Amino Acid Profiles, Chemical Scores, and Mineral Contents of Some Pearl Millet Inbred Lines

The grain from 14 inbred lines of pearl millet (*Pennisetum americanum* (L.) Leake) from the plant breeding program at Tifton, Georgia, was analyzed for protein, amino acid composition, and mineral assay. Protein content varied from 10.7 to 17.1%. Lysine and methionine were negatively correlated with total protein in these samples. Chemical scores on the amino acids showed lysine to be the first limiting amino acid and, in many cases, the only limiting amino acid. Mineral content varied considerably among the different hybrids. The predominant elements present were phosphorus and potassium along with substantial quantities of calcium, iron, zinc, copper, magnesium, and manganese.

The nutritive value of millet is well documented (Burton et al., 1972; Papli and Singt, 1972; Desikachar, 1975; Dendy, 1977), and its many virtues have been much heralded in recent years. Millets have been and continue to be the staple food for millions of people in Africa, India, and parts of eastern Europe. The cereal is prepared and eaten in a variety of ways, but, because of its strong flavor and its classification as a course grain, it has received little acceptance in the more affluent countries, which have access to the more bland grains such as wheat and rice. Millet is grown in the United States as a forage crop, and except for its use as a novelty item for health food enthusiasts, it has no commercial status as a food source for humans.

Pearl millet hybrids, especially the dwarf varieties, are receiving considerable attention in the United States with respect to improvement of essential amino acid balance and crop yield potential (Burton and Foston, 1966; Badi et al., 1976) to assist in the development of pearl millet as an economically viable grain crop. In the present report, selected pearl millet inbred lines were screened for their

Table I. Amino Acid Composition of Some Pearl Millet Inbreds (g/16 g of Nitrogen)

	amino acid content													
amino acid	Tift 2D	Tift 5D	Tift 6D	Tift 8D	Tift 9D	Tift 12	Tift 17	Tift 21	Tift 28	Tift 29	Tift 30	Tift 18DB	Tift 23DB	Tift 383D
alanine	8.9	8.5	8.6	8.2	8.4	8.6	8.5	7.9	8.5	8.5	8.4	8.0	7.5	8.8
valine	5.4	5.3	5.1	5.8	6.1	5.7	5.8	5.0	4.4	5.6	5.6	4.2	5.0	5.5
glycine	2.9	3.2	3.5	3.9	3.5	2.9	2.7	2.5	3.3	2.9	3.3	2.6	3.4	3.1
isoleucine	4.0	4.4	4.2	4.5	4.8	4.7	4.8	4.0	3.6	5.0	4.5	3.3	4.1	4.2
leucine	11.0	10.8	11.0	10.0	10.7	11.2	11.2	10.5	10.4	11.3	10.7	10.3	9.1	10.3
proline	6.8	6.8	7.1	6.7	6.8	6.6	6.8	6.2	6.3	7.3	6.8	6.0	6.5	7.1
threonine	4.1	4.2	4.3	4.4	3.0	4.3	4.0	4.0	4.0	4.0	4.0	3.9	4.4	4.3
serine	5.5	5.3	5.4	5.1	4.9	5.2	5.1	5.5	5.4	5.2	5.2	5.5	5.2	5.5
methionine	1.8	2.0	1.8	1.7	1.7	1.6	2.0	1.8	1.8	1.9	1.9	1.6	2.2	2.6
phenylalanine	5.3	5.9	5.3	5.7	5.8	5.7	5.9	5.1	5.0	5.8	5.7	5.0	5.8	5.8
aspartic acid	8.2	8.1	8.1	8.3	8.0	8.2	8.0	8.8	8.5	7.7	7.9	8.9	8.9	8.2
glutamic acid	19.9	20.1	20.0	18.5	19.7	20.0	20.0	1.0	19.4	20.0	19.7	21.5	17.6	17.8
tyrosine	2.0	3.1	3.0	3.3	3.5	3.1	3.2	3.3	4.0	3.3	3.2	2.4	3.5	3.4
lysine	2.0	2.9	2.4	3.6	2.9	2.8	2.6	2.8	2.9	2.3	2.5	2.9	3.6	2.7
histidine	1.6	1.7	1.5	1.5	1.6	1.3	1.3	1.5	2.0	1.2	1.7	1.8	1.7	1.7
arginine	4.4	3.0	3.8	3.6	3.2	3.9	2.9	4.0	4.7	2.6	3.0	5.1	5.1	3.2
cysteine/2	1.8	1.6	1.9	1.9	1.8	1.5	1.5	1.8	1.9	1.8	1.8	1.7	2.2	1.7
tryptophan	4.4	3.1	3.0	3.3	3.6	3.8	3.5	4.1	3.8	3.5	3.8	4.7	3.7	4.0
recoveries %	82	99	89	94	95	98	117	76	93	91	93	87	92	97
% protein in meal (N × 6.25)	15.1	17.1	16.7	13.9	15.5	13.5	14.0	16.1	12.2	17.1	15.8	12.4	10.7	10.7

