APPENDIX V-9

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Evaluation of the Protein Quality and Digestibility of Four Varieties of Sorghum in the Diets of Infants

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Sorghum is among the world's major cereal crops. Although it is used primarily as an animal feed crop or in making beer in most parts of the world, there is substantial direct human consumption in the less well-off areas of Africa and Asia. Sorghum production is increasing annually: between 1952 and 1968 there was an increase of 1288 percent in acreage and an 80% increase in yield of sorghum world wide (1).

In 1966, USAID began supporting research at Purdue University directed at the improvement of protein quality and content, digestibility and yield of sorghum. Wide variability in protein content, amino acid composition and digestibility of different sorghums offered the promise of improving all three aspects as they related to human nutritional requirements.

The current studies were undertaken to evaluate the protein quality and digestibility in children of 4 varieties of sorghum made available by Purdue University. Young children have the highest relative nutritional requirements and the most limited digestive capacities and consequently represent the major nutritional problem for most countries. If staple foods of improved protein quality and digestibility are to help in the alleviation of malnutrition around the world, they must be able to be consumed and utilized by this age group. For this reason, this form of human testing under the carefully monitored conditions of a metabolic ward is an important part of such breeding improvement programs. Although all our studies with sorghum are not yet completed, they are far enough along and the data are so striking that a preliminary report is warranted.

MATERIALS AND METHODS

Patients (Table 1): Children in recuperation from protein energy malnutrition participated in the studies with the consent of their parents.[1] Table 1 shows the ages, heights, weights and hospital day at the beginning of the study. Height and weight ages can be compared with chronologic age and with each other to give an indication of the stage of recovery. The children ranged in age from 6 to 27 months and weighed between 4.7 and 9.7 kg at the outset. All were free of infection and parasites and were gaining weight well prior to entering into the study. Initial serum albumin concentrations ranged from 3.6 to 4.8 g/dl.

Diets: Four samples of whole grain sorghum flour were submitted for evaluation: Sorghum 954114 was reported to have a protein content of 12.7% and a PER in rats of 0.80, P721-OP9 a protein content of 13.6% and a PER of 1.28. Number 954063 had been analyzed to contain 11.5% protein and a PER of 0.58 had been measured. IS11758 contained 15.8% protein and the PER was reported to be 1.67 (Casein control per 2.31). All four sorghums had been ground on an air-impact grinder through a 0.27" RHD screen by Quaker Oats Company, Barrington, Illinois. The proximate analysis of the four samples is shown in Table 2. Total N content in our laboratory was generally slightly lower than that reported by Purdue University. Our analyzed values were used in the diet calculations.

Based on these values, 4 sorghum diets were designed (Table 3). The amount of sorghum in each case was that needed to provide 256 mg N (1.6 g protein-N x 6.25) per 100 Kcal. A blend of soybean-cottonseed

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[1] The protocol for these studies was approved by the Joint Committee on Human Investigation, School of Medicine, The Johns Hopkins University, and the Committee on Human Subjects of the Instituto de Investigacion Nutricional.

oils (80:20) was added to provide 30% of calories. Sucrose completed the rest of calories. Na, K and Cl were adjusted to constant intakes. Major and trace mineral intakes were assured with a mineral mixture standard for the unit. Vitamins were supplied by Polyvisol (Mead · Johnson) 0.6 to 1.2 ml/day, and folic acid, 200 ug/day.

The sorghum flours were cooked for a period of 15-20 minutes and were then blenderized with the oil, sucrose, minerals and vitamins. Water was added to give a final energy density of approximately 0.67 to 0.8 Kcal/ml. Because of the very poor results obtained in the early studies, concern arose that cooking time was insufficient. In later studies, cooking time was increased to 45 minutes, at which time there was no question that gelatinization of the starch component had occurred. Cooking time (short or long) for individual studies is indicated in the presentation of results.

A control diet based on casein paralleled the sorghum diets. Protein supplied 6.4% of calories. Fat provided 30%. Corn starch, dextrimaltose, and sucrose provided carbohydrate. Minerals and vitamins were the same as in the sorghum diets. During preparation, the corn starch was cooked to the point of gelatinization and was then blenderized with the casein, oil, sucrose and dextrimaltose. Calorie density was the same as that of the sorghum diets.

