Crambe Seed Processing: Improved Feed Meal by Ammoniation[1]

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Abstract

An improved crambe meal has been developed by using an ammonia-heat treatment to give significantly better nutritive value and acceptability. The quantity of ammonia permanently bound as nitrogen in the processed meal was from 0.5 to 1.5% of the meal weight and varied with conditions of reaction. Destruction of the undesirable thioglucoside fraction of the meal was demonstrated by paper chromatograph changes and by the absence of the thioglucoside conversion product thiooxazolidone. Ultraviolet-absorbing compounds in the meal, at least one of which is associated with bitterness, were also modified. Feeding experiments with chicks and cattle show the improved palatability and nutritional quality. Incorporation of the ammonia reaction into desolventizer-toaster operations should be possible to provide an economical means of improving the feeding value of crambe meal.

Introduction

INDUSTRIAL INTEREST in *Crambe abyssinica* as a new oilseed crop is increasing as new potential markets for the oil develop; however, the economics of processing crambe for oil and meal depend on the meal serving as a successful protein supplement for livestock. Although crambe protein appears well-balanced in essential amino acids, conventionally prepared meal contains substances that are growth inhibitory and unpalatable to some animals.

Previously, we reported bench-scale filtration-extraction of crambe seed (9). Successful plant-scale processing of this oilseed by prepress-solvent extraction was also reported by Mustakas et al. (14). Both studies demonstrate that, provided reasonable precautions are taken, crambe seed can be processed by either prepress-solvent extraction or direct extraction to yield an oil low in free-fatty acids and with good refining and bleaching characteristics. The residue meal from both processes, however, presents problems for acceptance as an animal feed because it is growth inhibitory and goitrogenic to nonruminants (3,7,20) and unpalatable to ruminants (4). Since more than 1.25 tons of hull-free meal is produced per ton of oil, the overall economics of processing are influenced considerably by the value of this by-product.

Compounds in crambe meal responsible for the undesirable effects have not been clearly defined because pure fractions have not as yet been fed to test animals. Similarities between the major thioglucoside of crambe (6) and of rapeseed, however, leave little doubt that this meal constituent is responsible for crambe's goitrogenicity. This thioglucoside, *epi*-progoitrin, may also be responsible for crambe's growth inhibitory effects since Kirk et al. (9), VanEtten et al. (20) and Tookey et al. (17) have shown in rat-feeding experiments that growth is either much improved or normal on crambe meals treated to modify or remove the thioglucosides. Crambe meal also contains sinapine (2), a compound bitter to human taste. The reluctance of certain animals to eat the meal is probably related to the presence of this compound. Both of these undesirable compounds in crambe are destroyed by an ammonia-heat treatment of the defatted meal. The resulting meal has improved nutritive quality and acceptability. The ammoniation process described here is both economical and adaptable to existing oilseed plants.

Experimental

Materials, Methods and Equipment

The principal raw material for ammoniation was a prepress-solvent-extracted crambe meal prepared on a commercial plant scale (14). Pertinent analyses of the dehulled source seed and of the defatted press-cake product are given in Table I. The defatted flakes were prepared from the same source seed as the extracted meal. Separated hull contained 1.8% fat, 6.9% protein and 13.5% moisture. Hull-intact seed contained 31% hull; however, about 15% of the seed as received was free of hull.

Since crambe seed contains enzymes capable of converting *epi*-progoitrin to goitrogenic substances, the derived full-fat or defatted meals should not be ammoniated at moisture content above approximately 10% unless the enzyme system has previously been deactivated. This can be accomplished by steaming for 30 min at 212F. The prepress-solvent-extracted meal used here was enzyme-inactivated during production by the heat and steam employed in desolventization.

Gaseous ammonia was anhydrous compressed ammonia, and the aqueous ammonia reagent contained 29.6% ammonia.

