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## EVALUATION OF ENZYME-MODIFIED, SOLVENT-EXTRACTED CRAMBE SEED MEAL BY CHEMICAL ANALYSES AND RAT FEEDING<sup>1</sup>

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### ABSTRACT

A method is presented for the removal of thioglucosides (as their hydrolysis products) from crambe seed meal. Defatted meal is wetted to allow native myrosinase to hydrolyze the thioglucosides. Solvent extraction of the wet meal leads to a product free of thioglucosides, the organic aglycones (R)-goitrin and (+)-1-cyano-2-hydroxy-3-butene (and other C≡N compounds), and essentially free of the bitter substance sinapine. Similar meals may be prepared directly from autolyzed full-fat flakes by employing mixed solvents which remove the above products as well as the oil. The extracted meals range from 45 to 56% crude protein and contain approximately 6.0 g. lysine per 16 g. N, 2.0 g. methionine, and 2.2 g. cystine. Processed meal prepared from defatted crambe seed was fed to rats at 28% of the diet. The meal supports growth at 88% of the controls during a 13-week period. Organ weights of the test animals were not significantly different from those of the control animals. Histological examination of the organs revealed no abnormalities. Feeding autolyzed, but unextracted, meals at the 28% level caused death of all animals within 2 weeks.

Defatted seed meal of *Crambe abyssinica* Hochst ex R. E. Fries is rich in protein and contains relatively large amounts of lysine, methionine, and cystine (1). These constituents make it appear to be a favorable source of protein for livestock feeding. In common with other crucifers, crambe seed contains thioglucosides, which are hydrolyzed to goitrogenic or extremely pungent products; sinapine, which is somewhat bitter (2); and probably other undesirable substances which are as yet unknown. These materials should be removed or neutralized to make an acceptable feed, as indicated by preliminary trials of a meal freed of thioglucosides (1). That method removed intact thioglucosides by extraction of defatted meal with 75% acetone-

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25% water, but it resulted in a loss of more than 20% of the meal solids (1).

Another method of removing thioglucosides from crambe seed meal has been devised. This method depends upon enzymatic hydrolysis of the thioglucosides by myrosinase present in the seed. Hydrolysis of the major thioglucoside *epi*-progoitrin (3) produces glucose, inorganic sulfate, and either (R)-goitrin or a group of cyano compounds including (+)-1-cyano-2-hydroxy-3-butene (4,5). Either (R)-goitrin or the nitriles, along with sinapine and possibly unknown substances, can be removed from the meal by extraction with a mixed solvent containing a large amount of acetone and from 2 to 12% water. Such a procedure effectively detoxifies the meal without excessive loss of protein.

The processed meal is palatable to rats and supports normal growth for 13 weeks when fed at 28% of the diet. By contrast, hexane-defatted crambe meal is lethal when fed at 25% of the diet (1). Meals containing hydrolysis products of thioglucosides are similar to the defatted meal and are lethal at 28% of the diet.

### Materials and Methods

*Assay Methods.* Total thioglucosides are determined by titration of inorganic sulfate following enzymatic cleavage of the thioglucosides (1). A qualitative paper-chromatography test for the presence of thioglucosides is also employed that uses n-butanol-ethanol-water, 4:1:4, as a developing solvent, and alkaline silver nitrate as a color reagent. A neutralized hot-water extract of the meal is extracted four times with ethyl ether. (R)-Goitrin is measured directly in ether at  $\lambda = 248 \text{ m}\mu$  or transferred to absolute ethanol and measured at  $\lambda = 244 \text{ m}\mu$ . The molar absorptivity,  $\epsilon$ , of purified goitrin is 4.25 in either solvent and compares well with the value  $\epsilon = 4.23$  reported by Kruela and Kie-svaara (6) for (S)-goitrin. Because extraneous substances still are present in the ether, appropriate correction for background absorbance must be made.

Cyano compounds are recovered from the ethereal solution containing (R)-goitrin by evaporation of the solvent. The material is dissolved in chloroform and measured in the IR at  $4.4 \mu$  (4). This procedure determines the  $\text{C}\equiv\text{N}$  grouping and probably includes unknown component(s) as well as 1-cyano-2-hydroxy-3-butene.

Sinapine was measured by the method of Tzagalof as modified by Austin and Wolff (2).

Amino acids of a hydrochloric acid hydrolysate were measured in a Beckman-Spinco amino acid analyzer by the method of Spackman *et al.* (7).

