Urea Breakdown Activity in Cottonseed

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Abstract

Cottonseed meal, when mixed with urea, may cause breakdown of the urea to ammonia. Compounds in the meal which cause this reaction may be eliminated or greatly reduced in concentration by cooking the cottonseed prior to extraction, or by heat-treating the finished meal. Passage of the extracted meal through two desolventizer-toasters greatly reduced the urea breakdown activity and caused only a slight loss of nutritive value.

Introduction

UNTIL RECENTLY, there had been no problem of cottonseed products breaking down urea to release carbon dioxide and ammonia. However, due to the recent incorporation of larger quantities of urea into finished rations for animal feed, the problem has arisen, especially with cottonseed meal of low-heat treatment history. Investigations here have been developed to answer these questions: 1) what degree and kind of heat is necessary to deactivate the agent producing urea breakdown in cottonseed meal; 2) what damage does this heat do to the nutritive value of the meal; and 3) are cottonseed and soybeans the only source materials that show this activity.

A comparison is given of laboratory methods used to measure this activity. No attempt is made here to identify the causative agent. It is presumed to be urease, as is the case with soybeans and jackbeans. Because of limited facilities, the isolation, purification, and identification of the causative agent was not undertaken.

A study of the effect of sterilization of the cottonseed, and of handling the material in the laboratory under sterile conditions, is included.

Experimental

Preliminary investigations of a qualitative nature, using variously treated cottonseed meal, to which urea had been added, indicated that raw cottonseed kernels and uncooked cottonseed flakes caused the greatest degree of urea breakdown, as determined by amounts of ammonia released. Failure of the modified Caskey-Knapp test (4) to give reproducible results led to a search of other methods. A modified Kjeldahl procedure was used to measure the ammonia released by the activity of the various cottonseed fractions and meal treats with added urea. One procedure follows. One g of sample and 1 g urea are put in a Kjeldahl flask. Water is added (300 ml) and the mixture agitated. The flask is then tightly stoppered and placed in an incubator at 37.5C overnight. Then 1 g finely powdered magnesium oxide, and glass beads heavily coated with an antifoam compound (Dow Corning Type A) are added and the flask connected to a Kjeldahl distillation unit, using moderate heat. Ammonia is condensed in standard H_2SO_4 solution and is back-titrated with standard NaOH solution with methyl red as indicator.

An alternate procedure is the use of standard boric acid solution to collect the ammonia and titration with standard HCl solution, using a mixture of brom cresol green and methyl red as the indicator (3). This method works well and eliminates the fading end-point of the previously described procedure. A urea blank determination is run with each batch. The amount of ammonia released from urea alone is subtracted from the total amount measured from sample and urea.

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TABLE I

Survey of Variously Treated Cottonseed Meals

Sample no.	Description	% H_2O	% NH_3
I	Raw flakes (tempered only)	8.8	4.63
II	DT meal, two DT's[a]	8.9	1.66
III	DT meal, one DT	13.5	3.45
IV	Production meal, one DT 11-2-61	13.9	3.80
V	Production meal, one DT 11-4-61	14.4	2.11
VI	Production meal, two DT's	9.7	1.25
VII	Meal with gums added, one DT	8.8	2.93
VIII	Urea blank	0	.62

[a] Meal treated twice in a desolventizer-toaster.

Trial 2-hr incubation periods failed to give reproducible results and were abandoned in favor of the overnight incubation periods, which gave excellent checks.

Table I shows results of a survey of various cottonseed fractions and cottonseed meals. Table II shows the effects on urea breakdown activity of various cooking and drying treatments on meals. It should be noted that Sample IIC was the only one in which urea breakdown activity was reduced to zero. This sample was subjected to a severe cooking treatment, which caused the meal to darken appreciably, casting doubt on its nutritive value. Table III shows the effects of the various treatments on protein solubility and free gossypol content.

As urea breakdown activity decreased, so did protein solubility and free gossypol content. Dry heating or drying per se, either following a cook treatment or in lieu of it, had little effect on lowering urea activity. The temperature used (101C) was mild. The only successful treatment, as far as urea breakdown activity is concerned, was a wet cook at higher temperatures (Sample IIC).