Thioglucoside content of dehulled seed was determined by the sulfate method of McGhee (11) and total thiooxazolidone by the method of Wetter (21) at pH 5.9. The thiooxazolidone analysis was used to estimate residual *epi*-progoitrin thioglucoside in ammoniated meals. By this method *epi*-progoitrin thioglucoside is enzymatically converted to thiooxa-

TABLE I

Analyses of Crambe Seed and Meal, Per Cent

Constituent	Dehulled seed	Prepress-solvent extracted meal
Moisture	6.1	7.7
Crude fat	42.7	3.6
Protein (N × 6.25)	25.2	40.6
Thiooxazolidone content		
Free	.....	0.09
Total[a]	1.09	1.60
Thioglucoside	5.2	[b]
Sinapine	0.28	0.46
Reducing sugars as glucose	1.8	2.6
	(3.5[c])	(2.9[c])

[a] Includes both the free and the potential thiooxazolidone attainable after enzymatic hydrolysis.
[b] A comparison of thioglucoside UV absorption peaks at 220 mμ indicates that 90% of the original thioglucoside of the seed is retained in the process meal.
[c] Per cent reducing sugars as glucose on moisture- and fat-free basis.

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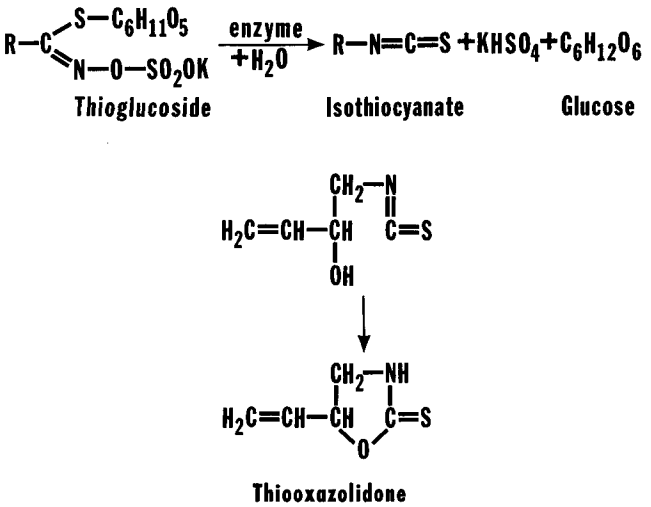

FIG. 1. Enzymatic hydrolysis of *epi*-progoitrin.

zolidone as illustrated in Figure 1. Meals were also checked for "free" thiooxazolidone by modifying Wetter's procedure so that the sample was initially enzyme-deactivated by boiling for 5 min and no new enzyme was added.

Reducing sugars were determined by the method of Munson and Walker (12) isolated from the meal as described by McGhee (10). Sinapine was determined by the method of Tzagoloff (18) with the modifications suggested by Austin and Wolff (2). Fat and nitrogen analyses were according to AOCS Official Methods (15).

Water extracts of crambe meals were chromatographed on paper by descending technique employing the top layer of an *n*-butanol:ethanol:water-solvent mixture in volume ratio 4:1:4 as developing solvent. The extract was prepared in the following manner: A 5-g meal sample was boiled 5 min in 60 ml of distilled water, held 10 min without additional heat and vacuum-filtered; the cake was re-extracted three times in 25 ml of hot water. The filtrates were combined and the total volume adjusted to 125 ml. A 10-μl sample of extract was spotted on the chromatogram. Thioglucoside and sugar spots were made visible by spraying or dipping first in a solution prepared by dissolving 0.5 ml of a saturated aqueous silver nitrate solution in 100 ml of acetone containing 2.5 ml of water and then in a solution prepared by the addition of a solution of four sodium hydroxide pellets (approximately 1 g) in 5 ml water to 100 ml of ethanol. Fluorescent spots were observed by exposure of the chromatogram to a "long-wave" UV lamp before spraying with silver nitrate.

Bound ammonia in the meals was determined by nitrogen analysis before and after ammoniation.

