

mixtures of the same meals were analyzed. The weights of saponins recovered from the mixtures were almost perfectly proportional to the percentages of the two meals in the samples.

Application of Saponin Determinations to Study of Chick-Growth Inhibition. An example of the utilization of saponin determinations in studying the influence of alfalfa saponins in diets upon growth of chicks is shown in Figure 1. In the figure, relative growth in 6 weeks is plotted against percentages of saponins in alfalfas fed at 20% levels in experimental diets. Each point on the graph represents the average for a group of 20 chicks.

NUTRIENTS IN SEED MEALS

Amino Acid Composition of Twenty-Seven Selected Seed Meals

Seed meals obtained from 27 genera of 13 botanical families were analyzed for their acid-stable amino acid content by the Moore, Spackman, and Stein ion exchange chromatographic method and for tryptophan by a modified Spies and Chambers procedure. Comparison of the amino acid compositions with the requirements for optimum growth of the rat and the chick showed some of the seed meals to be well balanced. The majority were deficient in methionine-cystine, lysine, or both. Lysine content varied from 2.5 to 6.9 grams and methionine from 0.5 to 2.3 grams per 16 grams of nitrogen. Three genera from the legume family contained canavanine, which was identified after isolation from *Sesbania exaltata*.

NEW CROPS research in the Agricultural Research Service, U. S. Department of Agriculture, has a major objective of discovering plants that may be advantageously grown by the farmer in place of commodities like corn and wheat now in surplus. One large part of this program consists of determining the chemical composition of a wide variety of plants. Special emphasis is placed on quantitative analysis for compounds of known practical significance, such as amino acids. Also important is the discovery of unknown compounds that may have economic potential, and industrial uses appropriate to the properties found or developed.

Ion exchange chromatographic methods of quantitative analysis for amino acids and related compounds are ideal for determining the amino acid composition of plant material. In addition, these methods detect unknown nitrogen-containing compounds which form a color with ninhydrin during analysis. This report contains analytical results from the determination of the amino

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acid composition of seeds from 27 selected plant species.

Materials and Methods

Seed Selection and Preparation. Factors considered in the selection of seed meals from different plant species for analysis were: high protein content; chemical composition, such as high or unique seed oil content; relatively high lysine or methionine, as determined by microbiological assay; lack of amino acid data in the literature for seed from the species reported; and favorable agronomic possibilities of the plant for the temperate zone. One sample from each species was analyzed. Amino acid composition might vary between varieties or with environmental conditions, but such variability is probably of a lesser magnitude than that to be found between species.

Mature, dry seeds were selected for analysis. Easily removable outer seed coverings (fruit or seed coat) were separated, using conventional seed-clean-

ing equipment. The sample was ground to pass a standard U. S. 40-mesh screen and oil was extracted with petroleum ether (boiling point 33° to 57° C.). The extracted meal was allowed to come to equilibrium with the moisture in ambient air, then sampled for total nitrogen determination and for hydrolysis.

Acid Hydrolysis. The seed meals were hydrolyzed for 24 hours under reflux with constant boiling hydrochloric acid, redistilled in glass. Excess acid, 250 ml. per 0.5 ± 0.2 gram of meal as recommended by Dustin *et al.* (2), prevented excessive humin formation in most cases. The acid was removed under vacuum to near dryness with a rotary evaporator, followed by evaporation of three small volumes of added water. The humin was removed by centrifugation; its nitrogen content was determined by the Kjeldahl method on the air-dried material collected in a weighed centrifuge tube. The clear supernatant was made to 50-ml. volume and held in a frozen state if not analyzed within 2 weeks.

Methods of Analysis. All the common amino acids except hydroxyproline and tryptophan were determined exactly as described by Moore, Spackman, and Stein (6) using their 150- and 15-cm. column with ion exchange resin particles classified according to the method of Hamilton (4). Tryptophan was estimated by the Spies and Chambers method (12) applied to a mild alkaline hydrolyzate of the seed meal.

Methods of Calculation. Nitrogen distribution based on ninhydrin color measurements and humin nitrogen determination by Kjeldahl analysis are given in Table I. Unaccounted nitrogen was determined by difference. When the unaccounted nitrogen was in excess of 5%, it was considered to be of non-protein origin. Amino acid content of the seed meals (Table II) is based on grams of amino acid per 16 grams of nitrogen in the original seed meal. This method of calculation is the one commonly employed by nutrition workers. Cystine values have been combined with methionine and tyrosine values with phenylalanine because these two amino acids partially replace methionine and phenylalanine as nutritionally essential amino acids.

