Crambe Meal as a Protein Source for Feeds[1]

K.D. CARLSON and **H.L. TOOKEY**, Northern Regional Research Center, Agricultural Research Service, US Department of Agriculture, 1815 North University Street, Peoria, IL 61604

ABSTRACT

Crambe abyssinica may be grown for its seed oil containing 55-60% erucic acid, which fills a long-term, technologically important US industrial market. The residual meal could serve in animal feeds, but, like other Cruciferae, crambe seed contains glucosinolates that limit the feed value of the defatted meal. Protein content, amino acid composition, protein efficiency tests and numerous animal feeding experiments show that crambe meal contains protein of good nutritional quality. Means of reducing, nullifying or removing the glucosinolates and their hydrolysis (aglucon) products have been the object of many studies, and crambe meals containing native levels of glucosinolates and/or aglucon products have been shown to be lethal to mice, rats and chicks when fed at significant dietary levels. Animal performance is inversely related to sublethal concentrations of these compounds in modified meals. However, water-extracted crambe meals have excellent nutritional quality when such extraction removes the glucosinolates and/or aglucon products. Feeding experiments suggest that these meals, although more costly to prepare, could be used in feeds for nonruminant animals. On the other hand, moist heat-toasting of crambe meals in conventional oilseed extraction facilities provides meals of value for supplemental protein in beef rations. For this use, specifications and FDA approval are in place for commercial exploitation of crambe meal. These studies and the status of crambe as a protein source in feeds are reviewed.

INTRODUCTION

Rapeseed oils have long been items of world commerce, with those highest in erucic acid being the primary sources for oil with industrial applications (1). Industrial sources suggest that as much as 20 million lb of this oil could be utilized annually in the USA. Recent years have seen a shift to double "zero" or low-erucic, low-glucosinolate types of rapeseed, both in Canada (as canola) and in Europe, having improved edible oil and feed meal characteristics. Consequently, concern over availability of economical supplies of high-erucic oil has increased. Domestically produced crambe oil could satisfy this market requirement.

Crambe abyssinica Hochst. ex R.E. Fries is an annual herb of the family Cruciferae. Commercial plantings have been made from time to time to test its agronomic and economic feasibility in several areas of the USA. Crambe has been evaluated over the years with three objectives in mind, to provide: (a) a domestic source of high-erucic oil; (b) an alternative feed protein source in certain geographical regions; and (c) an alternative crop for the nation's farmers.

Figure 1 shows the ranges in oil, erucic acid and protein contents in 75 samples of crambe seed grown in 17 states (2). Before oil extraction, protein content of dehulled seed averages 29%. Approximately 46% of the dehulled seed weight is oil, of which the erucic acid content at 56% is as high as any available seed source. Crambe is widely adapted and may be grown as an alternative crop in several major crop production regions of the USA (3-5). Crambe seed has a significant level of crude protein, which has been shown to be well balanced in amino acids and of good nutritional quality (6-9). Like other crucifers, however, crambe seed has enzyme-responsive constituents called glucosinolates, which prevent direct feeding of raw seeds or meal and that limit use of conventionally processed seed meal in feeds. In this review paper, we will look at some of the factors involved in enzyme-moderated reactions of the glucosinolates and how the products of these reactions affect the quality of byproduct meal, and then at the value of crambe meal as a protein source for feeds.

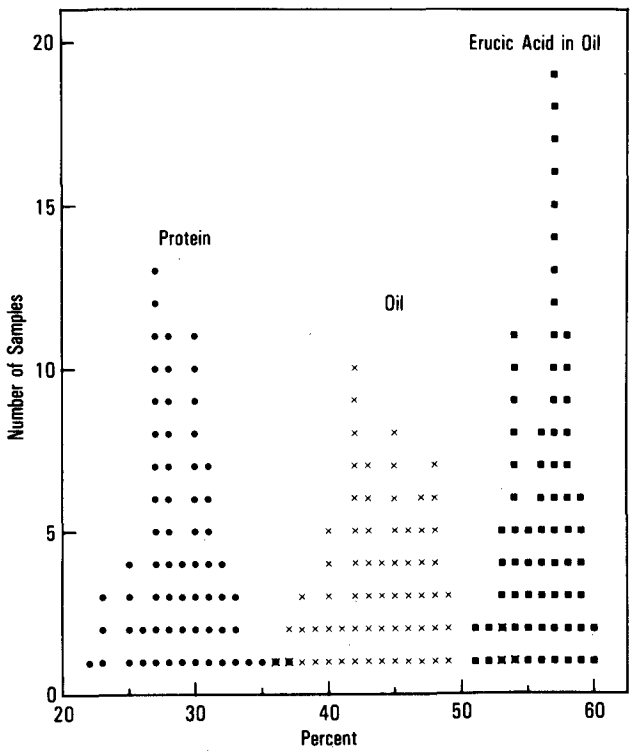

FIG. 1. Ranges of protein, oil and erucic acid in 75 samples of dehulled crambe seed from 17 states (adapted from Earle et al. [2]).

PROTEIN QUANTITY AND QUALITY

Average composition of seed grown in different geographical locations is shown in Table I, where crude protein (N × 6.25) levels range from 20% in the whole seed to nearly 50% in the dehulled, defatted meal (10-15). Seed hull accounts for ca. one-third of the seed weight and contributes significantly to the fiber content of the whole seed meal. However, seed can be dehulled easily to produce a higher protein, lower fiber meal.