When initial studies indicated that the apparent digestibility of nitrogen of sorghum by the children was extremely poor, the amount of nitrogen in the diet was increased for subsequent studies. In these later studies, N intakes from sorghum were 320 mg per 100 Kcal (protein = 2.0 g/100 Kcal or 8% of calories). Fat and carbohydrate calories were reduced proportionally. For these studies the N intake from the casein diet was not increased. In this way it was hoped that the amount of nitrogen absorbed from both diets would be more nearly equal. Because of the unequal N intakes (sorghum vs. casein) a bias against finding a difference between the two diets was introduced into the studies at this point.

Protocol: The planned sequence of dietary periods for the studies is shown in Table 4. Because of problems during these studies, not all sequences were used as planned. The departures from the protocol are evident in the presentation of the results. As planned, a 9-day control period (casein) was followed by a 9 day sorghum period and a second sorghum period of 7 days. A second 9-day casein period was followed by two further sorghum periods of 9 and 7 days and a final 9 day control period. The order of consumption of the 4 sorghums was varied with each patient, so as always to sequence one high protein-high lysine sorghum with one of the lower protein-low lysine varieties.

After either a 3-day or 1-day period of adaptation to each new diet, the child was placed in a metabolic bed where collections of urine and stool were carried out during the last 6 days of each dietary period, using methods standard for the unit (2). Three day adaptation periods always followed a change from casein to sorghum or sorghum to casein. A single day of adaptation to the second sorghum in each sequence was used to keep consumption of a protein of expectedly inferior quality to a minimum.

Blood samples for determination of hematocrit, serum total protein (Biuret) and albumin concentration (acetate gel electrophoresis), cholesterol and triglycerides were obtained on the first day of periods 2,4,5 and 7, i.e., just prior to and after completing the sorghum diets. On the last day of periods 3 and 6, blood for plasma free amino acids, fasting and 3 and 4 hours after the 8 a.m. feeding, was obtained. This meant that 4 sets of samples should be obtained with each of the 4 sorghums.

All stools were weighed wet and after drying. Fecal and urinary nitrogen were determined by the microKjeldahl method in 3 day pooled collections. Fecal fat was determined by the method of Van de Kamer et. al. (3). Energy intake and fecal energy losses were determined by bomb calorimetry (4). Apparent absorptions and retentions of nitrogen were calculated. Statistical analysis, where appropriate, was carried out by Student's t test and linear regression (5).

Plasma samples for amino acid analysis were separated, sealed in glass ampules and frozen to $-20^{O}C$ within one hour of being obtained. They were subsequently shipped to our laboratory in Baltimore for analysis by liquid column chromatography (6,7,8) using norleucine as an internal standard. These results are pending at the time of this preliminary report and will be submitted later.

RESULTS

Results of nitrogen balance studies are summarized in Tables 5 and 6. Because of the poor results obtained and the potential importance of these results, the studies of all children have been presented individually in Table 5. In Table 6, we have summarized results by sorghum variety.

Patient 525 had completed 4 dietary periods before it was decided to increase cooking time. Apparent absorption and retention of nitrogen were both very poor during sorghum consumption. Stool weights increased and serum albumin concentrations decreased in association with sorghum. Because of an intercurrent urinary tract infection, this patient could not be re-entered in the study.

Patient 549 had consumed only one sorghum variety before cooking time was increased. Apparent absorption and retention of nitrogen were very low. Problems unrelated to the studies precluded re-entrance in the study.

Patient 561 had consumed one variety of sorghum before cooking time was increased. Apparent N absorption and retention were very poor. Repeat studies were begun. Consumption of sorghum was again associated with poor absorption and retention of nitrogen. Stool weight and energy loss doubled when compared with casein. The study was stopped after one day of the second sorghum period because of loss of appetite and refusal to eat.

Patient No. 568 entered the study but excessive fecal fat losses during the control period were reported 4 days into the first sorghum period; the study was stopped. After further recuperation, studies were reinitiated. Two sorghum periods with short cooking time were markedly inferior to casein in apparent digestibility. Apparent N retention was zero in both cases. Repeat studies with longer cooking time were stopped when apparent N retention was again zero during sorghum consumption.

Patient 569 vomited repeatedly during the sorghum dietary period. For this reason the study was stopped. Apparent absorption and retention of nitrogen were inferior to casein but better than encountered in most of the other children.

