amounts of nonprotein nitrogen other than canavanine.

Recently Bell (*1*) reported canavanine in 16 species of the legume family other than those named above.

In the case of *Trigonella foenum-graecum* the canavanine peak following ammonia on the 15-cm. column was resolved into two peaks by rechromatographing on a 50-cm. column. One of these peaks had the same position as authentic canavanine. The second peak was not identified. No evidence of this unidentified material was found in *Sesbania exaltata* or *Coronilla varia*.

Discussion

The ion exchange chromatographic method of separation and analysis was tested by six runs of known amino acid mixtures by two different operators over a period of 9 months. Average recovery of 102 determinations on 16 different amino acids and ammonia was 100.2%, standard deviation 5.68. This accuracy is probably greater than necessary for finding seed meals of nutritionally desirable amino acid content. The method for estimation of tryptophan, including the hydrolysis step, was less accurate. Because recovery of added tryptophan averaged 86%, analytical results were corrected for a 14% loss.

The greatest source of error was the unpredictable destruction of labile amino acids during acid hydrolysis, especially in the presence of carbohydrate. This loss appears typical for each seed meal examined. To evaluate the loss properly or correct for it, a detailed study of hydrolyzing conditions for each seed meal would be required. The loss of cystine was believed the greatest, and for this reason the values reported are probably minimum. Tyrosine, phenylalanine, serine, theronine, proline, arginine, and methionine may have undergone some destruction also.

If the nitrogen distribution after hydrolysis is examined in Table I, better than 95% of the nitrogen is accounted for as amino acids and ammonia for 9 seed meals. In 22, the humin nitrogen was 3.1%, or less, of the total nitrogen. *Vernonia anthelmintica* and *Pentstemon albidus* gave high percentages of humin nitrogen, indicating significant losses of amino acids.

Excluding possible analytical errors, the unaccounted nitrogen obtained by difference is indicative of unknown nitrogen-containing substances. In *Coronilla varia*, *Sesbania exaltata*, and *Trigonella foenum-graecum*, 60, 80, and 28% respectively, of the unknown nitrogen was accounted for as canavanine. Because of the canavanine in these seeds, they are examples of natural products having a significantly lower protein than that assigned to them by multiplying their nitrogen content by 6.25.

Partial amino acid compositions of six seed meals are reported in the literature (*11*). Of these values, four recently obtained by microbiological assay, including *Daucus carota*, agree reasonably well with the values given here.

The essential amino acid requirements of an animal vary with physiological and environmental conditions and thus cannot be expressed by a single figure. Furthermore, the amino acid composition of a food sometimes does not reflect the type of response obtained when it is tested in feeding trials. Despite these objections the amino acid composition of a feed- or foodstuff is perhaps the most practical, initial way of predicting its value as a source of nutritionally high quality protein. This method is even more valuable when the proportions of amino acids present are compared with those required for optimum growth. Since work on the young rat was reported, other investigators have found that similar proportions of natural amino acids gave good growth when fed to weanling pigs (*8*).

The rating method indicates (Table II, Figure 1) the variability of the nutritional amino acid quality of seeds from the species examined. This evaluation of a small number of species suggests that analyses of large numbers from different families should reveal many seed meals that are well balanced in their nutritionally essential amino acid content. The complete picture of amino acid composition should aid in making the proper supplementation of protein sources deficient in methionine or lysine.

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NUTRITIVE QUALITY OF COTTONSEED

Dietary Evaluation of Cottonseed Protein from Cotton Bred for Low Gossypol Content

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GOSSYPOL, a toxic constituent of cottonseed, adversely affects utilization of meal and oil from the seed. The amount of cottonseed meal that may be fed safely to simple-stomach animals and to young ruminants is dependent upon the level of free gossypol in the meal (*3*). Although gossypol in the bound form is not considered toxic, it reduces the nutritive quality of the meal (*7*).

Developments in processing cottonseed have reduced the hazards from feeding the meal and improved the quality of the oil, but the undesirable effects of gossypol have not been eliminated entirely. Considerable improvement, however, may be effected by developing

Strains of cotton with a lower gossypol content than that found in varieties currently grown have resulted from crosses of commercial varieties with Upland cotton having the necessary genes for reducing the gossypol content. Feeding trials with rats showed good growth when meals prepared from three crops of these strains of seed were fed at the 10% protein level. The excellent growth when meals were prepared from certain genetic lines of cotton having relatively high levels of gossypol suggests that other components may have been improved through breeding.

varieties of cotton in which the gossypol content is less than 0.50%, which level yields meals containing less than 0.85% total gossypol (*4*).

Cotton geneticists have described genes which enable the gossypol content of the seed to be reduced in several strains without altering desirable characteristics of the cotton plant (*6*). The objective of this study was to evaluate the nutritive quality of the seed from these genetic strains of cotton.

