Nitrogen Balance in Infants Fed Formulas Containing Amaranth or a Soy-Oats Formula

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ABSTRACT

A nitrogen balance study of 10 infants five to 18 months old compared two infant formulas containing amaranth (*Amaranthus cruentus*) with a soy-oats formula. Analysis of covariance techniques were used to investigate significant differences between types of formula with respect to nitrogen absorption and retention. Infant weight and nitrogen intake were used as covariates in order to adjust the dependent variables to a constant level of each of these covariates. No significant differences ($P \leq 0.05$) were found among the three formulas with respect to either nitrogen absorption or nitrogen retention.

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Malnutrition among Tarahumara Indians living in the state of Chihuahua in Mexico continues to be very serious. The problem is most acute with lactating mothers and infants; because of malnutrition, the former do not produce enough breast milk, resulting in high morbidity and mortality among the latter. A relatively recent study conducted at Hospital Infantil del Estado de Chihuahua (HIEC) (Chihuahua Children's Hospital) revealed that 70% or more of all Tarahumara infants admitted were suffering from tuberculosis caused indirectly by malnutrition (H. Villanueva, HIEC, *personal communication,* 1979).

One possible solution to the problem would be to develop an infant formula, especially designed for the target population, that could be produced and distributed through either public or private channels. Such a product would have to 1) provide all nutrients required by infants, 2) be as inexpensive as possible, and 3) be acceptable to the target population. The first requirement would be satisfied by designing the formula in accordance with all recommendations established by the Committee on Nutrition (CON) of the American Academy of Pediatrics for infant formulas (CON 1976). The second requirement could be met by using high-quality, low-cost plant raw materials as protein sources; and the third requirement could be met by selecting raw materials that are already components of the Tarahumara diet.

Two such raw materials are oats and amaranth (*Amaranthus cruentus*). Since Aztec times, Indian groups have consumed amaranth, more recently in the form of popped seeds known as *alegria* (Early 1977, Sanchez-Marroquin 1980). Tarahumara Indians in Chihuahua also consume oats.

Del Valle et al (in press) developed and evaluated two infant formulas containing amaranth and oats. One product (amaranth 1-R formula) contained oats plus the protein-rich fraction of amaranth resulting from the air classification of amaranth seed (fraction 1-R); the other (amaranth 2-R formula) contained oats, dehulled soybeans, and the starch-rich fraction resulting from the air classification of amaranth seed (fraction 2-R) (Sanchez-

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Marroquin et al 1986). Both formulas were shown to satisfy all CON (1976) requirements and to equal a previously developed soy-oats formula (Del Valle et al 1981) in proximate chemical composition and calorie distribution pattern, with optimum essential amino acid complementation. The soy-oats formula was used as a reference or control because it was previously found to equal a commercial cow's milk formula in nutritive quality and because it has been manufactured and sold in Mexico since 1979 (Del Valle 1982, Mermelstein 1983). No significant differences were found between either amaranth formula and the soy-oats formula in terms of protein efficiency ratio (PER) and net protein utilization (NPU). Both amaranth products cost less than the soy-oats formula; the 1-R formula cost 18% less and the 2-R formula 5% less.

Del Valle et al (in press) did not conduct nutritional evaluations of the formulas directly with infants. The purpose of the work reported here, therefore, was to investigate nitrogen balance in human infants to compare both of the amaranth formulas with the soy-oats formula.

MATERIALS AND METHODS

Preparation of the Formulas

Both amaranth formulas (1-R and 2-R) and the soy-oats formula were prepared from the same protein raw materials and other ingredients used by Del Valle et al (in press), in the same manner described in that study. Table I shows the complete ingredient compositions of the three formulas. Amounts of vitamins and minerals added to the amaranth formulas met the CON (1976) recommendations (Table II).

Nitrogen Balance Study

The nitrogen balance study was conducted in the metabolic unit of HIEC. The unit, which was equipped for this purpose, contained two metabolic beds, all necessary equipment for formula preparation, and a freezer. A pediatric nurse was in attendance at all times.

Each metabolic bed had a mattress with a 20-cm hole in the middle. An infant taking part in the study was placed in the bed in such a manner that its buttocks, and hence its anus, were over the hole. A container was located below the hole to collect all fecal material produced by the infant. The urine output of each infant was collected by means of a catheter inserted into its bladder. The catheter drained into a urine collection bottle. All fecal and urine samples were collected quantitatively into wide-mouth jars, measured, and stored frozen until they were analyzed for nitrogen content by the Kjeldahl method (AOAC 1989).