Patient No. 570 was the only child to complete all 7 dietary periods. Two sorghum periods with shorter cooking time yielded poor results. During studies with longer cooking time, consumption of P721-OP9 was associated with apparent N absorption and retention that were markedly inferior to casein but higher than those from the lower lysine containing 954114. In the second sequence, the lower apparent digestibility of IS11758 (higher lysine) markedly prejudiced apparent N retention. Absorption and retention of 954063 (low lysine) were lower than casein. The results obtained in this child nearly equalled those we have seen previously with other staples, rice for examples, but were the exception rather than the rule and were not representative of our experience as a whole with sorghum.

Patient No. 571 developed infectious enteritis during his first sorghum period. He was never able to be re-entered in the study.

Patient No. 572 completed 4 dietary periods. Both sorghum diets were cooked 45 minutes. Depsite this fact, apparent absorptions of N were 43% and 25%, apparent retentions 8% and 9%. Stool weights were very high. Fecal fat, already slightly elevated during the first control period, increased dramatically during both sorghum periods. Fecal energy losses reflected the poor digestibility of sorghum and the losses of fat. Rates of weight gain decreased. This child had significant abdominal distention during sorghum consumption and because of this and the fecal fat losses, the second half of the study was not completed.

Patient No. 575 completed only one sorghum period. Absorption and retention of nitrogen were poor, stool weight was nearly 5 times that of control, and fecal fat and energy losses were one third of intake. For these reasons the study had to be stopped.

Table 6 summarizes the results of all the balance studies to date. The picture during sorghum consumption was one of very poor apparent N absorption and very low apparent N retention. Fecal weight and fecal energy losses were excessive. Rates of weight gain were apparently less although changes of body weight over periods of 7 to 9 days must be interpreted cautiously. Finally, it should be noted that apparent N retention during the post-sorghum casein control period was substantially higher than that during the pre-sorghum control period. This increased retention is further indication of the inferiority of sorghum and represents an attempt to compensate for poor N retention during the sorghum dietary periods.

DISCUSSION

The results of studies to date cast serious doubt on the usefulness of sorghum as a food for small children. Our experience with 18 sorghum dietary periods in 9 children indicates that for the two varieties predominantly studied the protein of sorghum is of poor digestibility and of low quality. The large fecal energy losses are more than can be explained on this basis alone and further indicate that sorghum is a poor source of energy in general.

Our initial studies were carried out with a short cooking time and the poor results caused us to worry that improper preparation was to blame. An analysis of results by cooking time showed no difference related to this factor. If anything, results of studies with shorter cooking time may have been slightly better. Furthermore, a recently published book on use of sorghum in Africa and Asia shows that in actual practice, the grain is usually cooked for only 15 or 20 minutes (9).

Consequently, all studies regardless of cooking time have been included in our analysis.

To place the present results in perspective, the mean values of selected parameters of these studies have been compared with similarly obtained data from four other staple foods, wheat, rice, potato and maize. These are also compared with representative values for casein. Nitrogen absorption from sorghum was only 75% of that from rice and potato--the lowest of the other four staples. The nitrogen retention from sorghum was less than half of that from wheat, a third of that from rice and maize, and a quarter of that from potato. Because of wheat's excellent digestibility, lysine supplementation markedly improves N retention. New varieties of maize (opaque 2 and sugary 2-opaque 2) have already yielded results superior to those presented in Table 7. Protein quality of the potato is obviously quite high. Our studies have suggested that the major drawback to the use of potato for child nutrition is its bulkiness and the relative indigestibility of the carbohydrate component. Yet, fecal energy losses with potato-based diets were less than half of those with sorghum.

Although further studies with varieties 954063 and IS11758 are under way, it is unlikely that their results will differ enough from those already obtained to change our initial assessment of sorghum. The protein digestibility and apparent protein quality of sorghum is lower than any food or food blend studied in our unit during the past 17 years. Bulkiness is a problem and overall digestibility is very poor. Whereas poor protein quality could have been predicted from the rat studies, the poor digestibility could not. One has to wonder to what extent it was responsible for the low PER's. Sorghum has been shown to be useful as a component of some food blends (Thriposha, for example) and it may be that with better processing methods some of the problems of digestibility can be overcome. We would be anxious to study sorghum further if improved milling or processing is developed. Until the problem of digestibility can be overcome, there is little benefit to be anticipated from amino acid fortification. In the meantime, consumption of significant quantities of sorghum by children cannot be expected to have a benficial nutritional effect and should be discouraged if any of the other major staples is or can be made available.

