AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY

1. SUBJECT CLASSIFICATION

A. PRIMARY: Public Health

B. SECONDARY: Nutrition

2. TITLE AND SUBTITLE

Critical evaluation of new or enriched protein sources for the prevention of malnutrition, progress report on whey-soy mix

3. AUTHOR(S)

Graham, G.G.; British-American Hospital, Lima, Peru

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1974	20 p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

British-American Hospital, Instituto de Investigacion Nutricional, Lima, Peru

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*

9. ABSTRACT

A whey-soy mix, designed to be used in preschool feeding programs, was compared to casein in eight nitrogen balance studies done with protein providing 6.4% of calories, and was studied as well as the prolonged source of protein (8% of calories) in the prolonged feeding of four other infants. Whey-soy proved to be significantly inferior to casein in nitrogen retention, partly on the basis of poor digestibility and partly because of a presumed biologic value. Stool weights and fecal fats did not differ significantly in the two diets. Children consuming whey-soy as the only source of protein showed good increases in weight age with lesser increases in length, a normal finding. Serum albumin concentrations generally were well-maintained. No intolerance to whey-soy was encountered. Possible limitations in the use of whey-soy in field situations must be taken into account, but it should prove to be of great value even in the diets of weaned infants.

10. CONTROL NUMBER

PN-AAC-579

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Food mixes
Milk
Proteins
Soy supplements

13. PROJECT NUMBER

14. CONTRACT NUMBER

CSD-2946 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

CSD-2946 RPS
PN-AAC-579

WHEY-SOY MIX

TO: Dr. Harold Rice
Science Research Officer
Office of Nutrition
Agency for International Development
Washington, D.C. 20523

FROM: Instituto de Investigacion Nutricional
Apartado 55
Miraflores, Peru
and
Dr. George G. Graham
Principal Investigator
615 North Wolfe Street
Baltimore, Maryland 21205

SUBJECT: Progress Report - April 15, 1974
Research Contract AID/csd-2946
Critical Evaluation of New (or Enriched) Protein Sources (For the Prevention of Malnutrition)

20 P

ABSTRACT

A whey-soy mix, designed to be used in pre-school feeding programs, was compared to casein in eight nitrogen balance studies done with protein providing 6.4% of calories, and was studied as well as the sole source of protein (8% of calories) in the prolonged feeding of four other infants. Whey-soy proved to be significantly inferior to casein in nitrogen retention, partly on the basis of poor digestibility and partly because of a presumed lower biologic value. Stool weights and fecal fats did not differ significantly on the two diets. Children consuming whey-soy as the only source of protein showed good increases in weight age with lesser increases in length, a normal finding. Serum albumin concentrations generally were well maintained. No intolerance to whey-soy was encountered. Possible limitations in the use of whey-soy in field situations must be taken into account, but it should prove of great value, even in the diets of weaned infants.

INTRODUCTION

For many years surplus non-fat dry milk has been the mainstay of supplementary feeding programs for impoverished children. Although adequate documentation is lacking, it has undoubtedly enhanced the protein nutriture of millions of school children and a smaller number of pre-school children. Because of its very low fat content, low calorie density, and very high solute load, it is not a proper substitute for human breast milk in the diet of weaned infants. Nevertheless, because of the lack of other alternatives, it has often been used in this fashion, with or without the addition of fat. In populations with high incidences of diarrheal disease and malnutrition, and associated secondary intestinal lactase deficiencies, its high lactose content represents a further objection to its use. With the disappearance of milk surpluses, these objections may prove to be academic. There is still a most urgent necessity to develop safe, inexpensive and nutritious substitutes for human breast milk, and to find additional substitutes for non-fat dry milk in supplementary feeding programs for school and pre-school children.

Infant formulas based on isolated soy protein enriched with DL-methionine match modified cow's milk formulas in quality (1) but are not yet a practical alternative for the underdeveloped world. Formulas based on soy flour have a much longer history, are of good nutritional value, and are being used in some areas where soy is readily available. Soy-cereal combinations are excellent alternatives for pre-school

and school children, less so for infants (2).