Results

Evaluation of Seed Meals for Nutritionally Essential Amino Acids. The essential amino acids considered most likely to be deficient in human diets and animal feeds are lysine, methionine-cystine, threonine, and tryptophan (3). Ranges of these amino acids for the seed meals analyzed were (in grams per 16 grams of nitrogen) lysine, 2.5 to 6.9; methionine, 0.5 to 2.3; methionine-cystine, 1.7 to 4.6; threonine, 2.2 to 4.6; and tryptophan 1.0 to 2.0 (Table II). The range indicates that some meals may be adequate while others are markedly deficient.

The proportions of each amino acid in the seed meals were compared with the amino acid proportions required by the rat for optimum growth (10). By this method each meal was rated (Table II). In computing these proportions (3), one amino acid was selected as unity, which, on inspection, was found to be close to the amount required by the rat. Tryptophan, histidine, lysine, and threonine were the amino acids selected in calculating the data. *Crambe abyssinica*, *Daucus carota*, and *Marah gilensis* were rated adequate. *Foeni-*

cum vulgare, *Limnanthes douglasii*, *Salvia columbariae*, *Trifolium incarnatum*, and *Valeriana officinalis* were only slightly deficient in lysine or methionine-cystine. Lysine and methionine-cystine were the most common limiting amino acids (Table II). *Clitoria ternatea* was low in threonine; *Coronilla varia*, *Lupinus luteus*, *Salsola pestifer*, and *Vernonia anthelmintica* were low in phenylalanine; and *Lupinus luteus* and *Trigonella foenum-graecum* were low in valine.

Seed meals were also rated on the basis of whether they could provide a balanced source of amino acids required to give optimum growth of the chick fed a 20% protein ration as reported by the National Research Council (7). By this criterion seed meal from *Daucus carota* was the only one rated adequate. Figure 1 shows its comparison with an unbalanced seed meal, *Lupinus luteus*. The sloping line shows minimum requirement of each amino acid for optimum growth rate. All the remaining seed meals were deficient in lysine or methionine-cystine or both. In addition, many of them were deficient for the chick in one or more of the following: phenylalanine-tyrosine, leucine, glycine, valine, and arginine.

Table I. Seed Composition and Nitrogen Distribution Data on Seed Meal Hydrolyzates

