Nutritional Adequacy of Soy Isolate Infant Formulas in Rats: Choline

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Protein quality and overall nutritional value of three concentrated liquid infant formulas containing isolated soy protein plus methionine were evaluated in rats. Although the formulas differed in levels of protein and added methionine, they were not significantly different in protein quality when fed at 10% protein (dry basis) and supplemented with vitamins and minerals. When the formulas were fed as the sole diet for 4 weeks, weight gains were related to protein levels, except that the rats fed the formula with the intermediate level of protein, Formula B, had the poorest weight gain, low serum protein levels, and enlarged fatty livers. Since Formula B did not contain added choline, a second experiment was conducted with choline added to this formula. The addition of choline prevented the development of fatty livers, but did not improve weight gains and caloric efficiencies. Formula B was also found to contain substantially more trypsin inhibitor than the other formulas.

Wide variation has been found in the protein quality of infant formulas (American Academy of Pediatrics, 1963; Gyorgy *et al.*, 1961; Harkins and Sarett, 1967; Sarett, 1961) and their vitamin adequacy (Cochrane *et al.*, 1961; Coursin, 1954; Davis and Wolf, 1958; Mann *et al.*, 1965; May *et al.*, 1950; Molony and Parmelee, 1954; Wilson and Clayton, 1962), especially those prepared as cow milk substitutes (American Academy of Pediatrics, 1963; Harkins and Sarett, 1967). The recent introduction of formulas made from soy isolate in place of soy flour makes it more important to evaluate fully the nutritional value of the formula, since the isolated protein contains very little of the accessory nutritional factors contributed by soy flour. Nutrients which need not be added to milk formula but which are known to be important in human nutrition must be included in soy isolate diets. An important example is the need for additional zinc in soy protein diets for rats and chicks (Forbes and Yohe, 1960; Morrison and Sarett, 1958). The findings of Cherry *et al.* (1968) that infants fed a synthetic formula based on isolated soy protein attained less growth than infants fed a milk formula may have been due in part to the lack of zinc or inadequacy of magnesium or methionine in this formula.

The present study compared the protein quality of three soy protein isolate infant formulas which have been introduced within the last few years, and evaluated their nutritional value as the sole source of food for growth of the rat.

METHODS

Approximate compositions based on the labels of the concentrated liquid soy formulas are shown in Table I. An adequate quantity of each formula was carefully lyophilized for use in the evaluation of protein quality in Experiment 1. The lyophilized formulas were then analyzed for protein and fat, and each used to prepare a 10% protein (1.6% N) diet as described by Harkins and Sarett (1967). One standard casein diet was prepared to contain 20% fat from corn oil, since this level was just about the fat level present when the lyophilized formulas B and C were diluted to 10% protein. A second standard casein diet was prepared to contain 14% fat from corn oil, since this was the approximate fat level present when lyophilized Formula A was diluted to 10% protein. Where necessary, small amounts of corn oil were added to bring the fat level in the experimental diet made with Formula A up to exactly 14%, and the fat levels in the experimental diets made with Formulas B and C up to exactly 20% fat.

In Experiments 2 and 3, the concentrated liquid formulas (134 kcal per 100 ml) were fed fresh daily in weighed sterilized drinking tubes as the sole source of food. Water was also available *ad libitum*. In Experiment 3, Formula B was fortified with choline to determine whether the fatty livers observed with this formula in Experiment 2 were due to choline deficiency. One milliliter of a 50% alcohol solution of choline chloride was added to each 13-fluid oz can of Formula B to provide the same amount of choline listed in the label claims of Formulas A and C; *i.e.*, 18.3 mg per 100 ml (85 mg per 16 fluid oz). The total choline content of each formula was determined (Glick, 1944).

Trypsin inhibitor in soy protein isolate (Edi Pro, Ralston Purina Co., St. Louis, Mo.) and the three formulas were assayed colorimetrically, using benzoyl-DL-arginine *p*-nitroanilide HCl (BAPA, Nutritional Biochemicals Corp., Cleveland, Ohio) as an artificial substrate (Erlanger *et al.*, 1961) for bovine trypsin (Sigma Chemical Co., St. Louis, Mo.). Trypsin inhibitor activity was calculated in μg of trypsin inhibited per 100 μg of protein in the sample.