The rate at which the meals sorbed ammonia at atmospheric pressure was determined from a material balance based on the addition rate and the rate at which excess ammonia left the reactor. With ammonia entering from the bottom of the reactor at a rate greater than that at which it could be sorbed by the meal, the excess gas passed into the reactor headspace and vented through the condenser system. By periodically closing the vent and measuring the rate of pressure rise ($\Delta P/\Delta t$) in the headspace, it was possbile to calculate the rate ($\Delta W/\Delta t$) at which excess ammonia was leaving the reactor during any stage of the ammoniation. The following ideal gas law relationship indicates the method of calculation: $\Delta W/\Delta t = (\Delta P/\Delta t) \times V/RT \times$ mol wt NH_3 where $\Delta W/\Delta t$ = rate of ammonia leaving reactor in pounds per min, $\Delta P/\Delta t$ = rate of pressure rise in inches of conc NH_4OH per min, V = reactor volume in in.^{3}, T = the absolute temperature in °R, and R = the gas constant equivalent to $5.72 \times 10^5 \frac{(\text{in. of } NH_4OH)\,(\text{in.}^3)}{(\text{pound mole})\,(^\circ R)}$. The rate of ammonia sorption by the meal was then calculated as equivalent to the rate of ammonia minus $\Delta W/\Delta t$.

The quantity of ammonia removed during steaming and drying operations was determined by absorption in excess hydrochloric acid and titration with N sodium hydroxide.

The pressure-ammoniation equipment consisted of a jacketed spherical cooker of 12-in. internal diameter and rotated at 1 rpm. A rotary steam valve permitted maintenance of steam pressure in the jacket during rotation; however, it was necessary to stop the reactor for addition of ammonia. This apparatus is shown in Figure 2. A thermowell in the center and a pressure indicator for measurement of internal temperature and pressure complete the apparatus. Ammonia was added through the valve shown at the bottom of the cooker. Pressure-ammoniated meals were moistened and steamed in the 5-gal steam-jacketed cooker described by Mustakas et al. (13). The unit was equipped with an air-driven meshing-rod agitation system, steam sparge coil, spray nozzle and condenser.

Equipment for atmospheric ammoniation was a 1-cu ft ribbon blender (Fig. 3). Gaseous ammonia was introduced through a 1/8-in. hole in the discharge plug at the bottom. The lid was fitted with a spray nozzle for introduction of water and with a vapor outlet having a globe valve and water condenser. The con-

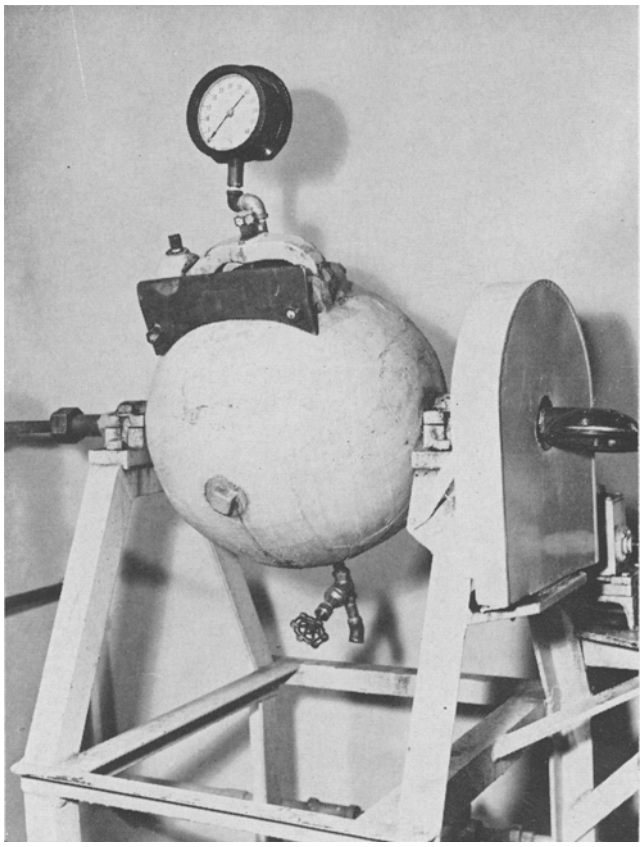

FIG. 2. Pressure reactor for ammoniation of crambe meal.

denser discharged through two scrubber bottles in series. The same equipment was used for aqueous ammoniations.