Total sulfur was determined by the oxygen flask method (8), and nitrogen by micro-Kjeldahl.

*Preparation of Flaked Seed.* Crambe seed was separated from the loosely adherent pericarp by passage through a burr mill, followed by sieving. The cleaned seed was flaked by passage between smooth iron rolls set at 0.003-in. clearance.

*Meal A, Hydrolyzed and Wet-Acetone-Extracted.* Crambe flakes were moistened with water (0.5 ml./g.). The resultant stiff paste was incubated 3 hr. at 25°–27°C. to allow completion of thioglucoside hydrolysis. Shorter incubation times are adequate at higher moisture levels. The paste was then stirred 0.5 hr. with sufficient acetone to make a mixed solvent of 98% acetone-2% water (v./v.) including the water present in the paste. The liquid was separated by filtration. A second extraction with acetone (14 ml./g. flakes) served to dehydrate the residual meal, which was then air-dried and ground to pass a 30-mesh screen. A third extraction with acetone followed by air-drying of the meal completed the process. A crude crambe oil can be recovered from the combined extracts by evaporation of the solvent.

*Meal B, Defatted and Hydrolyzed.* Crambe flakes were defatted by repeated extraction with pentane-hexane, air-dried, and ground to pass a 40-mesh screen. The powder was moistened with water (1.2 ml./g. powder) to form a paste which was incubated 1.5 hr. at 25°–27°C. The product was dried in a stream of warm air and reground to pass a 40-mesh screen.

*Meal C, Defatted, Hydrolyzed, and Dry-Acetone-Extracted.* Meal prepared according to the instructions for meal B was extracted with anhydrous acetone (8 ml./g. meal), then twice more with acetone (4 ml./g.), allowed to air-dry, and milled to pass a 60-mesh screen.

*Meal D, Defatted, Hydrolyzed, and Wet-Acetone-Extracted.* Crambe flakes were defatted by repeated extraction with pentane-hexane, air-dried, and ground to a powder. The meal was moistened with 1.2 ml. water per g. meal, mixed, and incubated at room temperature 1.5 hr. The wet paste was extracted with acetone (8 ml./g. meal), then twice with acetone (4 ml./g. meal); the slurry was stirred 1 hr. for each extraction. The meal was air-dried and reground as above.

*Meal E, Hydrolyzed and Extracted with Ternary Solvent.* Flaked crambe seed was moistened with water (0.5 ml./g. flakes), mixed, and allowed to stand 3 hr. at 25°–27°C. The wet paste was stirred 1 hr. with enough hexane-acetone to produce a ternary mixture (9) calculated to contain 53% acetone, 44% hexane, and 3% water (v./v.). The liquid was separated by filtration. The second extraction used the same ratio of acetone and hexane (53:44) to dehydrate the meal. After air-

drying, the meal was ground to pass a 60-mesh screen. A final extraction at the rate of 9 ml./g. flakes with acetone-hexane-water (54:45.5:0.5) was followed by air-drying of the meal.

*Feeding Experiments.* Weanling rats of the Sprague-Dawley strain weighing 40–50 g. each were fed a diet containing 28% crambe meal A and 72% basal ration containing 73% yellow corn meal, 10% crude casein, 10% linseed cake meal, 3% USP cod liver oil, 2% dehydrated alfalfa meal, 1.5% bone ash, and 0.5% NaCl (10). The control group was pair-fed the basal diet. At the end of 49 days the animals were sacrificed.

Meals B, C, D, and E were fed *ad libitum* to weanling rats. The above basal ration comprised 72% of the total diet and the test meal 28%. Testing was terminated at 7 weeks (meal E), 13 weeks (meal D), or at the death of the animals. Histological examination was performed on tissues fixed and stained by standard procedures.

## Results and Discussion

*Composition of Meals.* The proportion of (R)-goitrin and cyano compounds released by enzymatic action varies with the prior history of the seed and with processing conditions (5). Analytical data on small lots of meals indicate that such variation is of little consequence to the present studies, because both organic aglycones are extracted by wet acetone. Under the conditions used here, little or no (R)-goitrin was formed; the meals yielded predominantly cyano compounds from *epi*-progoitrin.

Two major variations of the processing procedures were employed: 1) hydrolytic cleavage of the thioglucosides in flaked seed, followed by extraction of the thioglucoside products simultaneously with the oil (meals A and E); and 2) extraction of the seed oil, followed by hydrolytic cleavage of the thioglucosides and their subsequent extraction from the fat-free meal. Analytical data on all meals are given in Table I.