Animals. Male weanling rats of the Wistar strain were used in all three experiments. Ten animals were allotted to each treatment on the basis of body weight and litter origin. Average starting weights for all groups ranged from 47.8 to 48.0 g in Experiment 1, 50.8 to 51.2 g in Experiment 2, and 48.0 to 48.3 g in Experiment 3. Each animal was housed individually in a galvanized screened-bottom cage in an air conditioned animal room maintained at 22° to 24° C. The animals were weighed weekly during these 4-week studies.

At the end of each experiment, the animals were anesthetized with sodium pentobarbital and bled by heart puncture. Total serum proteins were determined by the method of Kingsley (1942). In Experiment 1, the gastrointestinal tracts were removed and the rest of each carcass was autoclaved in sealed jars and analyzed for nitrogen by the Kjeldahl procedure. A group of 10 animals was similarly analyzed at the start of the study to calculate average apparent net protein utilization values for each group. In Experiments 2 and 3, pertinent organs were excised and weighed. Total liver lipids were determined by the method of Folch *et al.* (1957).

RESULTS

Experiment 1. The protein qualities of the three formulas were measured in terms of their apparent net protein utilization values (apparent NPU's) and protein efficiency ratios

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Table I. Composition of Concentrated Soy Isolate Formulas for Infants[a,b]

	Caloric Density kcal/100 ml	Proximate Composition Protein g/100 ml	Fat g/100 ml	Carbohydrate g/100 ml	Ash g/100 ml	Added DL-methionine g/100 ml
Formula A[c]	134	5.0	6.8	13.5	1.0	0.039[f]
Formula B[d]	134	4.0	7.2	13.5	0.8	0.020
Formula C[e]	134	3.8	7.4	13.6	1.1	0.053

[a] These figures are based on label information of these products. Each is designed to be diluted with an equal volume of water for feeding infants. [b] Each formula also contains added vitamins and minerals; the lists and levels of these differ somewhat in the three formulas, but the main difference appears to be choline. [c] ProSobee—Mead Johnson & Co., Evansville, Ind. [d] Similac Isomil—Ross Laboratories, Columbus, Ohio. [e] Neo-Mull-Soy—The Borden Company, New York, N.Y. [f] The total analyzed methionine levels in the formulas are Formula A, 0.08 g per 100 g (1.7 g per 100 g of protein); Formula B, 0.06 g per 100 g (1.6 g per 100 g of protein); and Formula C, 0.09 g per 100 g (2.5 g per 100 g of protein). Soy protein isolate also contains 1.1% cystine (Harkins and Sarett, 1967).

Table II. Protein Efficiency and Apparent Net Utilization Values of Soy Isolate Formulas in Male Weanling Rats

Diets: Dietary Protein Source	Dietary Fat Level %	4-Week Protein Evaluation: Weight Gain g	Apparent Net Protein Utilization %	Protein Efficiency Ratio g gain/g protein
Formula A	14	90 ± 16[a]	51.7	3.0 ± 0.1
Formula B	20	81 ± 20	49.4	3.0 ± 0.3
Formula C	20	91 ± 17	52.4	3.2 ± 0.3
Casein	14	100 ± 18	61.2	3.5 ± 0.4
Casein	20	78 ± 20	58.4	3.3 ± 0.3

[a] Means given with standard deviations.

(PER's) (Table II). The apparent net protein utilization values represent the average increase in carcass nitrogen over the 4 week period as a percent of the average nitrogen intake.

The protein efficiency ratios and apparent net protein utilization values of the three soy formula diets were quite similar and confirmed previous findings by Harkins and Sarett (1967) that the protein quality of a formula containing soy isolate supplemented with methionine is almost equal to that of casein. The small differences in absolute values obtained with the experimental diets may be related to the different levels of protein calories in these diets.

Experiment 2. When the isolated soy protein formulas were fed as the sole diet for 4 weeks, rats fed Formulas A, B, and C achieved weight gains of 204, 144, and 159 g, respectively, with caloric efficiencies of 107, 83, and 97 g of gain per 1000 kcal of formula consumed (Table III). The significantly higher weight gains with Formula A may have been due in part to its higher level of protein; *i.e.*, 15% of calories as protein compared to 12 and 11% for Formulas B and C, respectively. However, rats fed Formula B gained significantly less weight and had lower caloric efficiencies than rats fed Formula C, although Formula B was higher than Formula C in protein content. The rats fed Formula B also had significantly lower serum protein levels than those fed Formulas A and C.

