, including amino acids, required for the growth of bacteria has been widely accepted. The microbiological determination of amino acids gives data comparing favorably with the best chemical methods. This technique was, therefore, employed. The specific procedures used were those of Henderson and Snell (35) modified to be more applicable to our conditions.

A representative sample of solvent-extracted decorticated castor pomace was hydrolyzed by refluxing a one-gram sample with 150 ml of 6 N HCl for 24 hours. Most of the hydrochloric acid was removed by distillation at reduced pressure. The hydrolysate was then neutralized with 5 N NaOH, diluted to one liter and filtered to remove humin. All amino acids except tryptophane were determined on this hydrolysate. For this latter amino acid, alkaline hydrolysis was carried out as follows: a 0.5 gm. sample was suspended in 20 ml of 5 N NaOH and autoclaved at 15 pounds pressure for eight hours. The hydrolysate was then neutralized with 6 N HCl, diluted to one liter, and filtered to remove humin. This neutral hydrolysate was used for the assay of tryptophane.

The organisms used were as follows: Lactobacillus arabinosus, for glutamic acid, leucine, phenylalanine, valine and tryptophane; Streptococcus faecalis, for arginine, histidine, methionine, and threonine; and Leuconostoc mesenteroides P-60, for aspartic acid, lysine, proline, tyrosine, and isoleucine.

Cultures of the organisms were maintained as stabs in a complete agar medium. The same medium with agar omitted was employed for culturing the inocula. Inocula were prepared from the agar stab stock cultures and grown overnight at 37° C. The cells were centrifuged and resuspended in a volume of sterile 0.9 per cent sodium chloride solution five times greater in volume than that of the medium in which they were grown. One drop of suspension was used for each assay tube.

The composition of the basal media was essentially the same as that of Henderson and Snell (35) with the exception of the buffer salts. For the Lactobacillus arabinosus and Leuconostoc mesenteroides media, 10.0 grams of K_2HPO_4 was decreased to 1.0 gram, 2.0 grams of sodium acetate (anhydrous) was increased to 40.0 grams, 40.0 grams of sodium citrate was omitted, and 1.0 gram of KH_2PO_4 was added per liter of medium. For Streptococcus faecalis, 2.0 grams of sodium acetate (anhydrous) was increased to 6.0 grams, 10.0 grams of K_2HPO_4 was decreased to 1.0 gram, 40.0 grams of sodium citrate was omitted, and 1.0 gram of KH_2PO_4 and 20.0 grams of succinic acid added per liter. These changes were made in order to permit titrametric measurements of acid production by visualindicator methods.

For each assay the appropriate medium deficient in a single amino acid was prepared at twice its final concentration.

Quantities of pure amino acids were added to a series of uniform test tubes in amounts suitable for producing gradations between minimal and maximal growth. Contents of all tubes were diluted with water to 5 ml and an equal volume of medium was added. They were sterilized and inoculated with a previously washed suspension of cells of the proper organism. The tubes were then incubated in an air incubator at 37° C. for 60 to 72 hours. The acid production of the standards and test samples was then determined by titration of the acid produced with 0.2 N NaOH using bromthymol-blue indicator. The average acid production for each known level of amino acid was then plotted, giving a standard curve. The amount of amino acid present in the test sample at the various levels was then determined by inspection from this standard curve. The values obtained at the several levels were inspected to determine whether "drift" had occurred. If the values at widely differing concentrations were closely similar, the average was taken. All determinations were repeated until satisfactory duplicate values were obtained.

The results of the microbiological assays can be found in Table 10.

Table 10. Amino Acids Found in Casein and Castor Pomace in Per Cent

Results calculated to 16 per cent N on an ash-and moisture-free basis.

Amino Acid	Casein[1]	Castor Pomace
Arginine	4.3	11.0
Aspartic acid	6.1	4.6
Glutamic acid	23.3	18.0
Histidine	2.1	2.5
Isoleucine	6.3	5.3
Leucine	9.7	7.2
Lysine	7.6	3.1
Methionine	3.4	1.5
Phenylalanine	5.0	4.2
Proline	8.0	3.9
Threonine	3.8	3.6
Tyrosine	6.7	3.2
Valine	6.5	6.6
Tryptophane	1.2	0.62
Total	94.0	75.32

[1] Values in literature.

Examination of the amino-acid composition of castor bean protein shows it to be deficient in several of the essential amino acids. For comparison, the accepted values for the amino-acid composition of casein, a relatively high quality protein, are also presented. It can be readily seen that the protein of the pomace is particularly deficient in tryptophane and low in methionine. When it is recalled that casein is relatively low in the sulfur-containing amino acids, the castor protein

appears even more defective with respect to methionine.

Since biological tests had shown that castor protein was inadequate to provide for good growth in the rat, it was of interest to calculate the percentage of amino acids required by the rat as determined by Rose and co-workers (36) that would be provided by castor protein at the 25 per cent level. These computations are presented in Table 11. They likewise show the protein to be seriously deficient in tryptophane (42.5 per cent normal requirement) and methionine (50 per cent normal requirement). Lysine is also significantly below the required level for maximum growth. Further studies to determine the effect of supplementing castor protein with tryptophane and methionine, separately and together, appear in order.

