
**The Nitrogen Composition of Cereal Grasses.
II. Amino Acid Distribution in Clippings
From Greenhouse Cultures¹**

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The composition of the cereal grasses during various stages of growth is of interest not only in evaluating the physiological changes within the plant itself but also in making use of these plants as feed for livestock and poultry, particularly in the southern portion of the winter wheat belt. Extensive studies, therefore, have been carried out at this station to determine the composition of the cereal grasses during various seasons and at different

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stages of development. The proximate composition of cereals repeatedly clipped at grazing height has been reported by Heller (2), and selected varieties have been analyzed for carotene and certain vitamins of the B-complex by Moyer, *et al.* (8).

The high concentration of total nitrogen found in young, growing wheat plants prompted a more detailed study of the principal nitrogen fractions by MacVicar (7). In these studies, it was found that about 70 per cent of the total nitrogen was in the form of protein precipitable by trichloroacetic acid. Of the remaining 30 per cent, about two-thirds was present in the form of soluble proteins, polypeptides or amino acids. The remainder was primarily amide nitrogen, ammonia, or nitrate. The relatively large amount of the total nitrogen present in combined form made further investigation of the amino acid composition of the plant of more than usual interest. Studies were therefore instituted to determine the composition of five representative cereal grasses and annual ryegrass with respect to several of the more important amino acids for which suitable analytical methods were then available. The results of these investigations are presented in this report.

MATERIALS AND METHODS

Source of Material. Five representative cereal grasses, rye (*Secale cereale*, L., var. Balbo), oats (*Avena sativa*, L., var. Wintok), barley (*Hordeum vulgare*, L., var. Michigan Winter), hard wheat (*Triticum aestivum*, L. var. Tenmarq), and soft wheat (*Triticum aestivum*, L., var. Clarkan), and annual ryegrass (*Lolium multiflorum*, Lam.) were used in this study. The plants were grown in soil in flats under normal greenhouse conditions during three months from December to March. Hoagland's solution (4) was applied periodically to supply potassium, nitrogen, and phosphorus in addition to the nutrients already in the soil.

Collection of Material. Samples of the leaves of the plants were obtained by clipping the entire growth of plant to a height of about one inch whenever a total growth of from 3 to 4 inches had occurred. Depending upon the vigor of growth, 4 or 5 samples were obtained during the three-month period. The samples were rapidly dried in a current of air at 65° C., ground, and stored until analyzed for total nitrogen and amino acid composition.

Analytical Methods. Total nitrogen was determined by macro Kjeldahl procedure according to the A. O. A. C. (1). Amino acids were determined on alkaline or acid hydrolysates of the whole tissue. Hydrolysates for tryptophan analysis were prepared essentially according to the procedure of Kuiken *et al.* (5) by heating 2.5 gm. of dry material with 200 mg. cysteine and 32 ml. of 4N NaOH in an autoclave for 8 hours at 15 pounds pressure. These samples were then neutralized, made to a volume of 100 ml., and filtered. The filtrates were assayed for tryptophan with the test organism *L. arabinosus* 17-5 by essentially the procedure of Snell and Wright (9) for nicotinic acid except that nicotinic acid was added to the media and tryptophan was omitted. Hydrolysates for the determination of eight other amino acids were prepared by heating 2.5 gm. of dry material with 50 ml. of 3N HCl in an autoclave for 10 hours at 15 pounds pressure. The samples were neutralized and made to a volume of 50 ml. before filtering. *L. arabinosus* 17-5 was used for the assay of valine, leucine, isoleucine, phenylalanine, and glutamic acid. *S. faecalis* R. was used for the assay of threonine and *L. mesenteroides* P-60 for lysine and methionine. The procedure and media of Henderson and Snell (3) were employed for these assays.