The participants in the nitrogen balance study were malnourished infants who had been previously admitted to the HIEC for treatment of pathological conditions common for their age group (five to 18 months) and who had recovered from these conditions. The subjects selected for the study were those children found to be most malnourished, as judged by tables of height and weight for age for Mexican children (Ramos-Galvan 1975). Parents of all subjects were fully informed, in detail, about the experimental procedure involved and their right to withdraw from the experiment at any point; all gave their informed written consent, which was required by the human subjects review mandated by the National Council for Science and Technology funding agency.

TABLE I
Ingredient Composition of the Amaranth and Soy-Oats Infant Formulas

Ingredient	Amaranth 1-R Formula (%)	Amaranth 2-R Formula (%)	Soy-Oats Formula[a] (%)
Amaranth fraction 1-R	42.2	0.0	0.0
Amaranth fraction 2-R	0.0	14.5	0.0
Pearled oats	28.5	25.0	25.6
Dehulled soybeans	0.0	25.0	32.1
Sucrose	21.4	16.4	34.1
Corn starch	0.0	10.8	0.0
Vegetable oil	6.3	6.3	5.8
Tricalcium phosphate	1.2	1.2	1.2
Sodium chloride	0.5	0.5	0.5
DL-Methionine	0.0	0.3	0.2
Vitamin and mineral mix	0.1	0.1	0.1
Artificial flavoring	0.1	0.1	0.1

[a]Del Valle et al (1981, p. 194).

TABLE II
Vitamin and Mineral Content of the Amaranth and Soy-Oats Formulas (per 100 kcal)

Nutrient	Amaranth 1-R Formula	Amaranth 2-R Formula	Soy-Oats Formula[a]	Con (1976) Minimum	Recommended Maximum
Vitamins					
A, IU	377.0	377.0	377.0	250.0	750.0
D, IU	69.0	69.0	69.0	40.0	100.0
K, μg	5.0	5.0	···	4.0	···
E, IU	1.0	1.0	1.0	0.3	···
C, mg	10.0	10.0	7.3	8.0	···
B-1, μg	94.0	94.0	94.0	40.0	···
B-2, μg	146.0	146.0	146.0	60.0	···
B-6, μg	63.0	63.0	63.0	35.0	···
B-12, μg	0.21	0.21	0.21	0.15	···
B-3, μg	1,096.0	1,096.0	1,096.0	250.0	···
Folic acid, μg	5.2	5.2	5.2	4.0	···
Pantothenic acid, μg	418.0	418.0	418.0	300.0	···
Biotin, μg	2.0	2.0	···	1.5	···
Choline, mg	10.0	10.0	···	7.0	···
Inositol, mg	5.0	5.0	···	4.0	···
Minerals					
Calcium, mg	88.9	88.9	88.9	50.0	···
Phosphorus, mg	72.2	72.2	72.2	25.0	···
Magnesium, mg	10.4	10.4	10.4	6.0	···
Iron, mg	2.6	2.6	2.6	0.15	···
Zinc, mg	0.6	0.6	0.6	0.5	···
Copper, μg	63.0	63.0	63.0	60.0	···
Manganese, μg	5.0	5.0	···	5.0	···
Sodium, mg	24.0	24.0	24.0	20.0	60.0
Potassium, mg	110.0	110.0	110.0	80.0	200.0
Chloride, mg	77.0	77.0	77.0	50.0	150.0

[a]Del Valle et al (1981, p. 194).

Ten infants (eight males and two females) five to 18 months old participated in the study (Table III). All but one were severely underweight for their age; however, all were only mildly deficient in height for their age. All were free from apparent infection and other forms of pathology.

Each infant was tested with each of the three formulas, all with an equivalent level of nitrogen intake, as described by Hegsted (1974). Hence, each infant was his or her own control. To avoid bias as a result of the sequence of formulas tested, the sequence was varied as follows: four infants consumed soy-oats formula, amaranth 1-R formula, and amaranth 2-R formula, in that order; three were fed amaranth 1-R formula, amaranth 2-R formula, and soy-oats formula, in that order; and three consumed amaranth 2-R formula, amaranth 1-R formula, and soy-oats formula, in that order. Because the metabolic unit contained two beds, only two infants were tested at a time.