Corrugated rolls, dehulling equipment and smooth rolls for preparation of dehulled full-fat flakes were described previously (9). The flakes were defatted batchwise with hexane at 140F.

Procedure

Pressure Ammoniation with Anhydrous Ammonia. Six pounds of meal was charged to the reactor and the unit sealed. With the reactor rotated so that the gas entrance port was horizontal, ammonia was added to 10 psig internal pressure. The pressure was maintained at 10 psig for 5 min, after which the addition valve was closed and the ammonia tube removed. The reactor was rotated for 10 min and the procedure repeated until the desired quantity of ammonia was added (usually in three additions). After the last addition, steam at 20 psig was admitted to the jacket and the temperature raised to the desired level, generally 230F. After 15 min at temperature the jacket steam was vented and the reactor allowed to turn an additional 15 min without further heating. At the end of this period the reactor was vented and the ammoniated meal discharged. The meal from two ammoniations was placed in the 5-gal cooker, heated to 185F, moistened to 30%, steamed 30 min, dried 30 min and discharged to air cool.

Atmospheric Ammoniation with Anhydrous Ammonia. Fifteen pounds of meal was charged to the ribbon blender and the meal heated to the desired temperature with lid in place but with the vapor outlet open. If moisture was to be added, the lid was generally raised and the water heated to the meal temperature before addition. Addition of the calculated amount of water in this manner gave the desired moisture levels as determined by analysis of samples removed after a few minutes of mixing. With the reactor vent open, ammonia was added through the inlet port maintaining a constant rate of flow. The reactor vent was closed periodically for about 1 min to determine the sorption rate as already described. During these periods, the resultant pressure increase seldom exceeded 8 in. of water. After the desired reaction period of 60 min, ammonia flow was stopped and water was sprayed into the charge to 30%. Steam was admitted for 30 min through the ammonia inlet pipe and finally the charge was dried for 30 min before discharging. The condensate and vapor were collected in the vapor scrubber bottles. Where ammoniations were conducted at temperatures below 185F, the charge was heated to this temperature before addition of the steaming moisture to avoid the formation of tough pastes. Such pastes formed in defatted meal when the moisture level exceeded approximately 17%.

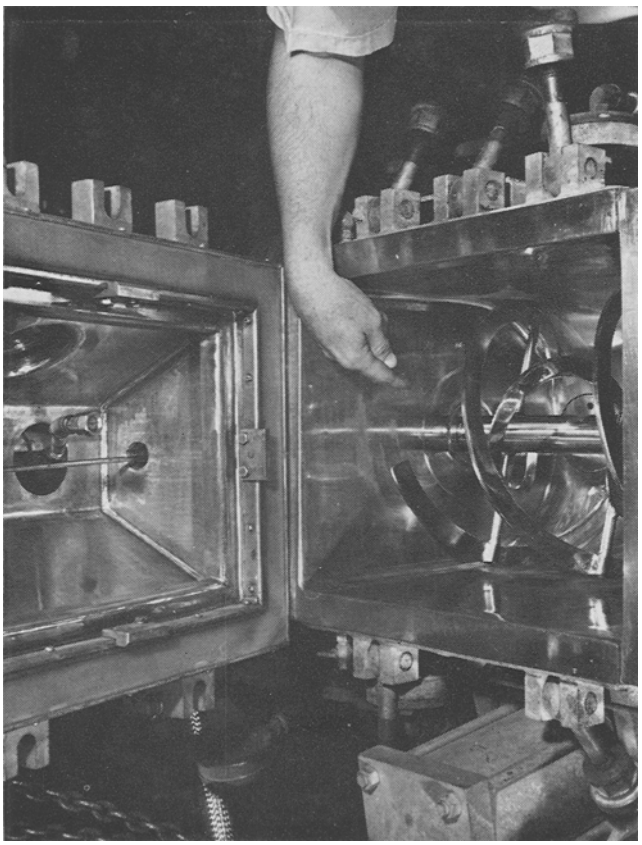

FIG. 3. Reactor for ammoniation of crambe meal at atmospheric pressure.