Procedure

Source of Cottonseed. Several genetic strains of Upland cotton having the necessary genes for reducing gossypol content in the seed were grown at Iguala, Mexico, during the winter of 1956–57. Sufficient seed was obtained for assaying the growth-promoting properties when incorporated in the diets of young rats (trial 1, Table I). Three strains used in trial 1 were also planted in North Carolina in the summer of 1957. From these plantings, seed was obtained by intercrossing within each strain and by crossing with commercial varieties of cotton. As a result of these procedures, strains 1a, 2a, and 3a of trial 1 and 1a, 2a, and 3a of trial 2 were different only in the location at which the seed were grown. For trial 3 seed was obtained from selections derived from further crossing of superior low-gossypol strains with commercial varieties. These selections were planted at Iguala in the 1957–58 season. A glandless-seed sample from Cali 38–6 was included in trial 3, Table I.

Preparation of Meals. After decorticating the acid-delinted seed in a Bauer mill, most of the hulls were removed by screening. The kernels were flaked in the laboratory by passing them twice between metal rolls. The flaked meats were extracted with hexane by percolation, which leaves the major portion of the gossypol with the seed residue. The dried hexane-extracted meats were ground coarsely in a burr mill and screened through a 20-mesh screen to remove the residual hulls.

Binding the Gossypol. A mixture of 1600 ml. of peroxide-free ether, 715 ml. of 95% ethyl alcohol, and 240 ml. of water was poured over 1000 grams of the ground and screened hexane-extracted cottonseed meats in the bowl of a mechanical mixer. Meats and solvent were mixed at room temperature and at low speed for 30 minutes. Then a water bath heated by steam to about 60° C. was placed under the mixing bowl. The mixing was continued for 3.5 hours as the temperature of the water bath was gradually raised to 83° to 85° C., in which process the meal reached a temperature of 73° to 75° C. The meal, which was thoroughly dry at the conclusion of the treatment, was stored immediately in a sealed container. As a safety measure during the binding process, a large exhaust fan was used to prevent the accumulation of solvent vapors in the laboratory.

The free and the bound gossypol contents of the meals and the corresponding diets in which they were fed are shown in Table I.

Bioassay of Meals. Meals from the selected strains of cotton were compared with ether-extracted cottonseed meats from a standard variety (control), after each had been subjected to the gossypol binding treatment, to evaluate the growth-promoting properties of the test meals.

Diets. The diets, test and control, contained 10% protein (N × 6.25) supplied by the respective meals; 5% fat supplied by the fat in the ingredients plus vegetable oil (Wesson); 3% Wesson salt mixture; 0.5% cod liver oil; and sufficient starch to bring the ingredient percentage to 100. Each kilogram of diet was supplemented with the following vitamins, in milligrams: thiamine hydrochloride, 5; riboflavin, 5; pyridoxine hydrochloride, 5; calcium pantothenate,

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Table I. Mean Gains of Rats Fed Meals from Different Experimental Strains of Cotton

Strain Identification	*Diet No.*	*Rats per Diet*	*Gossypol Content, %*				*Mean Gain, Grams*
			Meal		*Diet*		
			Free	*Total*	*Free*	*Total*	
Trial 1 (1956–57 Crop, Mexico)							
1a[a]	1	8	0.025	0.648	0.005	0.123	78
1b[b]	2	8	0.029	0.566	0.005	0.103	83
2a	3	8	0.021	0.555	0.004	0.103	90
2b	4	8	0.031	0.446	0.006	0.084	84
3a	5	8	0.044	0.894	0.008	0.172	87
3b	6	8	0.041	0.974	0.008	0.183	67
Control[c]	7	8	0.019	0.321	0.004	0.059	90
Casein	8	8	...	...	...	...	66
Trial 2 (1957 Crop, North Carolina)							
1a	9	6	0.029	0.754	0.005	0.131	83
1b × Coker	10	6	0.038	0.859	0.006	0.145	80
2a	11	6	0.025	0.513	0.004	0.090	85
2a × 3b	12	6	0.018	0.539	0.003	0.092	88
3a (repeat, 1956–57 crop)	5	6	0.040	0.926	0.008	0.175	93
3a	13	6	0.051	0.803	0.009	0.140	89
3b × 2b	14	6	0.034	0.803	0.006	0.139	79
3b × Fox	15	6	0.053	0.889	0.009	0.154	90
3b × Fox × others	16	6	0.039	0.866	0.007	0.149	79
Control[c]	17	6	0.019	0.320	0.004	0.059	89
Trial 3 (1957–58 Crop, Mexico)							
Cali 38-6	18	8	0.000	0.033	0.000	0.008	105
N. C. G. 9	19	8	0.014	0.484	0.003	0.096	85
10	20	8	0.015	0.408	0.003	0.084	91
11	21	8	0.027	0.564	0.006	0.117	94
12	22	8	0.017	0.522	0.004	0.109	93
13	23	8	0.022	0.636	0.005	0.131	91
14	24	8	0.024	0.586	0.005	0.114	93
15	25	8	0.005	0.164	0.001	0.033	99
Control[c]	26	8	0.026	0.438	0.005	0.080	84

[a] Indicates intercrossing by insects among plants within a strain.
[b] Indicates selfpollination of the plants of a strain.
[c] Ether-extracted commercial variety.

20; niacin, 30; *p*-aminobenzoic acid, 30; and choline hydrochloride, 1000.