Table II. Mineral Content of Some Pearl Millet Inbreds

	element[a]										
inbred	Ca	Mg	Fe	Sr	Cu	Zn	Mn	Ti	Br	K	P
Tift 2D	29	5	3	26	9.3	10.6	10.5	9.6	13	510	640
Tift 5D	28	8	2	nd[b]	11.3	11.4	7.2	8.4	10	860	870
Tift 6D	34	12	6	19	9.2	12.0	15.5	10.9	12	470	740
Tift 8D	28	7	4	60	9.2	11.4	10.3	12.9	17	470	530
Tift 9D	21	15	5	54	8.6	10.5	22.0	11.4	16	490	670
Tift 12	22	11	3	15	12.3	13.1	11.2	10.0	12	450	770
Tift 17	25	15	3	43	9.9	10.6	10.8	13.1	19	510	890
Tift 21	31	12	1	55	9.0	9.9	8.3	10.9	17	480	580
Tift 28	28	14	3	71	8.7	10.6	12.4	12.8	19	370	380
Tift 29	17	14	5	64	9.5	11.1	13.5	11.4	17	410	480
Tift 30	21	11	5	48	9.0	13.7	10.9	10.0	17	430	630
Tift 18DB	21	10	3	70	9.7	2.6	10.0	3.4	19	nd	420
Tift 23DB	22	12	3	70	21.0	5.3	20.0	4.2	19	nd	460
Tift 383D	19	nd	5	34	10.1	11.4	12.0	11.6	13	450	540

[a] Microgram/gram, dry basis. [b] Not determined.

Table III. Amino Acid Chemical Score of Some Pearl Millet Inbreds[a]

amino acid	suggested amino acid pattern, mg/g of protein[b]	Tift 2D	Tift 5D	Tift 6D	Tift 8D	Tift 9D	Tift 12	Tift 17	Tift 21	Tift 28	Tift 29	Tift 30	Tift 18DB	Tift 23DB	Tift 383D
lysine	55	36	53	44	65	53	51	47	51	53	42	45	53	65	49
threonine	40	103	105	108	110	75	108	100	100	100	100	100	98	110	108
methionine–cystine	35	103	103	106	103	100	89	100	103	106	106	106	94	128	123
leucine	70	157	154	157	143	153	160	160	150	149	161	153	147	130	147
isoleucine	40	100	110	105	113	120	118	120	100	90	125	113	83	103	105
valine	50	108	106	102	116	122	114	116	100	88	112	112	84	108	110
phenylalanine–tyrosine	60	122	150	138	150	155	147	152	140	150	152	148	123	155	153
tryptophan	10	440	310	300	330	360	380	350	410	380	350	380	470	370	400
first limiting acid		Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys
second limiting acid						Thr	Met/Cys			Val			Ile		

[a] Amino acid score = [(mg of amino acid in 1 g of protein)/(mg of amino acid suggested)] × 100. [b] Joint FAO/WHO Ad Hoc Committee (1973).

protein content, amino acid quality, and mineral uptake.

MATERIALS AND METHODS

Fourteen pearl millet inbreds grown under comparable agronomic conditions at the Georgia Coastal Plain Experiment Station, Tifton, Georgia, during the 1974 crop year were selected for screening. Eight of these were dwarfs and are designated with the letter D following the number of the breeding line. The grains were homogenized in a petroleum ether solvent and the dissolved lipids were removed by decanting off the supernatant. This procedure was repeated three times or until there was no color in the supernatant. The protein content of the lipid-free whole meal was estimated by the macroKjeldahl procedure with N × 6.25 as a conversion factor. Duplicate amino acid analyses were made by ABC Laboratories, Inc., Columbia, Missouri, by gas chromatography by the procedure described by Kaiser et al. (1974). Tryptophan was determined by the procedure of Villegas and Mertz (1971) as follows. Approximately 90–100 mg of the meal was weighed accurately, transferred to a 5-mL, wide-mouth, Teflon-cap vial, and 4 mL of papain solution (4 mg/mL in 0.1 N sodium acetate buffer at pH 7) was added. The solution was incubated for 24 h with occasional shaking. One milliliter of the hydrolyzate was combined with 2 mL of Fe^{3+}-HAc solution (270 mg of $FeCl_3 \cdot 6H_2O$ in 0.5 mL of H_2O dissolved in 1 L of glacial acetic acid) and 2 mL of concentrated H_2SO_4. The mixture was shaken vigorously and the color allowed to develop for 15 min at 65 °C. After cooling, the solution was transferred to a calibrated tube and the optical density determined. The tryptophan content of the sample was calculated from a standard curve. Mineral assays were made with an X-ray fluorescence spectrometer (General Electric XRD-6) according to the standard published technique of Piccolo et al. (1968).