It is recognized that currently available methods of hydrolysis of proteins, such as were employed in this study, result in losses, particularly when large amounts of carbohydrate are present. Values presented, therefore, should be regarded as minimal, particularly in the case of tryptophan and methionine. Values presented for these two amino acids are probably

less than the true value. Such minimal composition figures, however, permit a considerable degree of evaluation of nutritive properties. To conform to general usage, the content of the amino acids is given on the basis of per centage of total bulk protein, the data being calculated on the assumption that the mixed bulk proteins contained 16 per cent nitrogen and that all of the nitrogen by Kjeldahl analysis represented protein. That such is indeed not the case has previously been demonstrated (7). Nearly 70 per cent of the total nitrogen of these cereal grasses, however, has been shown to be precipitable with trichloroacetic acid (presumably protein). Much of the remaining soluble nitrogen was combined as amino acids, peptides, and trichloroacetic acid-soluble proteins. Therefore, even rather wide fluctuation in other nitrogenous constituents would introduce little error in computing amino acid composition on the basis of total nitrogen.

RESULTS AND DISCUSSION

The average compositions, with standard deviations, of various cereal grasses and annual ryegrass grown under greenhouse culture are presented in Table I. These data represented average values for four or five clippings obtained from the same plants at successive intervals through the winter and early spring. All six species were remarkably uniform, with only an occasional exception, in the pattern of distribution of the amino acids in the leaf tissue. This is in contrast to the differences among the seed protein compositions of these same plants. Even annual ryegrass, which in many respects differs from the cereal grasses, differs less from the general pattern of the cereals than certain of them differ among themselves.

Among the monocarboxylic group, leucine is quantitatively one of the more important amino acids in the leaf protein of cereal grass. With respect to this amino acid, leaf protein closely resembled the proteins of whole egg and muscle; it contained somewhat more leucine than the proteins of most plant seeds. Of the various cereals examined, the protein contained from 7.6 to 9.6 per cent leucine. Isoleucine followed essentially the same pattern as leucine, being found in the protein of cereal grass in an amount comparable to that reported as present in muscle tissue. There was a general positive correlation between leucine and isoleucine content; for example, Balbo rye contains the highest concentrations of both of these amino acids. The valine content of these leaf proteins also closely parallels the composition of muscle, having a range of from 4.9 to 5.7 per cent of total protein. There was no obvious relationship between valine content and any of the other monocarboxylic acids determined, although Wintok oats contained the least amounts of both valine and leucine. Comparison of these data with those summarized by Lugg (6) for the bulk proteins of a group of pasture grasses shows that, in general, the values obtained by microbiological procedures in this study of cereal grasses are somewhat higher than those obtained by chemical procedures.

One of the more striking differences between the composition of these grass proteins and proteins from such other sources as animal tissues and plant seeds is the relatively more dominant position of threonine. This amino acid accounted for 6.5 to 8.7 per cent of the total bulk protein, considerably more than is present in most bulk proteins which have been analyzed thus far. This amino acid is present in most plant seed proteins in concentrations considerably less than that needed for the synthesis of animal tissues. The high concentration is of importance in evaluating the supplemental value of these materials as forage for non-ruminant herbivores.

The diamino acid, lysine, was also present in the bulk proteins of the cereals and annual ryegrass in amounts only somewhat less than that found in total animal body protein; the lysine content varied from 5.3 to 6.3 per cent for various cereal grasses compared with values of 8 to 9 per cent in animal tissues. This amino acid is one which also tends to be present in amounts limiting growth on many rations compounded for non-ruminant

TABLE I
 Amino Acid Composition of Cereal Grasses and Annual Ryegrass from Greenhouse Culture