Design of Tests. The diets containing the respective meals (Table I) were fed ad libitum to individual weanling rats of the Wistar strain during a 4-week period. In trials 1 and 3 the design employed was a randomized block having eight replications in each trial, whereas in trial 2 the design was a balanced incomplete block, described by Cochran and Cox as plan 11.16 (*1*), having six rats receiving each diet. Rats were stratified on the basis of sex and initial weights.

Results

Trial 1. Statistical analysis showed that there were no differences ($P > 0.05$) in the mean gains from either diets 1 to 5 and control diet 7 or 1, 6, and 8. The greater gains from diets 3 and 7 *vs.* diets 6 and 8 and from diet 5 *vs.* diet 8, respectively, were highly significant ($P < 0.01$). The difference between diets 5 and 6 was almost significant at the $P = 0.01$ level.

Gains from all the cottonseed meal diets, except 6, were good. Gains from this diet were similar to those from a diet containing the ether-extracted control meal having sufficient gossypol bound to it to give a total content of 1.1% (*7*). The control meal used in diet 7 (Table I) was superior to a soybean meal used in an earlier study (*7*), when both were fed without added gossypol. Gains from diets 2 to 5 indicate that the meals used in them were equal to the control meal in growth-promoting characteristics.

Gains from the casein diet, 8, were poor in comparison with diets containing cottonseed meals; the one exception was diet 6, in which the meal had a total gossypol content of 0.972%. The growth response from a diet containing this meal was about what was anticipated from previous studies on the effect of bound gossypol (*7*).

The gains from diet 5 were significantly greater ($P < 0.05$) than those from diet 6 even though the meal in this diet contained only 0.080% more total gossypol than that in diet 5. This difference in total gossypol contents is too small to explain the differences in gains. Furthermore, the gains from diet 5 were either nearly equal to or greater than those from diets 1 to 4, all of which had markedly less gossypol than did diet 5.

Trial 2. Good growth was obtained from all diets, irrespective of the meals. None of the diets was significantly inferior to control diet 17, which contained ether-extracted meal. In most instances, however, the total gossypol content of the test meals was considerably higher than that of the control meal.

In trial 2, diet 5a was a repetition of diet 5, trial 1. The gains in the two trials were in good agreement, being 87 and 93 grams, respectively. The mean gain from diets containing meal from the new crop of 3a, grown in North Carolina and fed in diet 13, was near that from the previous crop of 3a, grown in Mexico and fed in diet 5a of trial 2: 89 and 93 grams, respectively. These results indicate that the location and the time grown did not affect the results, as the difference between diets 5a and 13 in total gossypol content was small, only 0.035%.

Trial 3. Statistical analysis of the results in this trial showed that the differences in gains between diets 18 and 25, or between diet 25 and diets 19 through 24 were not significantly different at the $P = 0.05$ level. The gain from diet 18 was significantly greater ($P<0.05$) than gains from diets 20, 22, 23, and 24 and was greater than the gain from 19 at the $P = 0.01$ level. The mean gain from diet 25 was greater than that from diet 26, which contained the ether-extracted control meal, at the $P = 0.05$ level and approached $P = 0.01$. The gains from all the test diets in trial 3 were greater than the control, diet 26; however, only those gains from diets 18 and 25 were significantly greater. The meals in these last two diets contained 0.033 and 0.164% total gossypol, respectively, and produced the greatest gains. The other meals, from the near commercial strains of cotton, fed in trial 3 contained only about one third to one half as much total gossypol as the meal from commercial cotton varieties commonly grown.

Discussion

The evaluation of the nutritive properties of cottonseed meal prepared from seed produced by experimental genetic strains of cotton, developed by addition of genes for low gossypol, showed in three feeding trials that it was either equal or superior to meal prepared from ether-extracted meats of a commercial variety. The gains from only one meal were significantly inferior to the control.

Of the 21 experimental meals from the cotton-breeding program, only five exceeded 0.85% total gossypol, the level below which Lyman *et al.* (*4*) found most cottonseed meals to be of high nutritive quality as measured by their Chick Growth and Chemical Index methods. Eleven of these meals contained less than 0.6% total gossypol. Only three of the assayed meals had a free gossypol content greater than 0.04%, the maximum recommended without restricting the amount of meal incorporated in diets of chicks, broilers, and swine (*5*). All of these meals could be incorporated in broiler rations to supply 20% protein with only two exceeding the 0.016% and none exceeding the 0.020% dietary free-gossypol levels, above which Heywang and Bird (*3*) found White Leghorn and New Hampshire chicks were affected. All of these meals were lower in free gossypol than the dietary level at which Couch *et al.* (*2*) found the growth of chicks was retarded.

The conditions under which these meals were prepared, although mild, bind the gossypol rather completely. The amount of residual free gossypol remaining in the meal after the binding treatment seems to vary with the total gossypol content of the meal.

An improvement in the nutritive value of cottonseed is the consequence of genetically lowering the gossypol content of the seed from which the meal is prepared. There is also the genetic possibility of improving the nutritive value of cottonseed by increasing the amount of some of the limiting constituents. The good growth from diets 5 and 5a, despite their 0.93% total gossypol, supports this view.

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