A typical amino acid (AA) analysis of defatted crambe meal shows that 85-87% of the meal nitrogen (N) is accounted for as AA (73%) and ammonia (13%). This can be increased to 95-97% if the meal is first given an acetone/water (98:2) extraction (8,16). The ranges in AA contents of crambe meal as reported by several groups are shown in Table II (2,6,7,17,18). In rating seed meals as a balanced source of AA for optimum growth, VanEtten et al. (6) and Miller et al. (7) judged the proportions of AA in crambe meal to be adequate for the rat and pig, but not for the chick. VanEtten et al. (8) compared the AA pattern of hen's egg to the AA patterns of a number of cereal grains and oilseed meals, and they concluded that the AA pattern and high protein content of crambe would make it a good supplemental source of protein to use with cereal grains in feeds.

[1] Presented in part at the symposium on New Protein Sources, 74th annual AOCS meeting, Chicago, 1983.

TABLE I

Average Composition of Crambe Seed and Meal (Dry Basis)[a]

Constituent	Whole seed (%)	Whole seed Defatted (%)	Dehulled seed (%)	Dehulled seed Defatted (%)
Oil	35.3	–	46.5	0.9
Protein (N X 6.25)	20.1	31.1	25.8	48.7
Crude fiber	14.3	22.1	3.6	6.7
Ash	4.8	7.4	4.5	8.6
Nitrogen-free extract	25.4	39.3	19.6	35.6
Glucosinolates (%)	–	4.5-7	–	8-10

[a]Adapted from Mustakas and coworkers (10,12,14); Kirk and coworkers (11,13) and Baker et al. (15).

VanEtten et al. (19) determined the solubility of crambe meal N as a function of pH. They showed that crambe N is much more soluble than soybean N at the pH of minimum solubility (pH 3.5-4.5); however, a higher pH is required for maximum solubilization of crambe N. Classified according to solubility in different aqueous solvents, ca. 12% of the crambe N is nonprotein and a large portion of the crambe proteins appears to be globulins.

Several researchers have evaluated crambe protein quality after glucosinolates were removed by extraction with aqueous acetone or with water. VanEtten et al. (9) determined protein efficiency ratios (PER) in rats fed crambe meals that had been once or twice extracted with acetone/water (89:11). PER of these unheated meals (Table III) were as good or better than the casein control. Similarly, Baker et al. (17) found that heat-treated, water-washed crambe meals had PER as good as casein controls (Table III). One concludes from the above evidence that a significant level of high-quality, well balanced protein is present in crambe meal.

GLUCOSINOLATES AND AGLUCON PRODUCTS

Dehulled, defatted crambe meal contains from 7 to 10% glucosinolates (19,20), 90% of which will be (S)-2-hydroxy-3-butenyl glucosinolate (21,22), commonly called *epi*-progoitrin and abbreviated in this paper as *epi*-PG (**I**, Figure 2). In crambe seed, *epi*-PG is accompanied by, but separated from, a glucosinolate-hydrolyzing enzyme system called thioglucosidase (abbreviated here as TGSase). This separation of enzyme and substrate is breached, and reaction may occur, whenever seed is crushed, when the seed germinates (23), or generally in crucifers when plant tissues are macerated. Enzymes of this type have been characterized as glycoproteins (24,25) with sulfhydryl groups essential to their activity (26,27). Crambe TGSase appears to be similar (27). Heat destroys the enzyme's ability to act on the glucosinolate. However, since TGSase activity is exhibited by some intestinal bacteria (28,29), ingested *epi*-PG still could be hydrolyzed to aglucon products in the digestive tracts of animals consuming crambe seed or meal.

Products of the *epi*-PG/TGSase reaction may be any of the four shown in Figure 2: (R)-5-vinyloxazolidine-2-thione (vinyl-OZT, **II**), 1-cyano-2(S)-hydroxy-3-butene (cyanobutene, **III**), and *erythro*- and *threo*-1-cyano-2(S)-hydroxy-3(R)(S),4-epithiobutanes (epithiobutanes, **IV** and **V**) (30-33). Although only one enzyme appears necessary to

TABLE II

Amino Acid Composition of Crambe Protein[a]

Amino acid	Range (g/16 g N)	Amino acid	Range (g/16 g N)
Arginine	5.7-7.3	Alanine	3.8-4.2
Histidine	2.2-2.7	Aspartic acid	6.0-7.6
Isoleucine	3.7-4.1	Cystine	2.6-2.8
Leucine	5.9-6.8	Glutamic acid	14.2-17.0
Lysine	4.9-5.7	Glycine	4.7-5.3
Methionine	1.6-1.9	Hydroxyproline	0.6-0.9
Phenylalanine	3.4-4.0	Proline	5.5-6.2
Threonine	3.1-4.6	Serine	3.5-4.1
Valine	4.5-5.6	Tyrosine	2.5-3.0
Tryptophan	1.0-2.0		

[a]Adapted from Earle et al. (2); Van Etten et al. (6); Miller et al. (7); Baker et al. (17); and Pereira et al. (18).

TABLE III

Protein Efficiency Ratios (PER) for Aqueous Acetone and Water Extracted Crambe Meals

Diet constituent	Protein in diet (%)	PER (g gain/g protein consumed) Average	PER (g gain/g protein consumed) Adjusted[a]
Casein control	11.8	3.18	2.50
Crambe, aq acetone 1X[b]	20.1	3.25	2.55
Crambe, aq acetone 2X[b]	20.1	3.50	2.75
Casein control	11.4	2.93	2.50
Crambe, water wash 1[c]	19.7	2.96	2.53
Crambe, water wash 2[c]	20.8	3.19	2.72

[a]Adjusted for casein = 2.50.