Through the combined efforts of the U.S. Department of Agriculture, U.S. Agency for International Development, cooperating industry, and scientists, a whey-soy mix has been developed for use in pre-school feeding programs, but also satisfying most of the requirements for infant feeding. It has been found to be generally well accepted by children and their mothers (3). Its main ingredients are full fat soy flour and sweet whey, a by-product of the cheese industry which has customarily been discarded, often serving as a pollutant, despite the very high biological value of its protein, primarily lactalbumin. It represents a further development of a previously described whey-soy mixture (4).

The present report covers our evaluation of the whey-soy mixture in convalescent malnourished infants.

MATERIALS

The blend used in these studies was prepared by the Foremost Foods Co. Research and Development Center (Dublin, California) and contained 41.38% sweet whey, 36.49% full fat soy flour, 12.13% soybean oil, 9.00% corn syrup solids and 1.00% vitamins and minerals. Its proximate composition was as follows: protein 20.0% (approximately 26.7% from whey, 73.3% from soy), fat 20.0%, mineral 5.0%, moisture 3.0% and fiber 0.9%. It yielded 435 Kcal/100 gm, approximately 18% from protein and 41% from fat. The following

amounts of minerals and vitamins were added per 100 gm of the final product: calcium 280 mg, zinc 1.0 mg, iron 17.6 mg, iodine 0.05 mg, thiamine mononitrate 0.3 mg, riboflavin 0.4 mg, pyridoxine HCl 0.17 mg, niacin 5.0 mg, calcium D-pantothenate 2.75 mg, folacin 0.2 mg, B_{12} 0.004 mg, vitamin A 1650 USP units, vitamin D 200 USP units, alpha tocopherol acetate 7.5 I.V., and ascorbic acid 40 mg. The PER in rats of the original whey-soy mixture was reported as 2.42, casein 2.50 (4).

METHODS

The methodology of these studies was that used in previous similar studies in our laboratory (5).

Comparative Studies: Eight sets of comparative studies were done in 7 infants, 14-30 months of age. Isocaloric isonitrogenous comparisons between casein and WS were made with protein (N x 6.25) providing 6.4% of calories. The addition of cottonseed oil and cane sugar achieved the desired dilutions and also ensured that the fat-carbohydrate distribution of calories remained constant. Vitamins and minerals were supplemented to both diets as needed.

In most studies 9 days on the control diet were followed by 9 days on WS, with an additional 6 day period on casein at the end. During the final 6 days of each period nitrogen balances and fecal fats were done. In 6 of the 7 infants an additional 6 days on WS followed the second casein period.

During these 6 days, WS was diluted only with a starch: sucrose (2:1) mixture so that 12% of calories in the final diet were derived from protein, 64% of non-protein calories being from carbohydrate. The diet was prepared in this manner because it seemed most likely that in actual use the product would be consumed with additional carbohydrate calories, rather than with fat. During these six days, stool weights and fats were of primary interest, as they allowed us to evaluate the digestibility of the fat in the product itself.

Long-Term Studies: In four infant girls (7-19 months) WS was fed as the sole source of protein (8% of calories) for periods of 9-11 weeks. Three of these infants had six-day collections for nitrogen retention done during the middle of their studies.

In all studies urinary, fecal, and dietary (weekly) nitrogens were determined by the micro-Kjeldahl method, fecal fats by the method of van der Kamer (6), and serum proteins by cellulose acetate electrophoresis.

RESULTS

Table I presents the results of the comparative studies. The age, height, and weight of each child at the onset of the study are given. The age in months after each measurement is the corresponding 50th percentile value on a well known U.S. scale (7) and gives a basic idea of the state of recovery.

Several explanatory comments should be made. Patient 399's calorie intake was higher in the first casein period than in the remainder of the study. This resulted from a diet error noted retrospectively. Because protein calories were constant at 6.4%, it should not significantly elevate the nitrogen retention. The second casein period in this patient includes only 3 days. The second three-day collection was mistakenly mixed with part of the subsequent WS period. Patient #404 had his calorie intake lowered after the first casein period as well. This was purposefully done because of excess weight gain at the higher level.

Nitrogen retentions in the 8 studies done at 6.4% protein calories were highly variable. WS appeared equal to casein in patients #396 and #400, and was inferior in patients #299, #403, #404, and #409. No fast conclusion can be drawn from the two studies done in patient #402. This child was clinically well during both sets of studies. In the first study nitrogen retention was good during the initial casein period and then quite low during the WS and subsequent casein periods. In the second study good nitrogen retention on casein was followed by low retention on WS. This parallels almost exactly the first study and without a final casein period one cannot be certain as to its meaning.