Presumably it is difficult to deactivate urea breakdown activity in finished meals. This is probably what gave rise to the problem in the first place. Perhaps a better approach is to treat the rolled flakes with considerable moisture and heat before extraction. Plains Cooperative Mill uses a low-heat treatment process. If this is coupled with high-moisture flake and meal, the problem of urea breakdown is accentuated. Because the mill does not cook prior to extraction, but only tempers the flakes with mild heat, it does not reduce urea breakdown activity sufficiently to prevent its occurring in finished meal if urea is added by feed manufacturers.

Considerable lowering of urea activity is accomplished when meal produced at Plains Cooperative

TABLE II

Comparison of Cook and Dry Treatments

Sample no.	Description	% H_2O	% NH_3
II	DT meal, two DT's[a] (control)	8.9	1.66
IIA	Cooked 1 hr at 186F	13.7	1.64
IIB	Cooked 90 min at 223F	15.9	1.35
IIC	Cooked 30 min at 260F	16.0	0
IID	Cooked 1 hr at 186F and dried 30 min at 101C	12.2	1.49
IIE	Dried 1 hr at 101C, no cook	0.97	1.34
IIF	Cooked 1 hr at 186F and dried 1 hr at 101C	0.86	1.32

[a] Meal treated twice in a desolventizer-toaster.

TABLE III

Effect of Cook Treatments on Nutritional Indices

Sample no.	Description	% H_2O	% NH_3	% Prot. sol.	% Free Gossypol
1	Raw flakes	8.8	4.63	79.60	.56
2	Control meal	8.9	1.66	77.00	.18
3	Cooked 1 hr at 186F	13.7	1.64	73.68	.13
4	Cooked 90 min at 223F	15.9	1.35	72.84	.08
5	Cooked 30 min at 260F	16.0	0	54.14	.06
6	Prod. meal, one DT	13.9	3.80	76.22	.13
7	Prod. meal, two DT's [a]	9.7	1.25	74.00	.16
8	Meal with gums added, one DT	8.8	2.93	73.92	.10

[a] Meal treated twice in a desolventizer-toaster.

Oil Mill is passed through two desolventizer-toasters (DT's) instead of one (Samples 6 and 7, Table III). In November, 1961, the mill added a second 100-in. 10-high DT to meet load requirements and to produce a dryer meal. The addition of the second DT also reduced hexane losses significantly.

The two DT's in parallel give more contact time with moist heat to the meal, thus reducing urea breakdown activity without significantly lowering nutritive indices, with the possible exception of a 2.2% lowering of protein solubility.

Table IV shows the effect of the various laboratory cooking treatments on total gossypol and lysine with free epsilon-amino groups. Again it will be noticed that the only significant lowering of nutritive value occurs in the severely cooked sample, No. 5.

Sample 8 has low free gossypol, but about the same total gossypol as all other meals. This sample was 3 months old and contained added cottonseed oil gums. Apparently free gossypol had been converted to the bound form.

It is necessary to explain that a second method for determining urea breakdown activity was used in the latter portion of this work. This is the colorimetric method of Ains (1) which is based on the reaction of *p*-dimethylaminobenzaldehyde with urea in a buffered, decolorized medium. Samples are agitated in a known amount of urea solution and incubated with occasional shaking for 30 min at 40C. The samples are then decolorized with potassium ferrocyanide, zinc sulfate, and charcoal in an acid medium, using mechanical agitation for 15 min. The solutions are then filtered, aliquots prepared, and *p*-dimethylaminobenzaldehyde is added to aliquots. A blank and a standard solution are also prepared. After 10 min color-development time at constant temp, 25C in all cases, samples are read in a spectrophotometer at 425 mμ. The reduction in urea concentration from the known value of the standard is determined from a standard curve and expressed in mg/l decrease. This figure, the amount of reduction, is shown as Urease number in Tables V, VI and VII.

Thus far the cause of urea breakdown activity in

TABLE IV

Effect of Cook Treatments on Total Gossypol and ε-Amino Lysine

Sample no.	Description	% Prot.	% Free gossypol	% Total Gossypol	Lysine with free e-amino groups	
					% Sample	% Protein
1	Raw flakes	30.88	.45	.68	.96	3.11
2	Control meal	41.75	.16	.70	1.41	3.38
3	Cooked 1 hr at 186F	41.44	.12	.78	1.38	3.33
4	Cooked 90 min at 223F	39.81	.09	.80	1.38	3.47
5	Cooked 30 min at 260F	41.75	.06	.78	1.18	2.83
6	Prod. meal, one DT	42.69	.14	.80	1.45	3.40
7	Prod. meal, two DT's	41.44	.18	.78	1.45	3.50
8	Meal, gums added, one DT	45.00	.12	.75	1.41	3.13

Courtesy C. M. Lyman, Dept. of Biochemistry, Texas A&M College.