[b]Meal autolyzed but not heated; washed once (1X) or twice (2X) with acetone/water (89:11). Adapted from VanEtten et al. (9).

[c]Meal moistened and crisped after defatting; washed on a continuous filter at two meal/water ratios. Adapted from Baker et al. (17).

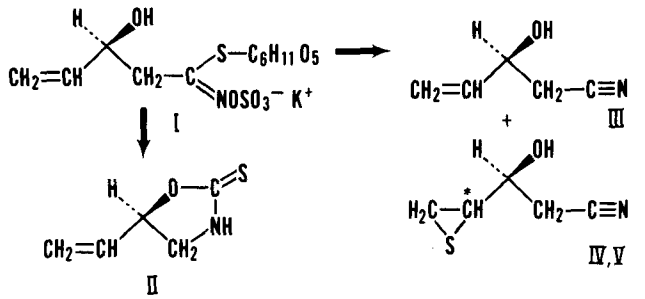

FIG. 2. Aglucon hydrolysis products (II-V) of *epi*-progoitrin (*epi*-PG, I) in crambe meal.

hydrolyze *epi*-PG, and glucose and acid sulfate ion are always formed, a number of factors are involved in determining which of the several aglucon products will predominate. And, as we shall see, which aglucon products are present in defatted crambe meal determines to a large extent the nutritional value of the meal.

For example, when fresh, unheated crambe meal is autolyzed at room temperature (pH 4-7), native TGSase hydrolyzes endogenous *epi*-PG primarily to a mixture of the three nitriles: 30-50% **III**, and 50-70% **IV** and **V** (*erythro/threo* ratio ca. 1.25) (Fig. 2) (20,27). Autolysis above 50 C or above pH 9 leads predominantly to production of vinyl-OZT (**II**), as does autolysis of meal from old seed stored under ambient moisture and temperature conditions. These observations, as well as the work of Tookey and Wolff (23) and Tookey (27,34), demonstrated that the TGSase system is labile and that vinyl-OZT is the likely product when the enzyme is altered by these factors. Indeed, Tookey (34) showed that a small, labile protein, which he named epithiospecifier protein (ESP), was required in concert with TGSase for the hydrolysis of *epi*-PG to produce the epithiobutanes (**IV, V**).

It appears that ESP moderates the transfer of sulfur from the S-glucosyl moiety of the glucosinolate (I) to the site of unsaturation in the aglucon portion. Such a specifier protein for epithioalkyl nitrile compounds also has been reported by Cole in turnip seed (35).

It is apparent that a number of products might be expected in crambe meal as a result of conditions prevailing during removal of the seed oil, and that the feed value of the residual meal will depend partly on the relative toxicities of intact *epi*-PG and the several aglucon products that may be present.

BIOLOGICAL EVALUATION OF CRAMBE MEALS

Raw Dehulled, Defatted Crambe Meal

Hesketh et al. (36) fed crambe meal as a protein source in broiler chick rations. Diets containing 5-42% crambe meal produced growth depression proportional to amount of crambe fed; they also decreased feed efficiency and enlarged thyroids. Similarly, VanEtten et al. (19) found that rats fed crambe meal as 15-25% of their rations lost weight and died within 90 days. Such toxicity is always observed in feeding raw crambe meals containing both intact glucosinolates and active TGSase (9,12-14,16-18,37). In contrast, VanEtten et al. (19) and Tookey et al. (16) showed that aqueous acetone extraction of dehulled, defatted crambe meal removed all but traces of *epi*-PG and aglucon products and effectively detoxified the meal. When fed to weanling rats at up to 28% of the ration, these meals were palatable and produced weight gains of 88-100% of the controls.

VanEtten et al. (9) further defined toxicity and feed value of crambe meals prepared in specific ways. In 90-day feeding studies, weanling rats were fed rations containing one of the following (Table IV, rations 1-10): (1) and (2) two levels of crambe meal autolyzed and aqueous acetone-extracted to remove all aglucon products; (3) crambe meal autolyzed to contain vinyl-OZT (II, Fig. 2); (4) added vinyl-OZT; (5) added *epi*-PG; (6) crambe meal with intact *epi*-PG without or (7) with TGSase activity; (8) crambe meal autolyzed to contain the mixed nitriles (III-V, Fig. 2); and (9) and (10) two levels of added mixed nitriles. Results are summarized in Table IV, where rations containing 10% crambe meal (or equivalents of *epi*-PG or aglucon) are listed in order of increasing toxicity. Clearly, growth and survival were poor on diets containing nitriles or crambe meals with intact *epi*-PG and active TGSase (rations 7-10, Table IV). In contrast, rations containing *epi*-PG (added or in autolyzed meal, rations 3-4) were less toxic, and rats fed these had final weight gains 77-85% of the control animals. Rats fed rations containing 10 or 30% of aqueous acetone-extracted meals (rations 1-2) grew normally. Rats receiving nitriles (**III-V**) had enlarged livers and kidneys with distinct and characteristic pathological changes compared with control animals. Rats receiving meal with *epi*-PG and active TGSase showed lesions in the liver and kidney similar to those in the animals fed nitrile-containing rations. Rats fed such crambe meals (e.g., ration 7) died when the level of *epi*-PG reached 0.5-1.0% or more of the diet. Those rats receiving isolated *epi*-PG, or meals containing *epi*-PG without TGSase activity, had milder lesions in all organs (rations 5,6). The goitrogenic effect of vinyl-OZT is shown by the enlarged thyroids of animals fed rations 3 and 4 (also rations 6 and 7). Generally, pathology indicative of the presence of both vinyl-OZT and mixed nitriles was associated with rations containing *epi*-PG. Organs were normal in animals fed rations containing the extracted crambe meals (rations 1 and 2).