Nitrogen retentions during the WS 12% periods were also variable. Retentions were generally adequate and in several

cases excellent. Because of errors integral to the balance technique at this level of nitrogen intake and without control periods, these retentions must be viewed cautiously.

Absorptions of nitrogen (and thus digestibility) on WS were similar to casein in three children but inferior in the rest. If one calculates biologic values (nitrogen retention as a percentage of nitrogen absorbed) for these studies, the values obtained for WS are better than or equal to those for casein in patients 396, 400 and 403, but are less than those for casein in the others. It is evident that poor digestibility alone is not the reason for inferior nitrogen retention seen in some children on WS.

Stool weights showed an expected slight increase while on WS in five of the studies but this was not uniform. Inspection of the fecal fat data gives the impression that for some children fat absorption was better on WS than while on casein. Yet as with stool weights this change was neither consistent nor of great magnitude. Stool weights and fecal fats on WS 12% did not differ significantly from those on WS 6.4%.

Rates of weight gain and changes in serum albumin concentration did not differ between the two diets. The weight loss in patient #396 while on WS 12% is unexplained. Changes in weight during short periods of time should always be interpreted cautiously. This child developed an upper respiratory infection during the final 6 days of the study and although

nitrogen retentions appeared excellent (possibly due to the inherent error at this level of nitrogen intake) serum albumin decreased 0.5% gm in six days, an alteration we generally consider to be significant.

Our experience with WS as the only source of protein in prolonged feeding studies is presented in Table II. In the first two children, a few days on WS 12% inadvertently preceded the bulk of the study at 8% protein calories. All children showed advances in weight age equal to or better than those in chronologic age. Height ages increased in all children but in none was the increase consistent with the length of the study. The significance of this finding is discussed below.

Serum albumin concentrations were well maintained, although in two children slightly below the level of 4.0% gm which we consider normal. Patient #413 showed a rise of serum albumin of about 0.5% gm during the study. Patient #407 decreased her serum albumin.

Nitrogen retentions for three of the four children are presented in Table III. These six-day collections were done at 8% protein calories mid-way through the long-term studies. Stool and urine were collected together and consequently nitrogen absorptions and biologic values cannot be calculated. Retentions of nitrogen are adequate and parallel in variability those in the comparative studies.

All children tolerated the diet well. The course of one, #407, was punctuated by three unexplained episodes of diarrhea and another, #405, suffered a urinary tract infection and otitis media during the latter part of the study. Patient #412 became anorexic during two of the last three weeks of the study but as can be deduced from her changes in height age and weight age, she had become quite obese. Calorie intake was decreased and she ate well for the final week of the study.

DISCUSSION

Although somewhat difficult to interpret because of the variability from child to child, the results of these studies allow some tentative conclusions. The comparative studies with casein were carried out with protein providing 6.4% of calories a level of protein intake considered to be the minimum acceptable for casein (8). Studies done at this critical level should accentuate any differences between casein and the protein being evaluated, the differences being reflected in nitrogen retentions and changes in serum albumin concentrations. In the present comparison, difference in nitrogen retention were most apparent.

If for each child nitrogen retentions on WS are expressed as a percentage of the mean retention on casein, patients #396, #400 and #403 yield values of 95%, 112% and 81% respectively. The remaining patients have values below 72% with three of these 30% or less. The inferior digestibility which was

encountered with WS (mean WS absorption 85.4% of casein) partially explains the poor retentions but the lower biologic values in five of the eight studies indicate that digestibility is perhaps not the most important factor. Neither of these findings was entirely unexpected, since most vegetable proteins or protein mixtures have inferior digestibility as well as decreased contents of total essential amino acids and of a specific essential amino acid.

Unfortified soy protein suffers from a deficiency of methionine. This imbalance is corrected if methionine is specifically supplemented or if soy protein is mixed with another protein containing adequate quantities of methionine. It is unlikely that approximately 25% whey protein could correct the deficiency. Although plasma amino acids were not done as part of these studies it seems reasonable to assume on the basis of the inferior biologic values in the majority of children that deficiencies in total or specific amino acids persist in the whey soy blend. These can be and were overcome by increasing protein intake above the level at which the comparative studies were done.