PLANT	NO. OF SAMPLES	AMINO ACID DISTRIBUTION (GRAMS FROM 100 GRAMS OF PROTEIN, N=16%)									
		NITROGEN	LEUCINE	ISOLEUCINE	VALINE	METHIONINE	LYSINE	THREONINE	GLUTAMIC ACID	PHENYLALANINE	TRYPTOPHAN
BALBO RYE	5	Ave. 4.3	9.5	6.4	5.3	1.2	5.3	8.7	8.7	4.2	0.8
		S.D.* 0.5	2.5	0.9	0.8	0.44	2.2	3.7	2.4	0.3	0.36
MICHIGAN WINTER BARLEY	5	Ave. 4.8	8.0	5.3	5.5	1.3	5.6	7.0	8.3	4.0	0.8
		S.D. 0.8	1.1	1.0	1.0	0.03	1.4	3.0	0.9	0.7	0.14
CLARKAN WHEAT	5	Ave. 3.8	8.5	5.5	5.7	1.3	6.1	7.3	7.6	4.5	0.9
		S.D. 0.5	0.9	1.3	1.0	0.32	2.2	3.0	2.0	0.7	0.22
TENMARQ WHEAT	5	Ave. 4.6	8.9	5.3	5.3	1.1	6.3	7.9	8.4	4.1	1.0
		S.D. 0.4	1.1	1.1	1.2	0.28	1.7	3.1	3.0	0.9	0.17
WINTOK OATS	4	Ave. 3.8	7.6	5.6	4.9	1.0	6.0	6.5	8.1	3.8	0.8
		S.D. 0.7	1.8	0.5	0.7	0.42	1.5	2.4	1.7	0.7	0.33
ANNUAL RYEGRASS	5	Ave. 4.2	8.8	6.2	5.0	1.1	5.6	7.8	8.7	4.5	1.0
		S.D. 0.7	1.7	0.7	1.0	0.48	2.7	3.0	0.8	0.6	0.34

* S. D. = Standard Deviation of the mean.

animals, and hence the proteins of these cereal grasses would tend to supplement such rations to advantage from the point of view of amino acid nutrition.

Methionine, on the other hand, was found to be present in amounts somewhat less than the concentrations in animal proteins but in amounts similar to that summarized by Lugg (6) as present in various spermatophytes. This situation, coupled with the generally low content of methionine in seed proteins, is generally unfavorable with respect to the use of plant leaf proteins as a source of this amino acid for animal feeding.

Glutamic acid was present in concentrations varying from 7.6 to 8.7 per cent, values which correspond reasonably well to those reported by Lugg (6) for a group of *Graminae* (5.6 to 7.8). When one compares the concentration of this amino acid in leaf proteins with the composition of the seeds of these cereals, it is obvious that a very dramatic shift in protein composition occurs in the development of the seed. More detailed study to determine the location of this transformation would appear warranted. Examination of the raw data reveals a general tendency in wheat, and to a lesser extent in oats, for glutamic acid to increase with the age of the planting. This suggests that the plant may be tending toward a protein metabolism typical of seed development, even though it was maintained in a continuously vegetative state by repeated clippings.

The aromatic amino acid, phenylalanine, was found in concentrations comparable to those present in other plant and animal proteins, ranging from 3.8 to 4.5 per cent. This amount is somewhat in excess of that summarized by Lugg (6), but can probably be accounted for on the basis of the marked improvement in assay procedure for this amino acid made possible by the introduction of microbiological methods. The reported tryptophan concentrations should be regarded as minimal values, due to the well recognized destruction of this amino acid which occurs in any hydrolysis procedure currently available for freeing this amino acid from protein. Even considering this factor, however, it would appear that these cereal grass proteins are not particularly rich in this amino acid. Thus, the average values of from 0.8 to 1.0 per cent are comparable with concentrations near 1.5 per cent reported for most animal tissue proteins and for many plant seed proteins as well.

SUMMARY

Leaf tissue of annual ryegrass and the cereals Tenmarq wheat, Clarkan wheat, Michigan Winter barley, Balbo rye, and Wintok oats, produced by greenhouse culture, was analyzed for the following amino acids in the bulk protein: leucine, isoleucine, valine, threonine, methionine, lysine, glutamic acid, phenylalanine, and tryptophan. No marked variation in amino acid distribution was observed among the various cereals, nor between them and annual ryegrass. Average values for all clippings of all cereals and for annual ryegrass respectively are therefore presented, the amino acid values being in per cent of total bulk protein: total nitrogen, 4.3, 4.2 per cent; leucine, 8.5, 8.8; isoleucine, 5.7, 6.2; valine, 5.3, 5.0; threonine, 7.5, 7.3; methionine, 1.2, 1.1; lysine, 5.9, 5.6; glutamic acid, 8.2, 8.7; phenylalanine, 4.1, 4.5; tryptophan, 0.86, 1.0.

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