The relative organ weights in the three groups were similar except for the liver (Table III). The relative liver weights of animals fed Formula B were significantly greater than those of animals fed the other two formulas. The liver lipid level in the Formula B group (15.8 g per 100 g of liver) was more than twice those in the groups fed Formulas A and C (7.1 and 7.6 g per 100 g, respectively). These findings suggested that Formula B may be deficient in choline; this was studied in Experiment 3.

Table IV. Findings in Male Weanling Rats Fed Concentrated Liquid Formulas as the Sole Diet for 4 Weeks—Experiment 3

	Formula Intake kg	Animal Growth Performance: Weight Gain g	Caloric Efficiency g gain/1000 kcal	Relative Liver Weight g/100 g body weight	Liver Lipids g/100 g liver
Formula A Protein, 5.0%[a] (15% of Calories) Choline, 27 mg/100 ml[b]	1.50 ± 0.35[αc]	187 ± 52[α]	97 ± 6[α]	4.4 ± 0.3[α]	6.6 ± 0.9[α]
Formula B Protein, 4.0% (12% of Calories) Choline, 5 mg/100 ml	1.21 ± 0.25[β]	125 ± 34[β]	81 ± 6[β]	4.7 ± 0.5[α]	12.5 ± 3.6[β]
Formula B + Choline Protein, 4.0% (12% of Calories) Choline, 23 mg/100 ml	1.20 ± 0.13[β]	126 ± 18[β]	82 ± 7[β]	4.4 ± 0.5[α]	7.0 ± 0.8[α]
Formula C Protein, 3.8% (11% of Calories) Choline, 23 mg/100 ml	1.22 ± 0.21[β]	140 ± 35[β]	88 ± 12[αβ]	4.6 ± 0.3[α]	6.7 ± 0.9[α]

[a] Label claim. [b] Total choline content determined by analysis. [c] Mean given with standard deviation. Values not superscript with the same letter are significantly different ($P < 0.05$).

Experiment 3. Relative findings with Formulas A, B, and C in this study were similar to those in Experiment 2, although all weight gains and caloric efficiencies were slightly lower than in the previous study (Table IV).

The addition of choline to Formula B lowered liver lipids to normal values but did not improve weight gains or caloric efficiencies. Other data on organ weights and plasma proteins were similar to those in Experiment 2 and were not affected by adding choline; these are not reported.

It was subsequently suggested that the lower weight gains with Formula B may be due to incomplete destruction of soybean trypsin inhibitor in this product. Assay of the three formulas for trypsin inhibitor content indicated this to be the case (Table V). On a protein basis, Formula B contained almost three times as much trypsin inhibitor as Formula C, and almost 15 times as much trypsin inhibitor as Formula A.

DISCUSSION

The results indicate that the protein qualities in the three methionine supplemented soy isolate protein formulas are equivalent, whereas the formulas themselves differ markedly in nutritive value. Each of these is designed as a complete formula for infants.

The results of the protein quality study are consonant with previously published results on Formula A (Harkins and Sarett, 1967) and show that the quality of the protein of all three soy isolate based formulas supplemented with methionine is approximately 90% or more than that of casein when measured in the rat. Methionine levels in the formulas differ, but apparently all of them contain enough of this amino acid to meet the needs of the rat.

In the evaluation of protein quality in the rat, complete mixtures of vitamins and minerals, including choline and other nutritional factors not always used as supplements in human nutrition, must be added to the diets to insure that the only limiting nutrient is protein. Thus, tests of protein quality of a diet or a formula do not uncover any inadequacy of vitamins or minerals.

When fed as the sole diet, the formulas differed markedly in overall performance. Formula A supported greater weight gains and caloric efficiencies than Formulas B and C. Markedly low values were found with Formula B. Although the addition of choline to Formula B prevented development of fatty livers, it did not improve the weight gains or caloric efficiencies. It is possible that choline may not be the only nutrient which is lacking or marginal in this formula. A higher trypsin inhibitor level in Formula B may be partially responsible for the poorer weight gains and caloric efficiency. The nutritional value of soybeans is inversely proportional to the trypsin inhibitor content, and heating results in destruction of the inhibitor (Liener, 1958). The level of trypsin inhibitor remaining in the soy product can serve as an index of the adequacy of heat treatment (Van Buren *et al.*, 1964).