Atmospheric Ammoniation with Aqueous Ammonia. The meal was heated to 185F or higher in the ribbon blender and aqueous ammonia was added (0.015 lb of ammonia/lb of meal). After holding 60 min at the reaction temperature, the charge was steamed 30 min, dried 30 min and discharged.

Ammoniations were conducted on defatted meals except for a single test with full-fat flakes.

Feeding Studies. Chick feeding studies were conducted by the Wisconsin Alumni Research Foundation, Madison, and cattle acceptance tests by the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln. Day-old chicks were fed ad libitum in groups of 20 for a 4-week period. The birds were weighed individually at the end of each week. Ten birds from each group were sacrificed at the conclusion of the experiment for gross examination of body organs and determination of thyroid weight.

Results and Discussion

Destruction of Thioglucoside

The destruction of *epi*-progoitrin accompanying the ammoniation of crambe meal was demonstrated by paper chromatograms taken before and after treatment. The thioglucoside spot near R_f 0.1 (Fig. 4) observed in untreated meal was not present after ammoniation. The lighter unidentified spot near R_f 0.12 visible in the ammoniated meal chromatogram was nearly masked in the chromatogram of untreated meal by the partially overlying thioglucoside spot. The spot near R_f 0.17 which appeared darker after ammoniation was also not identified. The fluorescent spots outlined in white are discussed later in connection with sinapine destruction.

An estimate of *epi*-progoitrin content was made by determining the amount of thiooxazolidone produced by enzymatic hydrolysis. Since other products of hydrolysis can be obtained with different conditions of moisture, pH and temperature (9,19), the conditions of hydrolysis specified in the methods section must be carefully followed. Because enzymatic hydrolysis of ammoniated meal by the prescribed method did not produce thiooxazolidone, destruction of the parent thioglucoside was demonstrated (see Table II). The trace detected in atmospherically ammoniated prepress-solvent-extracted meal was present before ammoniation (see Table I) and apparently exists in the free state. Since this trace was not detected in

TABLE II

Analyses of Ammoniated Crambe Meals[a]

	Pressure			Atmospheric				
	Prepress-solvent extracted	Prepress-solvent extracted	Defatted flakes	Prepress-solvent extracted	Prepress-solvent extracted	Prepress-solvent extracted	Prepress-solvent extracted	Prepress-solvent extracted
Moisture, %	7.7	7.7	7.7	7.4	16.0	15.0	17.0	8.0
Maximum temperature, F[b]	230	212	230	208	208	180	208	90
Ammonia	Anhydrous gas	Anhydrous gas	Anhydrous gas	Anhydrous gas	Anhydrous gas	Anhydrous gas	Aqueous *liquid*	Anhydrous gas
Reaction time, min	30	30	30	76	77	60	60	60
Ammonia added[c] (% of m.f.,f.f. meal)	....	....	....	2.02	2.05	1.35	1.51	0.64
Results, m.f.,f.f. basis								
Bond ammonia after steaming, %	1.41	0.79	1.35	1.30	1.12	0.35	0.79	0.61
Thiooxazolidone, total %[d]	0.00	0.04	0.00	0.00	.....	.....	0.00	0.14
Sinapine, %	0.053	0.12	0.058	0.063	0.064	0.12	0.12	0.32
Reducing sugar, %	1.1	2.1	.....	1.7	1.1	2.2	1.4	.....

[a] Important differences in control variables are underlined.
[b] Maximum temperature during ammonia addition.
[c] Moisture-free, fat-free meal.
[d] Includes both the free and the potential thiooxazolidone obtainable after enzymatic hydrolysis.

many ammoniated meals, apparently under some conditions thiooxazolidone was also destroyed by ammoniation. Thioglucoside appeared to be destroyed equally well under all conditions of moisture, temperature and pressure used.