Long-term dietary studies are done to uncover intolerance, toxicity or effects of amino acid imbalance not encountered in short-term studies. The present studies were carried out at 8% protein calories, a safe level for long-term feedings with casein but probably a marginal level for most vegetable proteins. All four infants had good increments of weight age.

Gains in height age were less than increases in chronologic age, strikingly so in three of the four. This lag in linear growth associated with good weight gain is not unusual in previously malnourished children during the early months of recuperation (8, 9) and should not be attributed to whey soy. The observed advances in height age and weight age are within the limits we would consider normal in our patient population.

Serum albumin concentrations remained constant in two patients. In one patient, #407, a fall in serum albumin occurred. This may have been related to periods of negative nitrogen balance during the intermittent periods of diarrhea she experienced, each of which resulted in 5 to 7 days of marginal protein-calorie intake before full diet could be resumed. A second possible explanation is excessive weight gain (10) during the study. This was clearly not the case with this child. If one accepts that negative nitrogen balance during the periods of diarrhea caused the decrease in serum albumin it is nonetheless important that during the relatively much longer periods on adequate intake the child was unable to regenerate her serum albumin to a normal level. Patient #405, although not experiencing a decrease in serum albumin, similarly did not increase her serum albumin to a level which we would consider normal. In contrast, #413, who began the study with a slightly low serum albumin immediately after her initial recuperation period, had a significant increase in

serum albumin from 3.59% gm to 4.07% gm. None of these changes is of major import but one is left with the impression that whey soy fed at 8% protein calories would be barely adequate for most infants and small children.

The results of both the comparative and the long-term studies should make one cautious about the use of whey soy in the field. The product is proposed as a supplementary beverage for pre-school children and when prepared undiluted with other calories it provides 18% of calories as protein. This leaves adequate room for dilution with non-protein calories. If whey soy were fed to infants under supervision at the point of distribution, it could be the main or only protein source in the diet.

The mean whey soy nitrogen retention being about 60% of the mean for casein, however, indicates that it would be unwise to decrease protein calories much below 10-12%. The results of long-term feeding at 8% protein calories confirm this impression. Experience in Peru with similar types of products suggests that if whey soy is consumed at home in an uncontrolled situation far greater dilution is likely to occur. Although this dilution might be accomplished with wheat or potato products, both good protein sources with the potential of improving the amino acid balance in whey soy, pure carbohydrates, because of cost, are more likely to be used. In this instance, the protein value of whey soy would

probably begin to approach that of the usually available vegetable protein sources. The main advantage of whey soy would then reside in its lower cost for the recipients.

The burden of this last criticism must be borne as much by supplementation programs in general, as they currently function, as by whey soy. The same excessive dilution of protein by non-protein calories would be as much a possibility if casein were distributed. Although casein allows for a greater dilution, the difference between 8% and 10 or 12% protein calories in practical terms is probably not significant.

In strictest terms whey soy is inferior to casein. Its ultimate value in food supplementation programs will depend to a large extent on how its distribution and consumption are carried out.

REFERENCES

1. Graham, G.G., R.P. Placko, E. Morales, et al: Dietary protein quality in infants and children. VI. Isolated soy protein milk. Am. J. Dis. Child. 120:419, 1970.

2. Graham, G.G. and J.M. Baertl: Nutritional effectiveness of soy-cereal foods in undernourished infants. J. Am. Oil Chemists' Soc. 51:152A, 1974.

3. Rodier, W.I., III, W.C. Wetsel, et al: The acceptability of whey-soy mix as a supplementary food for pre-school children in developing countries. Tech. Rep. 74-20-PR, U.S. Army Natick Laboratories, Natick, Mass., 1973.

4. Guy, E.J., H.E. Vettel and M.J. Pallansch: Spray-dried cheese whey-soy flour mixtures. J. Dairy Sc. 52:432, 1969.

5. Graham, G.G., E. Morales, G. Acevedo, et al: Dietary protein quality in infants and children. II. Metabolic studies with cottonseed flour. Am. J. Clin. Nutr. 22: 577, 1969.