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Table 5

Individual patient summaries of balance studies with sorghum

Pt. No.	Diet			Cooking Time[o]	No. Days	Apparent N Balance				Stool					Serum [Albumin]	
										Weight		Fat	Energy			
		Kcal/ Kg/d	% Protein Kcal			Δ Wt. g/kg/d	On Days	ABS* %	RET+ %	Wet g/d	Dry g/d	% Intake	Kcal/d	% Intake	Final g/dl	Δ g/dl
525	Casein	110	6.4		11	7.2	6-11	82	47	145	17.95	9.3	71.8	6.9	3.94	+0.11
	954114	110	6.4	S	9	1.8	4-9	64	17	208	27.17	3.7	107.2	8.9	—	—
	P721-0P9	110	6.4	S	7	2.3	2-7	72	24	193	26.70	3.9	93.2	—	3.43	-0.51
	Casein	110	6.4		9	4.9	4-6	82	51	123	13.90	9.2	—	—	3.74	+0.31
549	Casein	110	6.4		10	2.2	4-10	90	31	62	12.02	7.7	—	—	4.81	—
	954114	110	6.4	S	9	1.3	4-6	66	13	179	22.54	4.9	—	—	4.76	-0.06
	Casein	110	6.4		11	10.5	6-11	83	36	99	21.41	18.4	92.4	7.6	4.11	-0.65
561	Casein	110	6.4		11	0.2	6-11	76	30	122	15.56	14.7	—	—	4.38	—
	IS11758	110	6.4	S	10	1.9	4-7	48	7	204	29.37	5.4	—	—	4.09	-0.29
	Casein	110	6.4		9	5.6	4-9	81	33	123	17.08	9.7	65.4	6.2	4.11	—
	P721-0P9	110	6.4	L	13	0.9	4-10 12-13	58	11	262	34.06	11.6	136.4	—	—	—
	954114	110	6.4	L	1											
568	Casein	100	6.4		11	0.9	6-11	72	17	110	26.61	48.8	146.8	15.5	3.30	—
	P721-0P9	100	6.4	S	4	4.1										
	Casein	100	6.4		9	2.2	4-9	75	22	50	13.36	14.9	71.9	7.5	3.54	—
	P721-0P9	100	6.4	S	12	1.1	4-12	27	0	204	32.99	10.1	—	—		
	954114	100	6.4	S	5	0.0	2-4	19	0	166	36.66	12.2	—	—	2.58	-0.96
	Casein	100	6.4		9	1.2	4-9	73	28	127	24.37	18.6	99.6	9.1	3.77	-0.20
	954114	100	6.4	L	8	0.0	4-6	32	0	205	[illegible] 36	21.4	—	—	3.41	-0.36
569	Casein	125	6.4		9	6.1	4-9	84	49	111	21.70	22.8	—	—	3.75	—
	954114	125	6.4	S	7	-4.1	4-6	65	33	136	30.10	20.2	—	—	—	—

[o] Cooking time: S = short (15-20 minutes), L = long (45 minutes).

* Apparent absorption of nitrogen.

+ Apparent retention of nitrogen.

Table 5 (Cont.)

Individual patient summaries of balance studies with sorghum

	Diet					Apparent N Balance				Stool					Serum [Albumin]	
										Weight		Fat	Energy			
Pt. No.		Kcal/ Kg/d	% Protein Kcal	Cooking Time°	No. Days	Δ Wt. g/kg/d	On Days	ABS* %	RET+ %	Wet g/d	Dry g/d	% Intake	Kcal/d	% Intake	Final g/dl	Δ g/dl
570	Casein	125	6.4		14	5.4	6-14	87	43	47	13.69	12.4	64.9	6.7	3.48	—
	954114	125	6.4	S	9	3.0	4-9	63	8	98	26.68	5.8	120.9	11.1	—	—
	P721-0P9	125	6.4	S	7	3.9	4-6	56	17	163	30.04	5.6	—	—	3.32	-0.16
	Casein	125	6.4		9	9.7	4-9	84	52	104	15.63	10.0	67.3	6.0	3.48	+0.16
	954114	125	6.4	L	9	2.2	4-9	59	19	228	30.93	7.4	124.3	10.2	—	—
	P721-0P9	125	6.4	L	7	3.4	2-7	63	27	263	40.30	8.4	158.2	12.6	4.04	+0.56
	Casein	125	6.4		9	8.8	4-9	83	59	110	18.70	7.4	66.6	5.5	3.59	-0.45
	IS11758	125	6.4	L	10	0.9	4 6-10	45	20	281	45.50	9.1	194.0	14.7	3.62	+0.03
	954063	125	6.4	L	7	5.5	2-7	69	30	184	32.60	8.6	131.4	10.0	3.58	-0.04
	Casein	125	6.4		9	2.6	4-9	83	60	173	22.56	6.1	80.6	6.3	—	—
571	Casein	125	6.4		11	2.2	6-11	70	25	142	17.89	21.6	104.0	12.1	3.61	—
	P721-0P9	125	6.4	L	5	5.8	—									
572	Casein	150	6.6		11	8.5	6-11	81	42	82	14.06	14.1	68.3	7.6	3.51	—
	P721-0P9	150	8.0	L	10	3.3	4-6 8-11	43	8	302	49.01	31.6	241.6	24.2	—	—
	954114	150	8.0	L	7	0.0	3-7	25	9	349	67.22	42.6	364.2	34.0	3.24	-0.27
	Casein	150	6.6		9	10.3	4-9	78	58	197	20.93	19.0	103.4	9.7	3.50	+0.26
575	Casein	150	6.6		11	9.5	6-11	82	45	57	10.30	9.9	45.4	6.3	3.48	-0.28
	954114	150	8.0	L	10	0.0	5-10	22	9	276	57.24	34.9	287.0	34.6	3.61	+0.13
	P721-0P9	150	8.0	L	1	—										

