NOTE

Tryptophan Levels In Normal And High-Lysine Sorghums[1]

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Pushpamma et al (1972) obtained a significant growth response in rats when tryptophan was added to a lysine-supplemented sorghum diet. This suggests that tryptophan is the next limiting amino acid after lysine in sorghum. In maize these authors found lysine and tryptophan equally limiting. Because introduction of the *opaque-2* gene in maize increased both lysine and tryptophan 50% in the whole grain (Mertz et al 1975), it was of interest to determine whether high-lysine genes introduced into normal sorghum had the same effect.

Reliable tryptophan levels in sorghums were not available. The usual method for measuring tryptophan levels in cereals is to digest the cereal enzymatically, convert the tryptophan to a dye, and measure it colorimetrically. Low-tannin sorghums contain substances that interfere with color formation; therefore, to determine tryptophan, an improved version of the Hugli and Moore (1972) alkaline hydrolysis ion-exchange method for determining the tryptophan content of the whole grain was used. This method clearly showed that high-lysine sorghums had higher levels of tryptophan than normal sorghums.

MATERIALS AND METHODS

Low-tannin sorghum varieties from the 1980 harvest in Lafayette, IN, were used for seed. The seeds were ground in a Udy cyclone sample mill (Tecator, Inc., Boulder, CO 80302) to pass through a 0.4-mm screen, and the meal was defatted with hexane in a Soxhlet apparatus. A 100-mg sample of sorghum was placed in a Tefzel 120 × 27 mm screw cap tube (Scientific Products Co., McGaw Park, IL 60085)—replacing the two-tube system used by Hugli and Moore (1972)—and 0.5 ml of water and 5 ml of 5*N* sodium hydroxide were added. The mixture was degassed by aspirating the air out and replacing it with nitrogen. The cap was screwed on tightly, and the tube was placed in a forced draft oven at 110° C. After 24 hr the tube was removed and placed in ice, and 2 ml of chilled 6*N* HCl was added, followed by 5.5 ml of pH 4.2 (0.2*N* Na+) sodium citrate buffer. The mixture was centrifuged for 15 min at 20,000 × *g* at 4° C and filtered on a 0.2-μm Millipore filter. Filtrate (0.5 ml) was metered to the short column of a model 120C Spinco amino acid analyzer (Beckman Instruments, Palo Alto, CA) with 8 cm of PA35 resin (8-cm column). The elution buffer was pH 5.4 (0.2*N* Na+) sodium citrate at 52° C, and the buffer flow rate was 50 ml/hr. The peak was calculated by the height-width method (Beckman 1965), adding a 5% correction to compensate for adsorption of the tryptophan on the sorghum starch (Hugli and Moore 1972). With the modified Hugli-Moore method, we obtained values of 0.6% tryptophan on normal whole grain maize and 0.9% on its high-lysine counterpart, compared with values of 0.65 and 1.05% obtained by Hugli and Moore (1972) on samples of normal and high-lysine maize supplied by us in 1972.

The use of a Tefzel tube and nitrogen gas eliminates three steps in the Hugli-Moore method: 1) repeated application of vacuum to the cooled sample suspension to remove dissolved oxygen, 2) use of a sealed evacuated tube for hydrolysis, and 3) transfer of hydrolysate to a volumetric flask prior to centrifugation and chromatography.

Six of the eight sorghum varieties were also analyzed for tryptophan using the enzymatic-colorimetric method of Hernandez and Bates (Villegas et al 1984). The sorghum varieties were also analyzed for lysine content using the usual acid hydrolysis procedure (Beckman 1965) and a 5-cm resin column.

RESULTS AND DISCUSSION

Table I shows that the introduction of the high-lysine gene into normal sorghums increased the means of the lysine and the tryptophan contents about 55 and 50%, respectively. Sorghum thus resembles maize, which shows similar percentage increases (Mertz et al 1975). The data in parentheses (Table I) show that values for tryptophan in sorghum are lower using the Hernandez-Bates colorimetric method. However, the relative values (high-lysine sorghum was twice the value of normal sorghum) suggest that this rapid method could be used to screen high-lysine sorghum cultivars that have no intefering colors.

Normal sorghum has the level of tryptophan (0.8%) recommended by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Panel as ideal for the human infant (Mertz 1974). High-lysine sorghum, with a level of 1.2% tryptophan, has a 50% higher value than the recommended level of tryptophan, which would be converted into niacin in a child (WHO 1967). The World Health Organization (WHO 1967) proposed 60 mg of tryptophan as physiologically equivalent to 1 mg of niacin (where tryptophan is in excess of that needed as a protein component). A child consuming 100 g of high-lysine

TABLE I
Lysine and Tryptophan Levels in Whole Grain Sorghums

Variety	Protein[a]	Lysine[b]	Tryptophan[c]
Normal			
P954063	10.6	2.1	0.8 (0.4)
P721N	12.0	2.1	0.8 (0.5)
IS 0466	11.9	2.0	0.9
P121180	10.0	1.9	0.8
Mean[d]	...	2.0	0.8
High-lysine			
IS 11167	9.6	3.3	1.2
IS 11758	13.2	3.2	1.2
P721O	13.1	2.7	1.0 (0.9)
P850314	11.8	3.2	1.3 (0.8)
Mean[d]	...	3.1	1.2

[a]Grams protein (N × 6.25) per 100 g of meal.
[b]Grams amino acid per 100 g of protein.
[c]Values in parentheses were obtained with the enzymatic colorimetric method of Hernandez and Bates (Villegas et al 1984). Interfering colors prevented colorimetric assay of IS 11167 and IS 11758.
[d]Application of the *t* test showed that the mean of the tryptophan values for high-lysine varieties was significantly higher than the mean for normal varieties at the 1% level of significance; similarily, lysine values were higher at the 0.1% level of significance.

[1]Journal paper 10,325 of the Purdue Agricultural Experiment Station. Supported by the United States Agency for International Development, International Sorghum and Millet Collaborative Support Research Program Project Grant AID/DSAN/XII-G-0149.
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sorghum (10–13% protein) would have enough excess tryptophan from the sorghum protein to produce about 1 mg of niacin (based on the above conversion rate). In addition, according to Pant (1975), 100 g of IS 11167 or IS 11758 (Table I) contains 10.5–11.5 mg of preformed niacin, whereas the three normal sorghums that he assayed contained only 2.9–4.9 mg niacin per 100 g. Normal sorghum, therefore, has no excess tryptophan to serve as a niacin source, and in addition, does not contain enough preformed niacin to meet the daily requirement for this vitamin (6–10 mg recommended for the 1–9 year old age group). This may explain why pellagra was found to be endemic in the Deccan Plateau of India where sorghum was the major cereal (Gopalan and Srikantia 1960). Our data strongly suggest that substitution of high-lysine sorghum for normal sorghum in such areas of the world would help to eliminate pellagra, unless the niacin in high-lysine sorghum is not completely available. Carter and Carpenter (1981) showed that the niacin in cooked normal sorghum was only 34% available in rat bioassays. It is possible that high-lysine sorghum may have less bound niacin than normal sorghum. Tests on niacin availability from high-lysine sorghum are planned.

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[Received May 29, 1985. Revision received October 22, 1985. Accepted November 9, 1985.]