Toxicity of Aglucon Products

Consistent with the apparent chronic toxicities suggested by the above studies are the acute oral toxicities of the aglucons as reported by VanEtten et al. (33) (Table V). Nishie and Daxenbichler (38,39) and Gould et al. (40) have recently reported additional toxicological data for all the aglucon compounds (**II-V**). None of the aglucons are teratogenic (38,41).

Enlarged adrenals and partially necrotic livers were observed in pregnant rats treated with 175 mg/kg (subcutaneous) of cyanobutene (**III**) (38), and rats fed rations containing mixtures of the epithiobutanes (**IV,V**) at 75-300 ppm for 90 days had dose-dependent kidney and liver lesions (40). The epithiobutanes caused embryonal death and decreased fetal weights, whereas the cyanobutene and *epi*-PG (**I**) increased or decreased fetal weight depending on dosage (38). Once formed in crambe meal, the cyanobutene is nonvolatile and stable, whereas the epithiobutanes are less stable and easily polymerized (32).

DETOXIFICATION OF CRAMBE MEAL

Conventional Processing

Clearly, aglucon products, particularly the nitriles, are undesirable constituents of crambe meal from the standpoint of palatability, growth inhibition, pathological changes in body organs, and general toxicity at higher levels of consumption by rats, mice and chicks. Minimally, thermal inactivation of TGSase without hydrolyzing or decomposing the glucosinolates is necessary. Conventional moist heat treatments may be used, or microwave processing (18, 42-44) may provide the energy for enzyme inactivation. Meals thus processed give better growth responses than

TABLE IV

Performance of Rats Fed Rations Containing Crambe Meals (CM), *epi*-PG or Aglucon Products[a]

Ration constituent	% added	Body wt % of control	Relative organ wt (g/100 g body wt) Liver	Kidney	Thyroid
Controls[b]	–	100	2.7-3.5	0.61-0.65	4.4-8.5
1. CM, aq acetone-extracted	10	105	2.7	0.74	8.0
2. CM, aq acetone-extracted	30	97	3.4	0.68	8.4
3. CM, autolyzed to 1.3% vinyl-OZT	10	85**	3.7	0.68	20.8**
4. Added vinyl-OZT	0.23	85**	4.0	0.62	14.7**
5. Added *epi*-PG	0.85	85**	4.7	0.81**	9.0
6. CM, no TGSase, 7.6% *epi*-PG	10	77**	4.5	0.86	12.9**
7. CM, TGSase activity, 7.6% *epi*-PG	10[c]	41**	9.3**	1.54**	13.4**
8. CM, autolyzed to 0.8% nitrile mix	10	All animals died within 21 days			
9. Added nitrile mix	0.2	All animals died within 14 days			
10. Added nitrile mix	0.1[d]	17**	5.6**	1.50**	6.1

[a]Adapted from VanEtten et al. (9). *epi*-PG, vinyl-OZT and nitriles as in Figure 2. Differences significant at $*P < 0.05$, $**P < 0.01$.

[b]Range of organ weights for 3 sets of controls used in the experiments.

[c]Four of 5 rats died within 35 days.

[d]Two of 5 rats died by day 84.

TABLE V

Acute Toxicities of Aglucon Products from *epi*-PG in Crambe Meal

Aglucon product tested[a]	LD_{50} (mg/kg) Oral[b]	SC[c]
Vinyl-OZT (II)	1260-1415	–
Cyanobutene (III)	170	200
Erythro-epithiobutane (IV)	178	109[d]
Threo-epithiobutane (V)	240	

[a]Structures are shown in Figure 2.

[b]In mice, VanEtten et al. (33).

[c]Subcutaneous in rats, epithiobutanes isolated and tested as a mixture, Nishie and Daxenbichler (38).

[d]Mixture of IV, V.

meals with active TGSase and intact glucosinolates or meals containing aglucon products. Even so, these meals, as we have seen, are generally not suitable for monogastric animals at significant dietary levels, unless glucosinolates and aglucon products are first removed.

Processing with Chemical Additives

Many procedures have been reported for improving the palatability and feed value of crucifer seed meals. For crambe, these include chemical treatments of the meal during conventional processing steps with ammonia (37), with soda ash (sodium carbonate) (12), and with ferrous salts or other metal salts and alkalis (13). Glucosinolates and vinyl-OZT were reported absent from the ammoniated meal; the presence of nitriles was not investigated (37). Soda ash treatment left 2-3% *epi*-PG and 0.2-0.7% nitriles in the meal (14) and *epi*-PG (0.5-0.6%) and cyanobutene (III, 0.8-0.9%) were found in the ferrous sulfate-treated meals (13). The intent of these chemical treatments was to destroy glucosinolates and prevent aglucon products from forming. Substantial destruction of glucosinolates by some of these treatments was counterbalanced by the formation of significant amounts of cyanobutene (III) or other aglucons in the meals. Soda ash also had the disadvantage of lowering the lysine level in the meal. Improvements in feed value over untreated meals were achieved for both rats and chicks; however, at dietary levels of 20-30%, the meals limited growth to 70-80% of the controls, and thyroids, livers and kidneys were often enlarged relative to control animals.

Generally, conventionally or chemically processed crambe meals are not suitable for feeds for monogastric animals.