Each formula was fed for seven days; however, the first three days were considered the period of adaptation of the infant to the formula, so feces and urine were not collected during this time. The subsequent four days were considered the actual nitrogen balance trial period, and feces and urine were collected and processed as noted. For two weeks between test formulas, as well as before and after test periods, infants were fed a commercial cow's milk-based formula. The study was conducted over a 32-week period. Infant weights were measured and recorded immediately before the start of each test period.

The nitrogen parameters were calculated as follows:

$$\text{N (absorbed)} = \text{N (ingested)} - \text{N (fecal)}$$

$$\text{N (retained)} = \text{NB} = \text{N (ingested)} - [\text{N (urinary)} + \text{N (fecal)}]\,,$$

where N = nitrogen and NB = nitrogen balance.

TABLE III
Data on Infants Participating in Nitrogen Balance Study

Infant No.	Age (months)	Sex	Weight (kg)	Percentage of Weight for Age[a]	Length (cm)	Percentage of Length for Age[a]
1	18	F	7.150	62	69	97
2	7	M	4.950	55	63	98
3	12	M	6.000	60	63	85
4	6	M	6.200	79	61	95
5	5	M	3.350	44	58	94
6	5	M	5.000	66	62	97
7	5	M	4.240	59	56	82
8	14	M	5.300	51	64	82
9	14	F	5.620	56	65	87
10	5	M	2.600	39	55	87

[a]As determined from tables for Mexican children reported by Ramos-Galvan (1975).

TABLE IV
Summary of Data Obtained in Nitrogen Balance Study

Child	Period	Formula[a]	Weight (kg)	Nitrogen Intake (g)	Nitrogen Absorbed[b] (g)	Nitrogen Retained[c] (g)
1	1	1	8.200	205.6	169.6	86.9
1	1	2	8.840	243.1	215.7	125.8
1	1	3	7.700	135.8	103.7	42.3
2	1	1	6.560	183.1	127.4	48.9
2	1	2	6.880	173.8	141.4	49.5
2	1	3	6.540	158.5	124.3	33.2
3	2	1	6.380	213.6	170.1	82.3
3	2	2	7.140	202.9	172.5	76.3
3	2	3	5.600	330.7	288.8	143.4
4	2	1	7.310	194.4	142.4	35.8
4	2	2	7.620	169.5	125.3	42.4
4	2	3	6.400	140.9	91.2	25.1
5	3	1	3.375	109.7	63.4	34.1
5	3	2	3.375	193.1	155.9	78.8
5	3	3	3.483	205.2	171.6	82.1
6	3	1	5.250	134.2	93.1	57.0
6	3	2	6.600	195.4	153.8	63.8
6	3	3	7.410	195.9	159.3	64.5
7	4	1	4.660	116.7	81.7	12.5
7	4	2	4.460	175.5	119.4	68.6
7	4	3	5.640	215.0	188.6	109.8
8	4	1	5.460	120.7	87.0	9.2
8	4	2	6.290	159.1	124.5	50.2
8	4	3	6.200	148.5	158.9	20.6
9	5	1	6.300	208.0	171.7	64.3
9	5	2	5.800	171.5	135.7	55.6
9	5	3	7.160	215.7	171.4	75.1
10	5	1	4.060	165.5	128.0	59.8
10	5	2	3.280	118.5	83.7	63.8
10	5	3	4.880	166.2	128.5	33.7

[a]Formula 1 = amaranth 1-R; formula 2 = amaranth 2-R; formula 3 = soy-oats.
[b]N (absorbed) = N (ingested) − N (fecal), where N = nitrogen.
[c]N (retained) = NB = N (ingested) − [N (urinary) + N (fecal)], where N = nitrogen and NB = nitrogen balance.

Statistical Analyses

The experimental design in the nitrogen balance study was a replicated measures design with covariates. Analysis of covariance techniques were used to determine any significant differences between types of formula fed and between beds and to determine any interaction between these effects, with respect to nitrogen absorption and retention (P. Biemer, *personal communication,* 1990). Infant weight and nitrogen intake were used as covariates in order to adjust the dependent variables to a constant level of each of these covariates (P. Biemer, *personal communication,* 1990). The different feeding periods were used as replicates. To account for the repeated measures on the same infant and for the infant effect, nesting within replications was included in the model. The infant effect also served to account for differences between the beds used in the experiment.

Separate analyses were conducted for nitrogen absorption and nitrogen retention. In testing the significance of "Formula" in the model, the Rep × Formula interaction term was used. This test was indicated by the expected mean squares from the model.