Destruction of Sinapine

The sinapine content of prepress-solvent-extracted crambe meal was 0.52% (moisture-free, fat-free basis). This compound (R_f 0.45) was readily detected on paper chromatograms of meal extracts due to its fluorescence, whereas there was little or no fluorescence on the paper chromatograms of ammoniated meals (see Fig. 4—fluorescent compounds are delineated by white lines). The fluorescent spot at R_f near 0.1 was also removed by ammoniation. Since this compound has not been identified, the significance of its removal is not known. Although not completely destroyed under any of the conditions used, the sinapine content of crambe meal was reduced as much as 90% by ammoniation (see Table II). Destruction of sinapine was favored by: (a) addition of moisture, (b) elevated temperature and (c) higher levels of ammonia addition. Aqueous ammonia was not as effective in reducing sinapine content as gaseous, perhaps a matter of distribution.

Binding of Ammonia

Ammonia was retained by processed meals in two forms: as a sorbed ammonia which was vacuum labile and as chemically bound ammonia stable to vacuum. For example, a typical ammoniated-steamed meal retaining 1.12% ammonia initially was reduced to 0.8% in 20 hr under vacuum. Additional ammonia was not removed even after 120 hr under vacuum.

Vacuum labile ammonia was observed by Seehof and Benson (16) in ammonia-protein studies. They concluded that this ammonia sorbed to protein in a manner very similar to water and that the mechanisms responsible are "considerably complicated."

The remaining strongly bound ammonia undoubtedly represents ammonia that has reacted chemically with constituents of the meal. The most likely reactions are those with (a) thioglucoside, (b) sinapine, (c) reducing sugars and (d) proteins.

Preliminary studies of the ammonia-thioglucoside reaction have indicated an equimolar reaction between the two compounds. Such a reaction would account for 0.4% bound ammonia-based on the thioglucoside content of the meal.

The exact function of ammonia in the destruction of sinapine is not known, but sinapine is unstable under basic conditions (18). If chemical combination with sinapine does occur, the amount of bound ammonia resulting from such a reaction would be small, about 0.03% for an equimolar reaction.

Permanent binding of ammonia undoubtedly resulted from reaction with reducing sugars. The quantity of these sugars in crambe (as glucose) decreased appreciably during ammoniation as shown by a comparison of data in Tables I and II. The decrease in reducing sugars resulting from ammoniation in these runs accounts for 0.1–0.2% bound ammonia calculated on an equimolar basis.

Seehof and Benson (16) found that when proteins from various sources were contacted with ammonia gas, a small amount was permanently bound, presumably through reactions with free acidic groups of the amino acids in the protein chain. The actual quantity observed by these workers could account for a permanent binding of ammonia by the protein present in crambe meal of 0.1% ammonia.

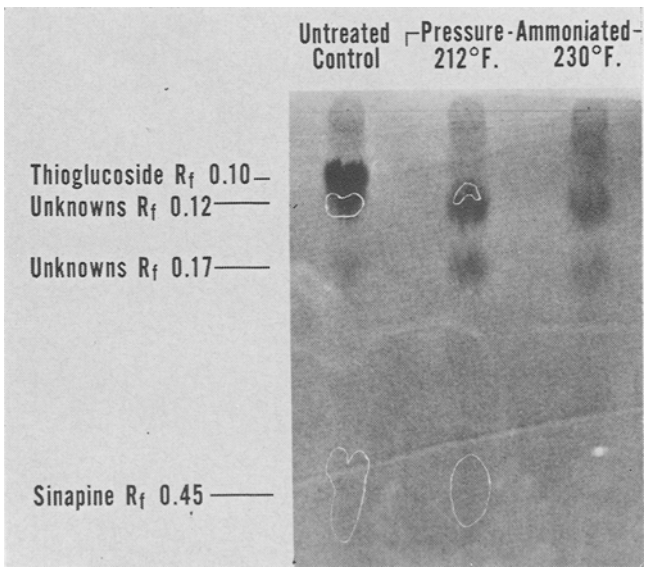

FIG. 4. Paper chromatogram illustrating changes in thioglucoside and in crambe meal achieved by ammoniation. Fluorescent compounds delineated by white lines.