Table 11. Comparison of Amino Acids in Castor Pomace with Levels Required for Maximum Growth

Results calculated on a 25 per cent protein level.

Amino Acid	Optimum Level	Castor Pomace
Arginine	0.20%	3.12%
Aspartic acid	0.20	1.20
Glutamic acid	2.00	3.78
Histidine	0.70	0.66
Isoleucine	0.80	1.38
Leucine	1.20	1.92
Lysine	1.20	0.83
Methionine	0.80	0.40
Phenylalanine	1.20	1.10
Proline	0.20	1.01
Threonine	0.70	0.94
Tyrosine	0.60	0.82
Valine	1.00	1.76
Tryptophane	0.40	0.17

DISCUSSION

It has been shown that the oral administration of solvent-extracted castor bean meals results in severe symptoms of toxicity and death in rats and chicks. A sample of expeller-process meal did not have a high order of toxicity. It may be that the higher temperatures employed in expeller-type extraction cause a denaturation of the toxic albumin. The commercial trend toward solvent extraction, however, will produce increasing amounts of a highly toxic protein concentrate. The partial decortication of the seed preceding extraction, which is at present being tested on a pilot-plant scale, will provide a meal of very high protein content. A suitable method of detoxication would provide a better market for this by-product, thus improving the economic feasibility of producing castor beans in the United States.

These studies clearly demonstrate that treatment of the meal by moist heat for periods of fifteen minutes cause satisfactory reduction in toxicity. Autoclaving for this length of time at 125° C. produced a meal of high palatability to rats and chicks which was non-toxic when fed at the forty per cent level. The practicability of this type of processing on a plant scale is beyond the scope of this study. It seems likely, however, that some modification of the "toasting" process used to improve the nutritive quality of soybean meal might be employed with castor pomace. Further investigations along these lines will be undertaken.

In common with the protein of most seeds, castor bean protein

is not of high quality. Both biological tests with rats and chicks and analysis of amino-acid composition show the protein to be of low biological value. Favorable rates of gain were obtained when a small portion of the total protein requirement was supplied by castor pomace; as the levels were increased, growth became progressively poorer. Under the conditions used, castor pomace gave somewhat better growth response with chicks than with rats. Whether this is due to an improved palatability of the ration due to the incorporation of the castor pomace, to the higher level of total protein in the chick ration, or to a difference in the amino acid requirements of the two animals requires further study.

Analysis of amino-acid composition of the whole protein of the seed showed that the tryptophane and methionine contents were lower than those required for optimum growth in the rat. Lysine was also present at a marginal level. While this is of importance in the utilization of this meal as the sole source of protein for a non-ruminant, it does not impair its value as a protein supplement in a mixed ration. Moreover, protein quality appears to be of less importance in feeds for ruminants because of the reconstitution of the amino acids by the rumen micro-flora. The detoxified meal should prove an acceptable high-protein feed for cattle and sheep. Further investigation in this regard will be necessary, however, in order to determine the nutritive value of the detoxified meal for these animals. Such studies are dependent on the development of a method of detoxication on at least a pilot-plant scale. It is hoped that arrangements can be made to continue these studies through

co-operation with the Northern Regional Laboratory of the United States Department of Agriculture.

In conclusion, it should be noted that the production of a high-protein supplement from castor bean meal has been achieved on a laboratory scale by methods which appear to offer commercial possibilities. The extension of these findings should provide a more economical use of castor pomace with resulting benefit to the farmer-producer and a new source of high-protein feed for the farmer-consumer.

SUMMARY

A toxicological study of castor pomace showed that it contained a highly toxic material, as previously reported. This toxicity was due to the presence in the untreated pomace of an albuminous protein, ricin. The symptoms of toxicity and the gross and microscopic pathological lesions were similar to those observed by previous investigators. In the rat the oral administration of the toxic pomace produced inanition, emaciation, nervous involvement, convulsions, and death by respiratory failure. Post-mortem examination showed a general inflammation and edema of the gastro-intestinal tract, with occasional punctiform hemorrhage. The contents of the small intestine were usually liquid and blood was sometimes found. A serous fluid was occasionally noted in the body cavity. The liver was hyperemic and the kidneys were congested. Ureates were noted in the cortex. Microscopic sections of small intestine and liver confirmed these gross pathological findings. Similar lesions were also found in the chick, although it appears that this animal is somewhat more resistant to the toxic protein.

Various treatments were investigated to determine their effect on the toxicity of the pomace. Moist heat (autoclaving for a minimum of 15 minutes at 125° C. and 20 pounds pressure) was effective in destroying the toxicity of the meal, both in the presence and absence of added water. Treating with either 0.2 N HCl or 0.2 N NaOH also produced nearly complete destruction of the toxin. Mild oxidation with three per cent hydrogen peroxide was likewise effective. Heating a slurry of pomace in

excess water at 60° C. for one-half hour and dry heating at 140° C. were ineffective in removing the toxicity.

The biological value of castor pomace protein was investigated with rats and chicks. The protein in castor seeds does not compare favorably in growth-promoting value for the rat with casein. Castor bean protein appears to be of higher biological value for the chick than for the rat.

The principal amino acids were determined in castor bean protein using microbiological assays. Methionine and particularly tryptophane were found to be low. This accounts for the poor quality of the protein found by biological tests.

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