Botanical Name	Common Name	Oil, %	Protein, % (N × 6.25)	Nitrogen Distribution after Hydrolysis as % of Total Nitrogen			
				Humin	Ammonia	Amino acids ^a	Unaccounted
Chenopodiaceae							
<i>Salsola pestifer</i>	Russian thistle	22.0	40.0	1.7	11.8	72.6	13.9
Compositae							
<i>Dimorphotheca aurantiaca</i>	Cape marigold	34.0	38.0
<i>Echinacea angustifolia</i>	Cornflower	37.0	49.0	0.7	13.5	82.0	3.8
<i>Liatriis spicata</i>	Blazing star	36.0	36.0	0.8	11.8	84.9	2.5
<i>Vernonia anthelmintica</i>	Ironweed	26.0	18.0	8.0	7.7	65.2	19.0
Cruciferae							
<i>Crambe abyssinica</i>	Abyssinian cabbage	36.0	32.0	2.3	12.6	73.1	12.0
<i>Matthiola bicornis</i>	Evening stock	29.0	35.0	1.7	13.5	75.7	9.1
Cucurbitaceae							
<i>Marah gilensis</i>	Bigroot	57.0	28.0	1.0	8.1	92.2	-1.3
<i>Momordica balsamina</i>	Balsam apple	40.0	30.0	1.0	8.4	76.5	14.4
Euphorbiaceae							
<i>Euphorbia heterophylla</i>	Mexican fireplant	37.0	25.0	3.4	9.2	79.5	7.9
Gramineae							
<i>Pennisetum typhoides</i>	Bulrush millet	5.6	17.0	2.5	15.1	82.1	0.3
Labiatae							
<i>Perilla frutescens</i>	Perilla	42.0	32.0	1.9	11.1	85.9	1.1
<i>Salvia columbariae</i>	Chia	34.0	20.0	2.1	9.8	80.5	7.6
Leguminosae							
<i>Clitoria ternatea</i>	Butterfly-pea	12.0	47.0	1.3	8.6	76.6	13.5
<i>Coronilla varia</i>	Crown vetch	8.0	32.0	1.9	6.4	66.4	25.3
<i>Cyamopsis tetragonolobus</i>	Guar	4.0	33.0	1.7	8.9	85.9	3.5
<i>Lespedeza stipulacea</i>	Korean lespedeza	7.0	52.0	2.0	9.0	87.4	1.6
<i>Lupinus luteus</i>	Yellow lupine	5.0	43.0	0.7	12.9	83.3	3.1
<i>Sesbania exaltata</i>	Hemp sesbania	4.0	40.0	1.5	7.1	69.4	22.0
<i>Trifolium incarnatum</i>	Crimson clover	4.0	44.0	2.3	8.6	83.7	5.4
<i>Trigonella foenum-graecum</i>	Fenugreek	5.0	26.0	1.9	8.2	78.8	11.1
Limnanthaceae							
<i>Limnanthes douglasii</i>	Meadow foam	24.0	25.0	3.1	13.2	80.3	3.4
Ranunculaceae							
<i>Nigella hispanica</i> , var.	Love-in-a-mist	43.0	26.0	2.6	14.6	75.1	7.7
Scrophulariaceae							
<i>Pentstemon albidus</i>	Beardtongue	20.0	15.0	6.0	10.1	80.9	3.0
Umbelliferae							
<i>Daucus carota</i>	Queen Anne's lace	27.0	25.0	1.8	14.8	83.7	-0.3
<i>Foeniculum vulgare</i>	Fennel	27.0	20.0	3.1	12.8	80.7	3.4
Valerianaceae							
<i>Valeriana officinalis</i>	Garden heliotrope	30.0	20.0	4.3	11.1	88.5	-3.9

^a Nitrogen present as those amino acids listed in Table II.

Nonessential Amino Acids. Composition of a seed meal with respect to its nonessential amino acids (Table II) was considered important because of the increasing evidence for amino acid imbalance (5) and evidence that small amounts of some considered nonessentials may be required (9).

The total amino acid composition is valuable in gaining insight into seed protein storage by the plant. Table II indicates that at least with some amino acids there exists little variation between species. Seeds from many members of various plant families will be analyzed for amino acids in order to determine if there is any significant correlation between amino acid composition and botanical relationship.

Identification and Quantitative Estimation of Canavanine in Leguminosae. Analysis of seed meals from *Sesbania exaltata*, *Coronilla varia*, and *Trigonella foenum-graecum* revealed an unidentified elution peak immediately following ammonia on the 15-cm. column. This peak was identified as canavanine, as seed from *Canavalia ensiformis* (jack bean meal, a common source of canavanine) contained material eluting in the same position. An authentic sample of canavanine sulfate likewise had this elution position. The compound was isolated from *Sesbania exaltata* through the flavinate according to a method described by Bell (7). Its identity was confirmed

by elementary analysis and by its well-defined x-ray pattern, which were both identical with those of an authentic sample of canavanine sulfate.

The compound gave a ninhydrin color yield on a molar basis of 1.01 related to leucine as 1.00. According to this color yield, the whole seed meals from *Sesbania exaltata*, *Coronilla varia*, and *Trigonella foenum-graecum* contained 8.7, 8.0, and 1.5 grams of canavanine per 16 grams of nitrogen, respectively. Canavanine was detected from the

properly positioned elution peak on the ion exchange column in alcohol extracts of seed from *Sesbania drummondii*, *S. macrocarpa*, *S. sonorae*, and *S. vesicaria*. None was detected in hydrolyzates from the remaining seed meals listed in Table I. Better than 95% of the canavanine was extracted in the free form from seed meal of *Sesbania exaltata* by repeated extraction with 50% alcohol. Results show that essentially all the compound is present uncombined with protein. The alcohol extract contained small