TABLE III

Effect of Ammoniation on Feeding Quality of Crambe Meal to Chicks[a]

Treatment	Mortality	Growth as % of control	Feed consumption as % of control	Feed eff. lb feed/lb gain	Condition of body organs thyroid size mg/100 g body wt	Other organs
None	11 of 20	68	....	....	84.4	Liver discolored
Ammoniation[b]	No deaths	82	91	2.02	11.4[c]	Normal
Soy-corn control	No deaths	100	100	1.81	10.5	Normal

[a] Prepress-solvent-extracted crambe meal.
[b] Meal is the same as column 1, Table II.
[c] Not statistically different from the control at the 95% confidence limit.

The total bond ammonia probably from these reactions (0.7%) checks well with the 0.8% determined by analysis of the vacuum-stripped meal (Table III).

Ammonia Sorption Rates and Efficiencies

Ammoniation efficiency is defined as the percentage of ammonia used which is retained in the steamed meal. Losses include: (a) ammonia not sorbed by the meal during introduction of the gas and (b) ammonia removed by steam stripping after reaction.

Except for volatile losses in steaming, ammonia losses in either pressure or aqueous runs do not occur. Efficiencies are high provided the quantity of ammonia used is not excessive. For example, a material balance of a gaseous pressure run in which 1 lb of ammonia was added per 100 lb of meal indicated an ammoniation efficiency of 79%.

In atmospheric runs where the reactor is not sealed, ammonia loss occurs if the gas is introduced at a rate faster than the rate of sorption by the meal. It was possible to minimize this loss by adding ammonia at the same rate as the rate of sorption. Figure 5 illustrates sorption rates for crambe meal at 208F and moisture levels of 7.4 and 16.1%.

Moisture and protein are believed the major factors in determining sorption rate and capacity. The average sorption rate during the first 60 min of the 16.1% moisture run was 0.0237 lb ammonia/min/100 lb of meal. This is the rate at which ammonia can be added to crambe meal under the stated conditions of moisture and temperature without loss to the atmosphere. Approximately 45 min are required to add ammonia to a level of 1 lb/100 lb of meal under these conditions.

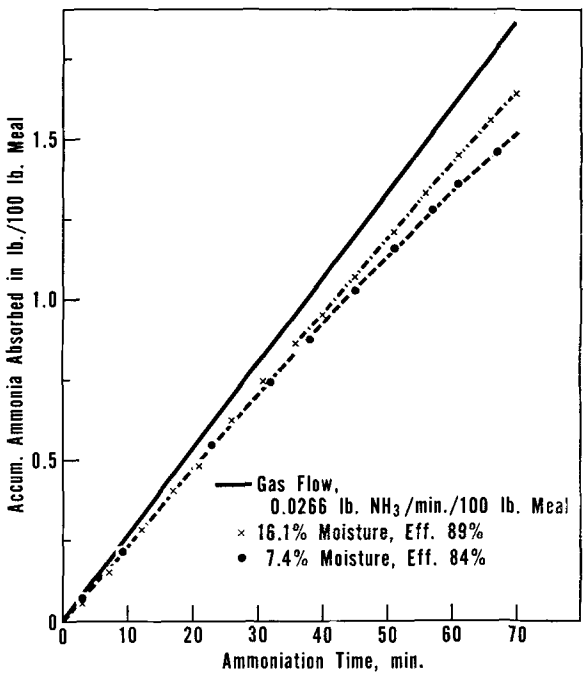

FIG. 5. Sorption of ammonia by crambe meals at atmospheric pressure and 205–210F.