The thioglucoside content of these meals ranged from 8 to 10%. Of this, the organic aglycones (goitrin or cyano compounds) would be expected to be removed and would account for 2.8–3.5% of the meal. Hence, the estimated weight recovery indicates substantial retention of protein. The sulfur content of the meal is largely accounted for by sulfur of amino acids (about 0.57% of the meal) plus the sulfate derived from thioglucosides (0.81%). Myrosinase in the meal is still active in cleaving added thioglucosides. Amino acid data are included to indicate the potential nutritional quality of the protein.

Table I shows that meal A is free from thioglucosides and (R)-

TABLE I  
COMPOSITION OF MEALS PREPARED FROM CRAMBE SEED

	CONTROL <sup>a</sup>	HYDROLYZED MEALS				
		A	B	C	D	E
Weight recovery, % <sup>b</sup>	100	88	99	97	91	90
Sulfur, % <sup>c</sup>	2.0	1.86	1.84	1.72	1.43	1.76
Ash, % <sup>c</sup>	8.0	..	8.0	8.2	8.5	8.0
Thioglucosides, % <sup>c,d</sup>	9.5	0	0	0	0	0
(R)-goitrin, %	0	0	0	0	0	0
Cyano compounds, % <sup>e</sup>	0	0.1	1.4	1.1	0	0
Sinapine (as thiocyanate), % <sup>e</sup>	0.45	0.05	0.31	0.42	0.06	..
Myrosinase activity	+	+	+	+	+	+
Crude protein, % <sup>c</sup>	46	53	44	46	45	56
Percent of N present as protein	87	97	..	..	97	..
Lysine, g./16 g. N	5.2	5.8	..	..	6.2	..
Methionine, g./16 g. N	1.6	1.9	..	..	2.0	..
Cystine, g./16 g. N	2.6	2.4	..	..	2.1	..

<sup>a</sup> Meal prepared by cold pentane-hexane extraction of seed.

<sup>b</sup> Air-dry defatted meal taken as 100%.

<sup>c</sup> Moisture-free basis.

<sup>d</sup> An estimated 90% of the thioglucosides consists of *epi*-progoitrin (1).

<sup>e</sup> As 1-cyano-2-hydroxy-3-butene.

goitrin, and nearly free from cyano compounds and sinapine. Meals B and C contain large amounts of 1-cyano-2-hydroxy-3-butene and other C≡N compounds, as well as of sinapine. Meal B was not extracted after thioglucoside hydrolysis, and therefore is expected to contain these materials. Since meal C differs little from meal B, anhydrous acetone removes almost none of these substances.

Although meal D was prepared starting with a defatted seed and meals A and E were prepared by thioglucoside hydrolysis in whole seed flakes, thioglucoside aglycones and sinapine were removed in all three cases by acetic solvents containing water.

*Feeding Tests.* The results may be conveniently divided into two parts: 1) feeding trials of meal A, which indicated nontoxicity of a

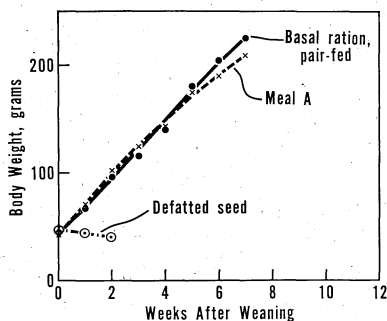


Fig. 1. Growth of weaning rats fed diets containing 28% crambe meals and 72% basal ration, or basal ration alone. For details, see text.

TABLE II  
FEED CONSUMPTION AND ORGAN WEIGHTS<sup>a</sup> OF RATS FED CRAMBE MEALS

DIET	FEED CONSUMED	THYROID	LIVER	PANCREAS	HEART	KIDNEY
	g.	mg.	g.	g.	g.	g.
At 7 weeks						
Meal A 28%, basal <sup>b</sup> 72%	668	14.4	11.3	1.42**	..	..
Pair-fed basal ration	653	11.7	10.8	1.16	..	..
At 6 weeks						
Meal D 28%, basal 72%	640	..	..	..	..	..
Meal E 28%, basal 72%	642	23.9	14.6	1.84	..	..
Basal ration, <i>ad lib.</i>	658	..	..	..	..	..
At 13 weeks						
Meal D 28%, basal 72%	1545	29.4	15.5	1.97	1.58	3.41
Basal ration, <i>ad lib.</i>	1644	27.2	17.2	2.01	1.54	3.07

<sup>a</sup>Weights are of fresh organs, except for pancreas which was fixed and trimmed of fat before weighing.