Processing with Water Extraction of the Meal

A practical approach to detoxifying crambe meal is first to inactivate TGSase and then to extract the water-soluble glucosinolates with a minimum of water. In the first such study by Mustakas et al. (14), dehulled, defatted crambe meal was steam-cooked to inactivate TGSase prior to water-washing in a batch process. Growth rates of chicks or rats fed these meals at 20-30% of their rations were not significantly different from controls. However, these meals were not totally devoid of both *epi*-PG and aglucon products, and growth and organ pathology correlated with the levels of these constituents.

Baker and coworkers (15,17) developed a process for water-washing crambe meals on a continuous filter that gave meals of 50% protein with good amino acid balance and PER equivalent to casein. Only traces of aglucon products and 0.6% or less of *epi*-PG were detected in the washed meals. Chicks and rats fed 20-30% of the washed meals in their diets had gains, feed consumption and feed efficiencies ranging from 85 to 100% of the controls. Performance again correlated with residual glucosinolate content of the meals.

Baker et al. (15) estimated that a practical water-extraction step would add significantly to the cost of a conventional oilseed extraction process. The potential benefits (speed, process control, energy efficiency) of applying microwave technology (45-48) to inactivation of TGSase in intact crambe seeds might be balanced against the added cost of water-washing to remove glucosinolates. This novel approach was demonstrated by Kirleis and coworkers (42-44) who prepared microwaved (MW), water-washed crambe meals with less than 1% total glucosinolates and 50-65% protein. Nitrogen solubility and the functional

properties of these meals were not different from water-washed meals prepared by hot water (HW) inactivation of TGSase in the whole seed (44). Compared to soybean meal, both washed meals gave superior gains and feed efficiencies when diets contained 10% crude protein from these sources (Table VI) (18). Increasing the levels of MW and HW crambe meals in the diets to provide up to 17.5% crude protein had no adverse effect on animal performance, indicating that detoxification of the meals by water-washing was effective. Performance of animals on HW-inactivated crambe and soybean meal diets at 12.5-17.5% crude protein did not differ, although for maximum gains on soybean meal, a higher level of crude protein (15% vs 12.5%) was required. The poorer performance of rats fed MW-treated crambe meals may be related to a significantly lower available lysine level (4.4%) compared to the HW crambe meal (5.3%), due to some overheating of the seed in the microwave step. Neither lysine nor methionine was first-limiting in the diet containing 10% crude protein from HW crambe meal (18).

Results of these studies suggest that water-washed crambe meal could be used at reasonable levels (15-30%) in feeds for monogastric animals. However, the added cost of producing these meals likely would have to be recovered in higher feed or oil prices. It is conceivable that *epi*-PG (I) and/or aglucon products (II-V) recovered from aqueous washes could find use as pesticides (22,49-52) or as specialty chemicals.

CRAMBE MEALS FOR BEEF CATTLE RATIONS

Early Palatability and Growth Studies

Ruminants are more tolerant than monogastric animals of conventionally processed crambe meals. In short-term palatability trials, Mustakas et al. (12) reported on the acceptance by steers of crambe meals that had been prepared by a variety of chemical treatments or by conventional moist heat toasting. Rations containing sodium carbonate-treated crambe meals were consumed at nonsignificantly lower levels than rations containing soybean meal, and ammonia-treated crambe meals were less palatable than carbonate-treated meals.

Lambert et al. (53) further explored the feed value of crambe meals for beef cattle in a 196-day finishing trial. They fed dehulled and conventionally moist heat-toasted crambe meal to replace $^1/_3$, $^2/_3$ and all of the soybean meal in rations formulated to contain 11% crude protein. The results are summarized in Table VII. Daily feed intake and daily gains decreased significantly ($P < 0.01$) as the amount of supplemental protein provided by crambe meal increased. Differences in feed efficiency, however, were not significant, showing that increased crambe meal in the ration did not reduce the nutritional quality of the ration even though palatability may have been lowered. In a complementary study, where the crambe hulls were left in the meal to provide fiber in the ration, animal performance was not different from that with dehulled crambe. There was a trend, however, for animals receiving hulls to be more feed-efficient (53).

In a 112-day feeding trial, both conventionally heat-toasted and soda ash-treated crambe meals were fed with or without hulls in comparison with soybean meal as the only supplemental sources of protein. The lower palatability of the crambe-containing rations was indicated by lower feed consumption. Animals fed either crambe-containing ration had significantly lower average daily gains (92-94%, $P < 0.01$) compared with the soybean controls. Performance differences between the two types of crambe meals were not significant, and presence or absence of crambe hulls was without effect (53). Thus, dehulling of crambe seed is not necessary for beef cattle rations.

Two digestion trials were run comparing soybean meal rations with those containing ammoniated or soda ash-treated crambe meals as the source of supplemental protein (53). Digestibility of dry matter, crude protein, energy and metabolizable energy in the soybean-supplemented ration was significantly higher ($P < 0.05$) than in the ammoniated crambe ration. Digestibility of dry matter and protein was

TABLE VII

Performance of Steers Fed Rations Containing Crambe Meals (196 Days)[a]

	Supplemental protein from crambe (%)			
	0	33	67	100
Ration ingredients (%)[b]				
Corn	62.1	61.8	61.9	61.9
Corn cobs	20.0	20.0	20.0	20.0
Soybean meal	9.1	6.0	3.0	–
Crambe meal	–	3.4	6.6	9.8
Protein	11.1	12.0	11.0	11.7
Daily gain (kg)**	1.18	1.12	0.98	0.86
% of control	–	95	83	73
Daily feed (kg)**	9.0	9.0	8.3	6.0
Per kg of gain	7.9	8.1	8.1	6.9

[a]Adapted from Lambert et al. (53). **Differences significant at $P < 0.01$.

[b]Premix of vitamins, minerals, etc., not shown (8.3-8.8%).