TABLE V

Results of Sterile vs. Unsterile Conditions

Sample no.	Description	% NH_3 by mod. Kjeldahl	Urease no. by colorimetric
16A	Raw kernels, unsterilized	3.68	21
16B	Cold extracted kernels	4.47	18
17A	Raw kernels, sterile technique	4.63	23
17B	Raw kernels, unsterile	4.63	23
18A	Gin seed, sterilized with steam	3.49	21
18B	Sterilized seed, 10 days later	3.49	21
18C	Sterilized seed, cold extracted	4.30	21

cottonseed has not been discussed. Presumably it is urease, but this report offers no conclusive or concrete evidence such as isolation, purification, and identification of the enzyme urease. However, a literature search fails to reveal anything other than urease which is capable of decomposing urea at ordinary temperatures.

Table V shows a comparison of sterile vs. unsterile techniques used in the laboratory, and of sterilized cottonseed vs. unsterilized seed. Seed used in this experiment was a single lot of 25 lb. The seed was from Paymaster 101-C variety cotton, machine-stripped, and brought directly to the gin the same day. The cotton had not lain on the ground and the weather was mild and dry. The seeds were caught as they fell from the gin stand in a sterile container and were handled in the laboratory under sterile conditions. They were hulled in a Bauer laboratory mill with plates, chutes, and sample container sprayed with 99% isopropyl alcohol. The kernels were caught in a sterile cup, screened over a sterile screen to separate hulls and kernels. Kernels fell through the ¼ inch screen holes into a pan filled with 99% isopropyl alcohol. The kernels fell less than 1 in. from screen top to pan of alcohol. The mill, screen, cup, and operator's hands were sprayed with alcohol after each opening of the mill as well as beforehand. The kernels were left in the shallow pan and the surplus alcohol was drained off and discarded. Kernels were then dried with mild heat on a large water bath under a stream of air. Dried kernels were ground in a Waring Blendor, weighed in an analytical balance scoop using a spatula, and transferred to a Kjeldahl flask. All apparatus used in contact with the kernels was sterile; also the water and urea used.

Sterilizing the urea reagent was unnecessary, as it is used as an antiseptic in treating bacterial infections, and in dentifrices (2,6). It was sterilized in an oven for 1 hr at 101C to remove surface moisture and any possible bacteria. This temp was too low to convert any urea to biuret (7). Total elapsed time from gin to incubator was 2 hr.

Part of the same lot of seed was allowed to remain open and exposed for 48 hr. Samples were drawn and prepared in the usual manner without sterile technique. Table V shows there was no difference in Sample 17A, prepared by sterile technique, and Sample 17B, prepared by unsterile methods.

The remaining seed was sterilized with steam in the original container. Samples were again prepared, using the same sterilizing procedures as previously described. The percentage of ammonia released from seed so treated was reduced as compared to the raw seed, but by no means eliminated. A second sample taken 10 days later, after the seed had been left open and exposed, showed no rise in amount of ammonia released, after being adjusted to the same moisture basis (Sample 18B). Presumably the decrease in ammonia release was due to heat when seed was steam-sterilized, and not by any bacterial action that was present

TABLE VI
Survey of Various Oilseeds and Meals

Sample no.	Description	% NH_3 by mod. Kjeldahl	Urease no. by colorimetric
13	Ground fresh peanuts	1.50	10
14	Ground fresh pecans	1.65	13
15	Ground fresh coconut	.40	8
19	Citrus meal with lysine	.50	....
25	Peanut meal	.50	8
26	Soybean meal	1.69	14

before sterilization, because in 10 days it did not recur or cause any rise.

A comparison of Samples I, 17A and 22 is of interest. One was from freshly harvested, freshly ginned seed analyzed the same day it was harvested. The other samples came from average mill production flakes from seed which could have been five months old, and had been stored on the ground; yet there is no difference in amount of ammonia released. All three samples have the same value; i.e., 4.63% ammonia released. This evidence tends to rule out any possibility that bacteria either initiate the urea breakdown process or increase it with time in storage, but indicate that raw cottonseed has a fixed level of urease content and that urea breakdown will proceed until this amount of urease is expended.