In the analysis of means following the *F*-tests, least squares means were used (SAS 1985) to determine differences (if any) and to construct confidence intervals. Significance was accepted at the $P \leq 0.05$ level in all cases.

RESULTS AND DISCUSSION

Table IV summarizes the nitrogen balance data (nitrogen intake, nitrogen absorption, nitrogen retention, and initial weight upon entering the study) for each infant-formula combination. Tables V and VI reflect results of the statistical analyses (analysis of covariance) performed on the data in Table IV, specifically the effect of type of formula on nitrogen absorption (Table V) and nitrogen retention (Table VI). As noted previously, infant weight and nitrogen intake were used as covariates to eliminate from the analysis of variance variations in the former parameters due to both of the latter factors (P. Biemer, *personal communication,* 1990).

Average nitrogen absorption ranged from 138 g in infants consuming the 1-R formula to approximately 142 g for infants

TABLE V
Analysis of Covariance for Nitrogen Absorption

Source of Variation	Degrees of Freedom	Type III Sum of Squares	*F*-Value	Probability > *F*
Replicates	4	175.6046	1.30	0.3468
Baby (reps)	5	294.3459	1.75	0.2302
Formula[a]	2	70.1956	1.04	0.3964
Rep × Formula	8	572.3573	2.12	0.1539
Intake	1	12,125.8620	359.67	0.0001
Weight	1	6.4387	0.19	0.6736

[a]Formula fed.

TABLE VI
Analysis of Covariance for Nitrogen Retention

Source of Variation	Degrees of Freedom	Type III Sum of Squares	F-Value	Probability $> F$
Replicates	4	511.5655	0.65	0.6403
Baby (reps)	5	2,527.6865	2.31	0.1402
Formula[a]	2	504.8051	1.29	0.3267
Rep × Formula	8	1,690.6965	1.08	0.4576
Intake	1	8,184.1183	41.85	0.0002
Weight	1	7.5984	0.04	0.8486

[a]Formula fed.

TABLE VII
Mean Values[a] (g) for Nitrogen Absorption and Nitrogen Retention

Formula	Absorption	Retention
Soy-oats	142.49	56.81
Amaranth 2-R	142.02	66.90
Amaranth 1-R	138.15	60.62

[a]Adjusted for weight and intake.

TABLE VIII
Confidence Intervals (95%) for Comparing Nitrogen Absorption and Nitrogen Retention in Formulas[a]

Formulas Compared	Nitrogen Absorption		Nitrogen Retention	
	Lower Limit	Upper Limit	Lower Limit	Upper Limit
Amaranth 1-R and amaranth 2-R	−9.62%	+16.88%	−37.43%	+16.88%
Amaranth 1-R and soy-oats	−9.95%	+3.79%	−20.92%	+33.38%
Amaranth 2-R and soy-oats	−7.22%	+6.55%	−10.66%	+43.67%

[a]Expressed as percentages of the experimental averages.

consuming either the soy-oats or the 2-R formula (Table VII). Nitrogen retention was highest (mean 66.9 g) in infants fed the 2-R formula and lowest (mean 56.8 g) in children consuming the soy-oats compound (Table VII).

The data in Tables V and VI show that the type of formula fed was not a significant source of variance in either nitrogen absorption ($P = 0.6296$) or nitrogen retention ($P = 0.3517$). However, some caution should be exercised in interpreting this finding as evidence that all three formulas had the same nitrogen absorption and retention characteristics when nitrogen intake was held constant. The proper interpretation is rather that the differences among the formulas, if any, were too small to be detected by the experimental design employed.

Table VIII gives 95% confidence intervals on the differences between formulas in absorption and retention, all expressed as percentages of the experimental averages. For example, the difference between the amaranth 1-R and 2-R formulas in absorption ranged from −9.62% to +16.88%; that is, the amaranth 1-R formula could have yielded 9.62% less or 16.88% more absorption than the amaranth 2-R formula. The limits in Table VIII thus indicate the degree of precision of the experiments.

The results of the nitrogen balance study complement those obtained by Del Valle et al (in press) in their determinations of proximate chemical composition, calorie distribution pattern, and PER and NPU; they found no significant differences among formulas with respect to any of these parameters. Both studies, therefore, tend to reinforce the conclusion that, from a nutritional point of view, the amaranth and soy-oats formulas are essentially equal. Our results also reinforce the conclusion of Del Valle et al (in press) that the amaranth 1-R formula would be a lower-cost alternative to the soy-oats formula.

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