TABLE VI

Performance of Rats Fed Rations Containing Water-Washed Crambe Meals or Soybean Meal

	Protein in diet			
	10.0	12.5	15.0	17.5
Average daily gain (g)				
Water-washed crambe				
Microwave	4.01^{b}	4.66^{c}	$5.03^{c,d}$	4.85^{c}
Hot water	$5.46^{c,d,e}$	$6.33^{e,f}$	$6.18^{e,f}$	$6.30^{e,f}$
Soybean meal	3.24^{a}	$5.75^{d,e}$	6.66^{f}	6.99^{f}
Feed/gain (g/g)				
Water-washed crambe				
Microwave	3.58^{B}	3.15^{C}	$2.82^{D,E}$	$2.88^{C,D,E}$
Hot water	$2.95^{C,D}$	$2.62^{E,F}$	$2.58^{E,F}$	2.50^{F}
Soybean meal	4.06^{A}	$2.98^{C,D}$	$2.79^{D,E}$	$2.62^{E,F}$

Adapted from Pereira et al. (18).

[a-f]Average daily gain (ADG) means without common superscripts are different ($P < 0.05$).

[A-F]Feed/gain means without common superscripts are different ($P < 0.05$).

not different between the soda ash-treated crambe and soybean rations.

Lambert et al. (53) observed that lower palatability of the crambe rations caused cattle to sort rations in some trials, and this led to lower feed consumption. This problem was partly overcome by pelleting rations (53-55), and blending crambe meal with soybean meal essentially eliminated the problem. Histological examination of the abdominal and thoracic viscera and the adrenal and thyroid glands of animals fed crambe meals revealed no gross differences in steers fed the different diets. It is concluded from the work of Lambert et al. (53) that crambe could replace without consequence up to $^2/_3$ the supplemental protein provided by soybean meal in these rations.

Studies to Support a Petition to FDA

Between 1972 and 1977, four long-term feeding studies (152-182 days) were conducted to obtain data needed for petitioning the US Food and Drug Administration (FDA) to permit the use of crambe meal as a source of supplemental protein in beef cattle rations (54-55). The meals were prepared with hulls in three commercial oilseed-extraction facilities using only conventional moist heat-toasting to inactivate TGSase, and oil was extracted by solvent or prepress/solvent extraction processes. The finished meals contained 25-31% crude protein, 22-26% crude fiber, 1.1-3.7% *epi*-PG (**I**), and 0.6-1.5% cyanobutene (**III**). They may be considered as typical of crambe meals produced in suitably sized and equipped oilseed mills the first few times they run crambe seed. Processing objectives were to inactivate the TGSase enzyme system totally (achieved), while hydrolyzing or thermally destroying as little glucosinolate and generating as little aglucon product as possible in the finished meal (partly achieved).

Experimental feeding protocols were designed in consultation with FDA, and results of the four feeding experiments are summarized in Table VIII. In the first study, experiment A, crambe meal replaced $^1/_3$, $^2/_3$ and all the soybean meal in a high-energy diet containing 10.3% crude protein. Rate of gain and daily feed intake decreased with increasing level of crambe meal in the rations, but these differences were not significant even when 12.6% crambe supplied all supplemental protein in the diet. Furthermore, feed efficiency did not change. Based on processing objectives outlined above, this meal is judged to be the lowest quality of the four (Table VIII), because it has the lowest *epi*-PG (**I**) and highest cyanobutene (**III**) contents and lowest nitrogen solubility, all indicators of excessive heat treatment during processing. The results, however, are in general agreement with the similar experiment conducted by Lambert and coworkers (53) (Table VII).

Experiment B tested whether crambe meal would be as efficient as soybean meal in fortifying a urea-containing 9% protein diet to a 10.3% crude protein level. Animals consuming the two diets did show equivalent nonsignificant increases in feed consumption and rates of gain as well as improved feed efficiencies (Table VIII). This meal was judged to be of intermediate quality, similar in cyanobutene content to meals in experiments A and D, but with higher nitrogen solubility than either.

Experiments C and D further studied the quality of crambe protein by adding crambe meal to a low-protein diet formulated with urea. In experiment C, nonsignificant increase in rate of gain and significantly improved feed conversion ($P < 0.05$) were observed on adding crambe meal to raise the total protein to 11.0%. This meal was the best quality of the four—having the highest nitrogen solubility, highest *epi*-PG content, and the lowest cyanobutene level. It gave the best feeding results of the four meals.

In experiment D, with a lower-to-intermediate quality meal, neither rate of gain nor feed efficiency was significantly affected by increasing levels of crambe protein in the ration. Levels of *epi*-PG, cyanobutene and nitrogen solubility all reflect more severe processing conditions. Lower palatability of this meal, compared with C and as observed with A, is suggested by declining feed consumption as the level of crambe is increased (Table VIII). Smaller animals (initial weights) used in experiments B and C maintained daily feed consumption with increasing crambe meal in the rations, whereas the larger animals of experiments A and D showed declining feed consumption. Thus, crambe meal may be more acceptable to younger animals, but in these experiments, they fortuitously received the higher quality meals.

In connection with these studies, cattle were fed rations containing 10% crambe meal for up to 30 days to determine whether *epi*-PG or aglucon products (**I-V**) appeared in their fat or in muscle, liver and kidney tissues. None of these compounds were detected in body tissues by methods sensitive to 1 ppm (56). In unpublished results (C. H. VanEtten et al.), no *epi*-PG or aglucon products were found in rumen fluid from a number of these animals, which suggests that they are quickly destroyed or converted to unknown products on ingestion by beef cattle.