TABLE IV

Chick Ration

Ingredient	Ration, %
Ground yellow corn	58.7
44% Soybean oil meal	12.5
Crambe meal	20.0[a]
$CaHPO_4 \cdot 2H_2O$	2.0
$CaCO_3$	2.0
NaCl	0.5
$MnSO_4$	(15 g)
Vitamin supplement	1.0[b]
Added corn and soybean meal	3.3[c]

[a] In the control ration, all crambe was replaced with soybean oil meal.
[b] Vitamin mix per kilo of ration: thiamin, 10.1 mg; niacin, 100.0 mg; riboflavin, 16.0 mg; Ca pantothenate, 20.0 mg; menadione, 1.0 mg; B_{12}, 0.02 mg; B_6, 6.0 mg; biotin, 0.6 mg; folic acid, 4.0 mg; vitamin E, 10 units; choline, 2 units; vitamin A, 10,000 units; vitamin D, 1200 units.
[c] Additional corn and soybean oil meal as necessary to balance ration to 20% protein.

Steaming loss in atmospheric ammoniations as in gaseous pressure and aqueous ammoniations is dependent on the amount of ammonia added and becomes excessive when the quantity added exceeds that which can be permanently bound by the meal.

Figure 6 illustrates this relationship for meals ammoniated at atmospheric pressure at two moisture levels and various temperatures.

Feeding Results

In Table III are shown the results of feeding prepressed-solvent-extracted crambe meal before and after pressure ammoniation. Composition of the ration is given in Table IV. Chicks consuming the untreated meal developed enlarged thyroids, grew poorly and suffered high mortality. The treated meal was slightly less palatable than the soy control; however, there were no deaths in the 4-week feeding period. Growth was significantly improved over crambe meal without ammoniation, and all body organs including thyroids appeared normal on autopsy. Additional feeding studies are needed to determine the feeding value of gaseous-ammoniated meals prepared at atmospheric pressure and to determine the protein quality of am-

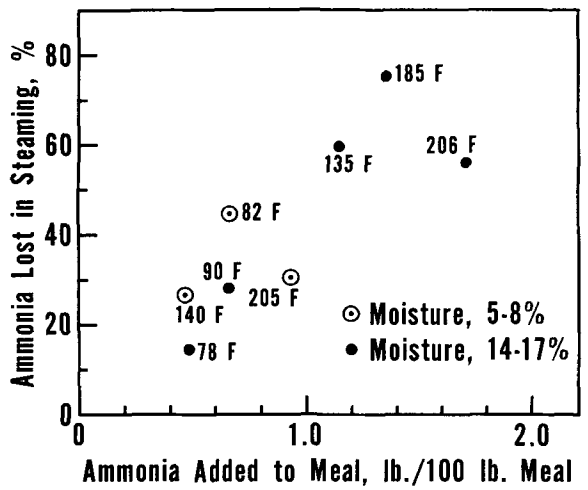

FIG. 6. Ammonia loss in steaming as a function of ammonia used in reaction at varying temperature and moisture levels.

moniated crambe meal. Since total growth was almost proportional to consumption, palatability may be the major factor responsible for the lower growth observed with ammoniated crambe meal. Sinapine is probably not the major factor affecting palatability in chick feeding since rapeseed oil meals which contain this compound and control diets to which sinapine has been added have been fed with no palatability problems reported (3).

In a short-term acceptance test of prepress-solvent-extracted crambe meal before and after pressure ammoniation, mature cows refused the untreated meal but consumed the ammoniated meal readily. Ammoniation apparently overcame the palatability problem with these animals, possibly associated with the sinapine content.

Workers have reported good results in the feeding of many ammoniated products to cattle including citrus pulps, bagasse, and beet pulp (1,8); however, the significance of some of this work his been disputed by Davis et al. (5). The nitrogen added in ammoniation is equivalent to the nitrogen in 2.5 to 8% protein, so that a significant increase in meal value to ruminants might be achieved if the additional nitrogen is utilized. Possible benefits of this increased nitrogen can only be decided, however, by actual feeding experiments.

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