Table V also shows results of cold-extraction of the various treats of kernels with petroleum ether by the method for determining FFA in seed oil (5). The urea breakdown activity is concentrated in the solid or protein portion of the seed, as in all cases using the modified Kjeldahl method, with a considerable rise in activity. However, a curious thing is noted concerning the colorimetric method. In all cases, no differences are observed between unextracted and extracted kernels. Considerable time elapsed between the time these samples were analyzed by the modified Kjeldahl method and the receipt of the colorimetric method.

Table VI shows a survey of other protein source meals in order to determine if presence of urea breakdown activity is restricted to soybeans, jackbeans, and cottonseed. All meals and seed tested showed some degree of urea breakdown activity regardless of the method used, except that Sample 19 could not be decolorized and therefore could not be tested colorimetrically. This was a citrus meal with 24% L-Lysine added (Pfizer). It could not be decolorized but was the only source of citrus meal available. By using excessive amounts of charcoal this sample was eventually decolorized, but failed to give any meaningful results after repeated attempts. Presumably there remained enough color to interfere, or there was some interfering ion present. At any rate, the colorimetric method failed on this sample. However, as can be seen by the modified Kjeldahl method, apparently citrus meal shows a mild degree of urea breakdown activity.

Other samples in the series responded well to both methods, and showed good correlation between the two methods.

Table VII illustrates further the comparison of the two methods. The samples are all of cottonseed or cottonseed products.

Table VII also shows the effect of adding various substances to finished cottonssed meal to increase fat content and /or to control dustiness, and facilitate mixing and pelleting operations.

It was noted in preliminary qualitative investigations that fresh meal, with cottonseed oil gums added, had less ammonia released than the same meal without added gums. This was verified by both quantitative methods.

TABLE VII
Comparison of Methods

Sample no.	Description	% NH_3 by mod. Kjeldahl	Urease no. by colorimetric
20	Meal with gums, one DT	2.93	24
21	Production meal, two DT's	1.66	13
22	Raw flakes	4.63	27
23	Production meal, two DT's	1.25	8
24	Meal from one DT	2.11	18
20A	Meal with 3% soybean foots added	1.88	16
20B	Meal with 3% acidulated c/s soapstock	1.89	16
III	DT meal, one DT (control)	3.45	29
IV	Production meal, one DT	3.80	31
V	DT meal, one DT	2.11	18

Samples of this same meal were given additions of 3% soybean oil tank settlings or foots, and 3% acidulated cottonseed oil soapstock. Niether of these samples were related weightwise to the amount of gums customarily added to meal in the Plains Cooperative Oil Mill degumming process. The gums added to meal by this process is about 1%w/w.

In all cases, (Samples 20,20A and 20B) reduction of ammonia released was noted in both quantitative methods. Also, the amount of reduction is roughly proportional to the amount of fatty material added, as shown by comparison with Sample no. III, the control meal. Hence, the added material, regardless of origin, acts simply as a diluent and has no inhibiting effect upon urea breakdown activity.

The data in Table VII also show at least two samples of each type of DT meal; i.e., two samples from meal produced using two DT's in the mill, and two samples produced with only one DT, as was true of all meals produced prior to November, 1961.

Samples 24, III, and IV are meals produced using only one DT and without the addition of gums or soapstock. Note the wide ranges of ammonia release activity, and indeed, the high values. These values are compared with those from samples of meals produced using two DT's in parallel (Samples 21 and 23). Not only is urea breakdown activity reduced, but results are more uniform. Therefore, it is to be concluded that increased toasting capacity reduced urea breakdown activity in finished meals. This premise is supported by experience in the soybean meal industry which has adopted the practice of thorough cooking to deactivate urease.

Formerly at Plains Cooperative Oil Mill when only one DT was used, it was difficult to control the temperature and moisture content because of overloading. Control now is easier as the load is split between two DT's. Also, moisture and temperature control equipment has been installed on the meats prep cooker (conditioner). These improvements have contributed to greater product uniformity and considerable reduction of urea breakdown activity, without appreciable impairment of nutritive quality of the meals.

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