A food-additive petition was filed with the FDA (57) in November 1979, proposing that the Code of Federal Regu-

TABLE VIII

Crambe Meal as Supplemental Protein for Beef Cattle[a]

Treatment	Experiment A				Experiment B			Experiment C				Experiment D			
	1	2	3	4	1	2	3	1	2	3	4	1	2	3	4
Protein (%)	10.3	10.3	10.3	10.3	9.0	10.3	10.3	9.0	9.6	10.3	11.0	9.0	9.6	10.3	11.0
Crambe meal (%)	–	4.2	8.4	12.6	–	5.5	–	–	2.5	5.4	8.3	–	2.6	5.4	8.5
Soybean meal (%)	7.5	5.0	2.5	–	–	–	3.1								
Daily gain (kg)	1.10	1.08	1.05	0.93	0.97	1.05	1.03	1.00	1.05	1.13	1.14	0.96	0.87	0.85	0.87
Daily feed (kg)	8.3	8.0	7.8	7.4	6.9	7.2	7.1	7.8	7.9	8.0	7.9	7.9	7.7	7.2	7.3
Per kg gain	7.6	7.4	7.4	8.0	7.2	6.9	6.9	7.9*	7.6*	7.1*	6.9*	8.3	8.8	8.4	8.5
epi-PG (%)[b]		1.1				1.6				3.7				1.9	
Cyanobutene (%)[c]		1.5				1.4				0.6				1.4	
N sol., 0.5 M NaCl (%)[d]		0				50				72				36	

[a]Adapted from Perry et al. (55). *Differences significant at $P < 0.05$.

[b]2-Hydroxy-3-butenyl glucosinolate (**I**, Fig. 2).

[c]1-Cyano-2-hydroxy-3-butene (**III**, Fig. 2); calculated as % *epi*-PG.

[d]Percent total N solubilized; a measure of heat damage to protein during processing.

lations (CFR), Part 573, "Food Additives Permitted in Feed and Drinking Water of Animals" (58) be amended to provide for the safe use of crambe meal in the feed of beef cattle. The proposed amendment was published June 5, 1981, in the Federal Register (59) for comment, and subsequently accepted and added as paragraph 573.310 to Title 21, CFR. The paragraph reads as follows:

21CFR573.310 Crambe Meal, Heat Toasted

(a) The additive is the seed meal of *Crambe abyssinica* obtained after the removal of oil from the and hull. The oil may be removed by prepress solvent extraction or by solvent extraction alone. The resulting seed meal is heat toasted.

(b) The additive conforms to the following percent-by-weight specifications: moisture, not more than 11 percent; oil, not more than 4 percent; crude protein, not less than 24 percent; crude fiber, not more than 26 percent; glucosinolate calculated as *epi*-progoitrin, not more than 4 percent; goitrin, not more than 0.1 percent; nitrile calculated as 1-cyano-2-hydroxy-3-butene, not more than 1.4 percent. At least 50 percent of the nitrogen shall be soluble in 0.5 M sodium chloride. Myrosinase enzyme activity shall be absent.

(c) The additive is used or intended for use in the feed of feedlot cattle as a source of protein in an amount not to exceed 4.2 percent of the total ration.

Thus, crambe meal produced by conventional oilseed processing techniques, having no TGSase activity and composition encompassed in 21CFR573.310, may enter, and is regulated in, interstate commerce for use as a source of supplemental protein in beef cattle rations. Such utilization would be a significant factor in the economics of producing crambe oil in the USA as a domestic source of erucic acid (60). Future producers or marketers of the meal should consult with feed registration officials within specific states for assistance and advice regarding state requirements for feed additives and feed formulations.