° Cooking time: S = short (15-20 minutes), L = long (45 minutes).

* Apparent absorption of nitrogen.

+ Apparent retention of nitrogen.

Table 6

Summary of balance studies with sorghum[a]

Diet	n	Δ WT	ABS[b]	RET[c]	Stool: Weight Wet	Stool: Weight Dry	Stool: Fat	Stool: Energy	Stool: Energy	Serum [Albumin] Final	Serum [Albumin] Δ
		g/kg/d	%	%	g/d	g/d	% Intake	Kcal/d	% Intake	g/dl	g/dl
Casein Pre	11	5.3 ±3.4	81.4 ±5.1	38.4 ±9.9	93.6 ±35.2	15.97 ±4.15	13.1 ±4.5	69.3 ±14.8	7.6 ±1.7	3.88 ±0.44	-0.05 ±0.22
954114	9	0.5 ±2.1	46.1[d] ±20.9	12.0[d] ±10.2	205.0[d] ±75.0	37.99[d] ±15.20	17.0 ±14.0	200.7[e] ±117.4	19.8[e] ±13.3	3.52 ±0.79	-0.30 ±0.41
P721-OP9	6	2.5 ±1.3	53.2[d] ±15.9	14.5[d] ±10.2	231.0[d] ±53.0	35.52[d] ±8.01	11.9 ±10.1	157.4[e] ±62.3	12.3[e] ±12.1	3.60 ±0.39	-0.04 ±0.55
954063	1	5.5	69.0	30.0	184.0	32.60	8.6	131.4	10.0	3.58	-0.04
IS11758	2	1.4	47.0	14.0	243.0	37.44	7.3	194.0(n=1)	14.7(n=1)	3.86	-0.13
Casein Post	5	7.4 ±3.5	81.8 ±2.2	52.8 ±10.0	140.0 ±42.0	19.50 ±3.43	12.0 ±6.2	85.8[e] ±15.8	7.3 ±1.8	3.74 ±0.27	-0.13 ±0.49

[a] Values are mean ±SD.
[b] Apparent absorption of nitrogen.
[c] Apparent retention of nitrogen.
[d] Significantly less than corresponding casein (pre) control value, $P<0.001$.
[e] Significantly less than corresponding casein (pre) control value, $P<0.05$

Table 7

Comparison of various results of sorghum studies with corresponding data from other staple foods

	Apparent N Balance		Stool Weight		Stool Energy
	ABS %	RET %	Wet g/d	Dry g/d	Kcal/d
Sorghum[1]	50	9	217	36.8	180.9
Wheat[2]	81	20	95	13.3	59.6
Rice[3]	66	26	67	11.6	58.0
Potato[4]	66	34	165	20.3	77.8
Maize[5]	73	27	133	28.6	116.6
Casein[6]	81	38	94	16.0	69.3

[1]Mean values of all 18 sorghum periods.

[2]Studies of all wheat pasta, J. Nutrition , in press.

[3]Data from studies of two varieties of rice, J. Nutrition, 108:1740, 1978.

[4]Data from 9 balance studies with potato , report in preparation.

[5]Data from 6 studies with whole kernal normal maize, report in preparation.

[6]Data from the 11 preceding casein control periods of these studies.