RESULTS AND DISCUSSION

The data from the protein and amino acid analyses of the 14 pearl millet inbreds are presented in Table I. Wide variations, ranging from a low of 10.7% to a high of 17.1%, are observed in their protein content. Lysine content varied from a low of 2.0% in Tift 2D, which contained 15.1% protein, to a high of 3.6% in Tift 8D and Tift 23DB, which contained 13.9% and 10.7% protein, respectively. Methionine varied from 1.6% in Tift 12 and Tift 18DB to 2.6% in Tift 383D. Both lysine and methionine correlated negatively with the total protein content for those hybrids analyzed. The regression line for the lysine–protein relationship in these hybrids is defined by

$$y = -0.097x + 4.18$$

and the line for the methionine–protein relationship by

$$y = -0.047x + 2.56$$

where x is the protein content and y is the amino acid content. The chemical scores for the protein quality of these hybrids were calculated from their amino acid composition and the amino acid scoring pattern prepared by the FAO/WHO expert committee (1973).

The data are presented in Table III. Lysine is shown to be the most limiting amino acid in each of the inbreds, with the second limiting acid alternating between threonine and isoleucine. With the exception of lysine, many of the inbreds exhibited no other deficiency in essential amino acids. This is in agreement with the accepted fact that millet is one of the better cereals in overall protein quality (Burton et al., 1972).

Mineral assays were determined for the 14 inbreds and

show (Table II) marked differences in the uptake for minerals of the different inbreds of pearl millet. Phosphorus and potassium were the predominant elements in all of them, along with substantial quantities of calcium, iron, zinc, copper, magnesium, and manganese, all of which are necessary for good nutrition in man and animals (Jones, 1977; Tuman and Doisy, 1975). The phosphorus to calcium ratio, however, is poor since it is of the order of 20:1, whereas comparable quantities would be preferable.

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A Novel Antagonistic Effect to the Toxicity in the Rat of *O,O,S*-Trimethyl Phosphorothioate by Its Phosphorothionate Isomer

O,O,S-Trimethyl phosphorothioate (thiolate isomer) has a rat oral LD_{50} value of approximately 15 mg/kg, with death occurring up to 22 days following administration. However, coadministration of the isomeric *O,O,O*-trimethyl phosphorothioate prevents this intoxication. Poisoning by the thiolate isomer, when given at either the 60 or 200 mg/kg level, is completely blocked by 1.0% (w/w) of the antagonist (thionate isomer). At the 60 mg/kg dose, 0.5% of the antagonist gave partial protection, and ~50% of the treated animals recovered. Administration of the antagonist 24 and 48 h after treatment by the thiolate did not affect any reversal of the poisoning symptomology or prevent mortality.

In a previous communication (Mallipudi et al., 1979), we described the delayed toxicity in rats that ensued following single doses of *O,O,S*-trimethyl and *O,O,S*-triethyl phosphorothioates (thiolate isomers). Both compounds are potential impurities in technical grade organophosphorus pesticides and they represent a far greater hazard, particularly for the methyl derivative, than suggested by their 24–48-h acute toxicity mortality date. Subsequent work has revealed an unusual specific antagonism effect to the action of the trimethyl compound, elicited by coadministration of minor amounts of *O,O,O*-trimethyl phosphorothioate (thionate isomer), and we now report our preliminary results defining this observation.

EXPERIMENTAL SECTION

Rat oral LD_{50} data were determined as previously described (Mallipudi et al., 1979) using 95–130-g female albino rats (Sprague-Dawley derived) from Simonsen Laboratories, Gilroy, CA. *O,O,S*-Trimethyl phosphorothioate was synthesized from trimethyl phosphite and methylsulfenyl chloride (Morrison, 1955). Initial product purification was by vacuum distillation, with further cleanup by either preparative TLC [solvent system, hexane–ether (1:1)] or column silica gel chromatography (solvent system, hexane–ether gradient). Structural assignments were verified by NMR and mass spectroscopy and product purity was determined by TLC [solvent system, benzene–ethyl acetate, 1:1; spray reagent, 2,6-dibromoquinone-4-chloroimide (Menn et al., 1957)] and GLC using 2.5% EGSP-Z (Applied Science Laboratories, State College, PA) on Chromosorb 750, 80/100 mesh, surface modified (Aue et al., 1973), and alkali flame ionization detection.

RESULTS AND DISCUSSION

In a continuing investigation of the toxicity of *O,O,S*-trimethyl phosphorothioate to rats, we observed that a sample of the compound which was purified by vacuum distillation could be administered at doses up to the 200 mg/kg level without mortality and symptoms of intoxication (diarrhea, excessive urination, and bleeding), except

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