REFERENCES

1. Nieschlag, H.J., and I.A. Wolff, JAOCS 48:723 (1971).
2. Earle, F.R., J.E. Peters, I.A. Wolff and G.A. White, JAOCS 43:330 (1966).
3. White, G.A., and J.J. Higgins, Culture of Crambe . . . A New Industrial Oilseed, Production Research Report No. 95, ARS, USDA, Washington, DC, 1966, pp. 1-20.
4. Christmas, E.P., K.J. Lessman, C.B. Southard and M.W. Phillips, Crambe: A Potential New Crop for Indiana, Bulletin AY-168, Purdue University, West Lafayette, IN, pp. 1-11.
5. Downey, R.K., JAOCS 48:718 (1971).
6. VanEtten, C.H., R.W.. Miller, I.A. Wolff and Q. Jones, J. Agric. Food Chem. 9:79 (1961).
7. Miller, R.W., C.H. VanEtten, C. McGrew, I.A. Wolff and Q. Jones, Ibid. 10:426 (1962).
8. VanEtten, C.H., W.F. Kwolek, J.E. Peters and A.S. Barclay, Ibid. 15:1077 (1967).
9. VanEtten, C.H., W.E. Gagne, D.J. Robbins, A.N. Booth, M.E. Daxenbichler and I.A. Wolff, Cereal Chem. 46:145 (1969).
10. Mustakas, G.C., G. Kopas and N. Robinson, JAOCS 42:550A, 552A, 554A, 594A (1965).
11. Kirk, L.D., G.C. Mustakas and E.L. Griffin, Jr., JAOCS 43:334 (1966).
12. Mustakas, G.C., L.D. Kirk, E.L. Griffin, Jr., and D.C. Clanton, JAOCS 45:53 (1968).
13. Kirk, L.D., G.C. Mustakas, E.L. Griffin, Jr., and A.N. Booth, JAOCS 48:845 (1971).
14. Mustakas, G.C., L.D. Kirk, E.L. Griffin, Jr., and A.N. Booth, JAOCS 53:12 (1976).
15. Baker, E.C., G.C. Mustakas and V.E. Sohns, JAOCS 54:387 (1977).
16. Tookey, H.L., C.H. VanEtten, J.E. Peters and I.A. Wolff, Cereal Chem. 42:507 (1965).
17. Baker, E.C., G.C. Mustakas, M.R. Gumbmann and D.H. Gould, JAOCS 54:392 (1977).
18. Pereira, J.A.A., A.W. Kirleis and T.R. Cline, J. Anim. Sci. 53:1278 (1981).
19. VanEtten, C.H., M.E. Daxenbichler, J.E. Peters, I.A. Wolff and A.N. Booth, J. Agric. Food Chem. 13:24 (1965).
20. VanEtten, C.H., M.E. Daxenbichler, J.E. Peters and H.L. Tookey, Ibid. 14:426 (1966).
21. Daxenbichler, M.E., C.H. VanEtten, F.S. Brown and Q. Jones, Ibid. 12:127 (1964).
22. Tookey, H.L., C.H. VanEtten and M.E. Daxenbichler, in Toxic Constituents of Plant Foodstuffs, edited by I.E. Liener, 2nd edn., Academic Press, New York, 1980, pp. 103-142.
23. Tookey, H.L., and I.A. Wolff, Can. J. Biochem. 48:1024 (1970).
24. Bjorkman, R., and J.-C. Janson, Biochim. Biophys. Acta 276:508 (1972).
25. Lonnerdahl, B., and J.-C. Janson, Ibid. 315:421 (1973).
26. Tsuruo, I., and T. Hata, Agric. Biol. Chem. 31:27 (1967).
27. Tookey, H.L., Can. J. Biochem. 51:1305 (1973).
28. Oginsky, E.L., A.E. Stein and M.A. Greer, Proc. Soc. Exp. Biol. Med. 119:360 (1965).
29. Tani, N., M. Ohtsuru and T. Hata, Agric. Biol. Chem. 38:1623 (1974).
30. Daxenbichler, M.E., C.H. VanEtten and I.A. Wolff, Biochemistry 4:318 (1965).
31. Daxenbichler, M.E., C.H. VanEtten, W.F. Tallent and I.A. Wolff, Can. J. Chem. 45:1971 (1967).
32. Daxenbichler, M.E., C.H. VanEtten and I.A. Wolff, Phytochemistry 7:989 (1968).
33. VanEtten, C.H., M.E. Daxenbichler and I.A. Wolff, J. Agric. Food Chem. 17:483 (1969).
34. Tookey, H.L., Can. J. Biochem. 51:1654 (1973).
35. Cole, R.A., Phytochemistry 17:1563 (1978).
36. Hesketh, H.R., C.R. Creger and J.R. Couch, Poult. Sci. 42: 1276 (1963).
37. Kirk, L.D., G.C. Mustakas, E.L. Griffin, Jr., JAOCS 43:550 (1966).
38. Nishie, K., and M.E. Daxenbichler, Food Cosmet. Toxicol. 18:159 (1980).
39. Nishie, K., and M.E. Daxenbichler, Food Chem. Toxicol. 20: 279 (1982).
40. Gould, D.H., M.R. Gumbmann and M.E. Daxenbichler, Food Cosmet. Toxicol. 18:619 (1980).
41. Khera, K.S., Ibid. 15:61 (1977).
42. Medeiros, H., A.W. Kirleis and R.J. Vetter, JAOCS 55:679 (1978).
43. Lessman, K.J., and A.W. Kirleis, Crop Sci. 19:189 (1979).
44. Kirleis, A.W., and H. Medeiros Brown, J. Food Sci. 45:1367 (1980).
45. Stuchly, S.S., and M.A.K. Hamid, J. Microwave Power 7:117 (1972).
46. Fanslow, G.E., L.L. Gittins, W.F. Wedin and N.P. Martin, Ibid. 8:83 (1973).
47. Hamid, M.A.K., N.J. Mostowy and P. Bhartia, Ibid. 10:109 (1975).
48. Okabe, T., M.T. Huang and S. Okamura, J. Agric. Eng. Res. 18:58 (1973).
49. Lichtenstein, E.P., Adv. Chem. Ser. 53:34 (1966).
50. Eckenrode, C.J., and H. Arn, J. Econ. Entomol. 65:1343 (1972).
51. Hicks, K.L., Ann. Entomol. Soc. Am. 67:261 (1974).
52. Erickson, J.M., and P. Feeney, Ecology 55:103 (1974).
53. Lambert, J.L., D.C. Clanton, I.A. Wolff and G.C. Mustakas, J. Anim. Sci. 31:601 (1970).
54. Perry, T.W., W.H. Tallent, W.M. Beeson and M.T. Mohler, Ibid. 37:353 (1973).
55. Perry, T.W., W.F. Kwolek, H.L. Tookey, L.H. Princen, W.M. Beeson and M.T. Mohler, Ibid. 48:758 (1979).
56. VanEtten, C.H., M.E. Daxenbichler, W. Schroeder, L.H. Princen and T.W. Perry, Can. J. Anim. Sci. 57:75 (1977).
57. Anon., Fed. Regist. 44:65190, November 9 (1979).
58. Anon., Code of Federal Regulations, Title 21, Part 573, Government Printing Office, Washington, DC, 1980.
59. Anon., Fed. Regist. 46:30081, June 5 (1981).
60. Mustakas, G.C., and K.D. Carlson, JAOCS 56:29 (1979).

[Received July 14, 1983]