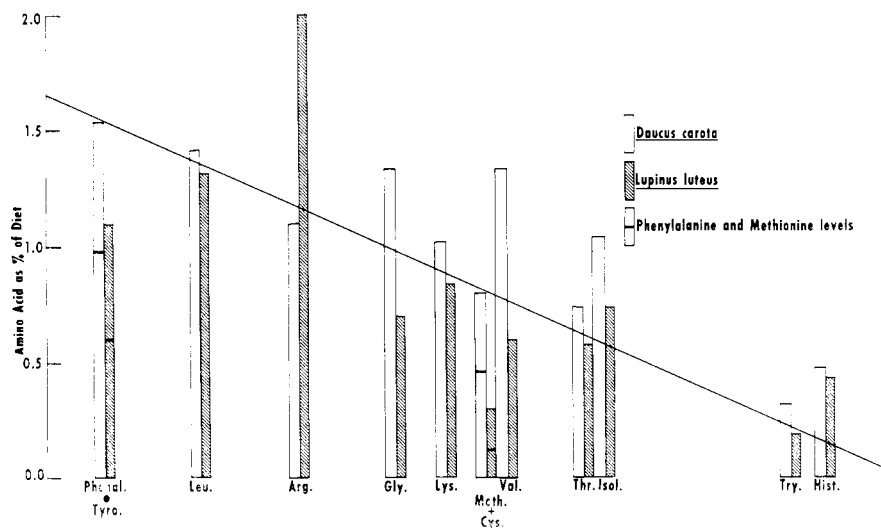


Figure 1. Comparison of amino acid content of seed from *Daucus carota* and *Lupinus luteus* with chick requirement

Table II. Amino Acid Composition of Seed Meals

(Grams of amino acid per 16 grams of nitrogen)