The low level of choline from the soy isolate and the added methionine in Formula B were apparently sufficient to prevent severe choline deficiency symptoms; *i.e.*, enlarged hemorrhagic kidneys, even though fatty livers were produced (Griffith, 1941).

Although fatty livers due to choline deficiency developed in rats fed Formula B, it is not known whether a similar effect would occur in the human infant. Wilson and Clayton (1962) attributed the severe extensive skin eruption in some phenylketonuric English infants receiving a low phenylalanine food made in England to a lack of choline and other vitamins in the formula.

Table III. Findings in Male Weanling Rats Fed Concentrated Liquid Soy Isolate Formulas as Sole Diet for 4 Weeks—Experiment 2

	Formula Intake kg	Animal Growth Performance		Relative Organ Weights (Per 100 g Body Weight)						Serum Proteins g/100 ml	Liver Lipids g/100 g liver
		Weight Gain g	Caloric Efficiency g gain/1000 kcal	Liver g	Kidney g	Heart g	Adrenals mg	Thyroids mg	Epididymal Fat Pads g		
Formula A Protein, 5.0%[a] (15% of calories) Choline, 31 mg/100 ml[b]	$1.49 \pm 0.17^{\alpha c}$	$205 \pm 27^{\alpha}$	$107 \pm 5^{\alpha}$	$4.1 \pm 0.3^{\alpha}$	$0.72 \pm 0.07^{\alpha}$	$0.37 \pm 0.04^{\alpha}$	$13 \pm 2^{\alpha}$	$7 \pm 1^{\alpha}$	$1.3 \pm 0.2^{\alpha}$	$5.7 \pm 0.2^{\alpha}$	$7.1 \pm 1.0^{\alpha}$
Formula B Protein, 4.0% (12% of calories) Choline, 6 mg/100 ml	$1.39 \pm 0.20^{\alpha\beta}$	$144 \pm 27^{\beta}$	$83 \pm 12^{\gamma}$	$4.6 \pm 0.5^{\beta}$	$0.72 \pm 0.04^{\alpha}$	$0.37 \pm 0.04^{\alpha}$	$14 \pm 2^{\alpha}$	$8 \pm 2^{\alpha\beta}$	$1.3 \pm 0.3^{\alpha}$	$5.2 \pm 0.2^{\beta}$	$15.8 \pm 4.0^{\beta}$
Formula C Protein, 3.8% (11% of calories) Choline, 23 mg/100 ml	$1.29 \pm 0.17^{\beta}$	$159 \pm 33^{\beta}$	$97 \pm 10^{\beta}$	$4.2 \pm 0.3^{\alpha\beta}$	$0.74 \pm 0.07^{\alpha}$	$0.37 \pm 0.04^{\alpha}$	$15 \pm 3^{\alpha}$	$9 \pm 2^{\beta}$	$1.1 \pm 0.4^{\alpha}$	$5.5 \pm 0.3^{\alpha}$	$7.6 \pm 1.0^{\alpha}$

[a] Label claim. [b] Total choline content determined by analysis. [c] Mean given with standard deviation. Values not superscript with the same letter are significantly different ($P < 0.05$).

Table V. Trypsin Inhibitor Content of Soy Isolate Formulas

Formula	Sample	Trypsin Inhibitor Content: Values (μg of trypsin inhibited/100 μg protein)	Average
Formula A	1	0.00, 0.21[b]	0.05
	2	0.00, 0.00	
Formula B	1	0.78, 0.84	0.80
	2	0.76, 0.84	
Formula C	1	0.43, 0.27	0.29
	2	0.16, 0.32	
Isolated Soy Protein[a]	A	4.46, 4.46	3.92
	N	3.41, 3.33	

[a] Edi Pro, Ralston Purina Co., St. Louis, Mo. [b] Duplicate values for each sample.

Teleologically, choline is present in all mammalian milks. The average choline content of human milk is considered to be about 89 mg per l. (Macy, 1949). When diluted for feeding infants, Formulas A and C contained levels of choline somewhat above this, whereas Formula B was found to contain only 23 to 29 mg of choline per l. The manufacturer of Formula B was promptly informed of these findings, and is now supplementing Formula B with choline.

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