<sup>b</sup>Composition of the basal ration: 73% yellow corn meal, 10% crude casein, 10% linseed cake meal, 3% USP cod liver oil, 2% dehydrated alfalfa meal, 1.5% bone ash, and 0.5% NaCl (10).

processed meal; and 2) the remainder of the experiments in which the animal response to such meals was more fully explored.

Figure 1 shows the average weekly weights of the test animals fed meal A at 28% of the diet and the pair-fed control animals. That untreated crambe meal is lethal is shown by the loss in weight and death of the animals fed 28% untreated meal. Table II gives the average feed consumption and selected organ weights of animals fed meal A. Statistical analysis showed no significant differences between groups regarding body, thyroid, or liver weights. The average pancreas weight was significantly higher in the test animals. If a possible outlier in the test group is eliminated, the remaining data suggest thyroid enlargement. (The thyroid weight of the suspected outlier is 50% that of the mean of the other four thyroids.) The experiment demonstrates that meal A will support satisfactory growth in rats over a 7-week period. Their rate of gain indicates that no serious palatability problem exists.

Because rats fed meals B and C at 28% of the diet did not survive 2 weeks, one concludes that meals containing hydrolysis products derived from thioglucosides are at least as toxic as untreated meals.

The rats fed meal E survived 6 weeks with no apparent toxic effects (Fig. 2). At 6 weeks their weight gains were equivalent to those of rats being fed meal D (91% of the control group). A comparison of the organ weights at 6 weeks of rats fed meal E with those of rats fed meal A at 7 weeks (Table II) suggests hypertrophy of the thyroid and pancreas. This observation, coupled with the possibility of thyroid and pancreas enlargement in the meal A group, may reflect a poorer extraction of organic aglycones when oil is present.

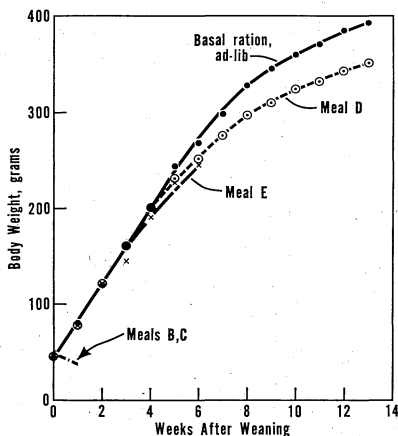


Fig. 2. Growth of weanling rats fed diets containing 28% crambe meals and 72% basal ration, or basal ration alone. For details, see text.

Rats fed meal D grew normally throughout a 13-week period (Fig. 2). Feed consumption was normal (Table II) and demonstrated an acceptable level of palatability. A comparison of organ weights per animal shows no significant difference between test and control groups (Table II). Analysis of organ weights *per se* was chosen because the smaller size of the test animals (88%<sup>\*\*</sup> of the controls) leaves comparison of relative organ weights open to question (11). Nontoxicity of the meal is shown by normal appearance of the organs upon histological examination and the normal value of bone ash (66.5%). Brain, heart, lung, liver, kidney, pancreas, spleen, stomach, intestine, lymph (mesenteric), submaxillary glands, adrenal, gonad, bladder, bone marrow, and uterus were all examined histologically and found normal. Unfortunately, the thyroids were not included in the microscopic examination. They may be assumed normal because thyroid tissue from similar feeding trials is microscopically normal even when hypertrophied.

Meal D, prepared by enzymatic hydrolysis of the thioglucosides and followed by extraction with 12% water in acetone, is essentially detoxified and is palatable at a high dietary level. The extracts contain sinapine and the known organic aglycones derived from the thioglucosides. If this extracted material is not removed, the meal remains toxic. *d,l*-Goitrin is known to inhibit iodine uptake by the thyroid (12), and the nitrile is suspect because of the toxic nature of related unsaturated nitriles and cyanohydrins (13). Sinapine has little toxicity for chickens (14) at levels comparable to its concentration in untreated crambe meal; sinapine has, however, a bitter flavor to humans. Although

present experiments are not conclusive, they suggest strongly that thioglucoside products are the toxic materials and that sinapine may contribute to antipalatability. Further feeding studies are under way to establish the role of these separate components in animal nutrition.

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