Seed Meal	Lysine	Methionine	Methionine cystine	Phenylalanine	Phenylalanine Tyrosine	Arginine	Glycine	Histidine	Isoleucine	Leucine	Threonine	Tryptophan	Valine	Alanine	Aspartic Acid	Glutamic Acid	Proline	Serine
<i>Clitoria ternatea</i> ^a	6.1	1.0	3.5	3.6	6.9	7.4	4.1	2.4	4.2	7.4	2.2	1.2	4.4	3.5	9.3	15.6	3.3	5.0
<i>Coronilla varia</i> ^b	4.1	0.8	1.9	3.1	5.9	6.5	4.8	2.4	3.0	5.6	2.9	1.3	3.9	3.8	8.6	13.7	3.2	4.4
<i>Crambe abyssinica</i> ^c	4.9	1.6	4.2	3.5	6.0	5.7	5.3	2.2	3.9	6.2	4.6	1.5	5.1	4.2	7.6	17.0	5.5	4.1
<i>Cyamopsis tetragonolobus</i> ^{b,d}	4.0	1.4	2.0	3.7	7.0	12.5	5.1	2.5	3.2	5.9	2.8	1.9	4.2	4.2	10.2	20.1	3.1	4.9
<i>Daucus carota</i> ^{c,d}	5.1	2.3	3.7	4.8	7.8	5.4	6.8	2.4	5.3	7.2	3.7	1.6	6.7	4.9	12.8	19.1	5.5	5.2
<i>Dimorphotheca aurantiaca</i> ^b	4.1	1.2	3.2	3.4	8.1	7.0	5.7	2.4	4.6	6.1	3.6	1.5	6.0	3.9	8.0	16.8	3.8	4.0
<i>Echinacea angustifolia</i> ^a	2.5	1.7	4.0	3.7	5.8	10.7	4.6	2.1	2.9	5.9	2.7	1.4	4.9	3.6	9.9	24.9	3.3	4.4
<i>Euphorbia heterophylla</i> ^a	4.3	1.8	2.6	3.8	6.0	7.8	5.4	2.2	3.4	5.4	2.8	1.2	4.6	4.0	9.4	16.8	3.2	5.6
<i>Foeniculum vulgare</i> ^a	4.8	1.9	3.3	4.1	6.7	4.3	7.0	2.1	4.4	6.3	3.8	1.6	5.8	5.0	11.6	18.7	5.7	5.7
<i>Lespedeza stipulacea</i> ^a	5.3	1.0	2.5	4.3	8.5	11.9	4.1	2.6	3.9	7.2	3.0	1.4	4.2	3.8	11.3	16.7	3.7	5.9
<i>Liatriis spicata</i> ^a	4.1	1.8	3.5	4.0	6.6	9.1	5.4	2.1	4.7	6.4	3.5	1.1	5.2	3.8	10.0	21.4	5.7	4.9
<i>Limnanthes douglasii</i> ^a	6.9	1.4	2.8	3.7	5.9	7.5	6.1	2.2	3.8	6.9	4.3	1.6	5.0	4.4	8.0	16.3	4.2	4.4
<i>Lupinus luteus</i> ^{b,d}	4.8	0.5	2.0	3.6	6.3	9.9	4.0	2.5	3.9	7.5	3.1	1.0	3.5	3.4	10.1	24.4	3.2	4.9
<i>Marah gilensis</i> ^c	5.6	1.8	3.1	4.2	7.0	13.9	5.8	2.3	4.3	8.2	2.9	1.9	4.4	4.3	8.5	18.1	3.7	4.9
<i>Matthiola bicornis</i> ^c	3.0	1.8	3.2	3.7	6.3	9.1	6.8	2.7	3.4	6.4	3.0	1.6	4.9	4.4	7.2	17.3	4.4	3.8
<i>Momordica balsamina</i> ^a	3.5	1.8	4.6	4.3	7.5	9.7	4.3	2.1	4.0	7.3	2.6	1.7	5.1	3.9	7.4	15.4	3.3	4.1
<i>Nigella hispanica</i> ^a	4.3	1.9	3.5	4.0	8.1	5.1	5.1	2.5	3.7	6.1	3.7	1.7	5.1	4.3	9.5	18.8	4.4	4.5
<i>Pennisetum typhoides</i> ^{d,e}	2.8	2.1	3.2	5.0	8.3	4.7	3.3	2.1	4.9	11.3	3.8	2.0	6.0	8.8	9.9	21.2	6.6	4.6
<i>Pentstemon albidus</i> ^c	3.9	1.4	4.1	3.8	6.7	8.7	4.9	2.0	4.6	7.1	3.9	...	5.0	5.2	9.1	15.6	3.8	5.6
<i>Perilla frutescens</i> ^{d,e}	3.8	2.2	3.4	5.0	8.6	11.3	5.1	2.8	3.9	5.8	3.6	1.2	4.7	4.6	9.1	19.3	3.3	5.1
<i>Salsola pestifer</i> ^b	4.3	1.4	2.9	3.1	6.0	8.4	4.9	3.0	3.4	5.3	3.0	1.3	4.5	3.4	7.8	14.8	2.8	4.6
<i>Salvia columbariae</i> ^a	4.4	2.0	2.8	4.7	8.4	8.3	5.0	2.5	4.2	7.0	3.8	1.2	4.9	4.7	9.0	17.2	3.4	5.9
<i>Sesbania exaltata</i> ^b	4.7	1.3	1.7	3.9	6.8	7.6	4.3	2.4	4.0	6.2	2.8	1.7	4.2	3.2	8.2	14.6	3.0	4.0
<i>Trifolium incarnatum</i> ^a	6.5	1.4	2.1	3.9	6.7	9.8	4.1	2.7	4.2	7.3	3.1	1.8	4.4	4.1	11.0	16.5	3.4	4.7
<i>Trigonella foenum-graecum</i> ^{a,d}	6.0	1.3	2.5	3.8	6.3	9.2	4.4	2.0	4.5	6.8	3.0	1.6	3.4	4.0	10.9	15.8	4.6	5.2
<i>Valeriana officinalis</i> ^c	4.9	2.1	2.8	5.0	7.7	9.6	5.3	2.4	5.3	7.8	4.4	2.0	5.2	5.6	10.6	20.7	3.3	4.6
<i>Vernonia anthelmintica</i> ^{b,d}	4.4	1.3	2.2	3.5	5.7	8.2	5.9	2.4	3.8	6.3	3.8	...	4.4	3.9	6.5	18.5	3.7	3.4

^a Methionine limiting for optimum rat growth. ^b Methionine and lysine limiting for optimum rat growth. ^c Adequate proportions of amino acids for optimum rat growth. ^d For literature values see (17). ^e Lysine limiting for optimum rat growth.

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, threonine, 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 am-

monia 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 (17). 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

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 depen-

dent 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 cotton-

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seed 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