6. Van der Kamer, J., H. Huimink and A. Weyers: Estimation of fat in feces, food, and other biological materials. J. Biol. Chem. 177:347, 1949.

7. Stuart, H.C. in Pediatric Methods and Standards, F.H. Harvie, Ed., Phila., Lea & Febiger, 1958, pp. 21-24.

8. Graham, G.G., A. Cordano and J.M. Baertl: Studies in infantile malnutrition. II. Effect of protein and calorie intake on weight gain. J. Nutr. 81:249, 1963.

9. Graham, G.G.: Effect of infantile malnutrition on growth. Fed. Proc. 26:139, 1967.

10. Graham, G.G., A. Cordano and J.M. Baertl: Studies in infantile malnutrition. IV. The effect of protein and calorie intake on serum proteins. Am. J. Clin. Nutr. 18:11, 1966.

Table I

Comparisons between whey-soy and casein in balance studies done at 6.4-6.7% protein calories. Final whey-soy periods, at 12% protein calories, designed to measure digestibility of fat in mixture and the effect of the greater lactose intake on stool weight.

Patient No.	Protein Source	Protein % Kcal	No. Days	Nitrogen Balances On Days	mg/day Intake	mg/day Ret.	% of Intake Abs.	% of Intake Ret.	Stool Wt gm/d	Stool Fat % Intake	Body Wt Change gm/kg/d	Serum Albumin gm/100 ml Change	Serum Albumin gm/100 ml Final
396[a]	C	6.6	9	4- 9	2323	902	89	39	55	4.3	2.8	-0.10	3.79
	WS	6.6	9	4- 9	2324	678	80	29	97	3.4	2.8	-0.01	3.78
	C	6.6	6	1- 5	2324	515	73	22	72	7.9	3.3	+0.16	3.94
	WS	12.0	6	1- 6	4224	1885	88	45	64	0.5	-1.3	-0.49	3.45
399[b]	C	6.6	10	5-10	2752	1124	86	41	93	12.6	1.2		(3.85)*
	WS	6.6	9	4- 9	2271	583	78	26	64	4.2	0.9		4.08
	C	6.6	7	1- 3	2270	723	83	32	66	8.9	0.4	-0.38	3.71
	WS	12.0	7	2- 7	4058	1256	78	31	150	5.2	0.8	+0.68	4.39
400[c]	C	6.7	8	3- 8	2880	637	86	22	109	14.7	+6.5	-0.25	3.53
	WS	6.7	9	4- 9	2872	921	85	32	130	5.0	+6.5	+0.36	3.89
	C	6.7	6	1- 6	2876	996	86	35	109	12.2	+8.0	-0.21	3.68
	WS	12.0	4	1- 3	5184	1639	85	32	112	8.6	+4.6		
			8	3- 8	5924	1035	78	17	196	4.4	+2.3	+0.37	3.57
402[d]	C	6.4	9	4- 9	2592	630	88	24	49	11.4	+9.3	-0.13	3.80
	WS	6.4	9	4- 9	2592	139	63	5	142	10.8	+7.9	+0.07	3.87
	C	6.4	6	1- 6	2592	231	79	9	81	13.1	+3.5	-0.04	3.83
	WS	12.0	6	1- 6	4860	1110	78	23	120	5.5	+6.2	+0.12	3.95
402[e]	C	6.4	9	4- 9	2592	568	83	22	159	7.2	+0.1	-0.05	3.69
	WS	6.4	9	4- 9	2592	135	67	5	131	9.0	+1.1	-0.11	3.58
403[f]	C	6.4	17	12-17	3008	367	86	12	100	8.1	+2.8	-0.06	3.74
	WS	6.4	9	4- 9	3008	382	64	13	133	10.1	+2.8	-0.18	3.56
	C	6.4	6	1- 6	3008	591	82	20	131	17.4	+1.9	-0.07	3.49
	WS	12.0	6	1- 6	5452	1766	80	32	164	7.8	+2.9	+0.31	3.80

Table I (Cont'd)

Patient No.	Protein Source	Protein % Kcal	No. Days	Nitrogen Balances On Days	mg/day Intake	mg/day Ret.	% of Intake Abs.	% of Intake Ret.	Stool Wt gm/d	Stool Fat % Intake	Body Wt Change gm/kg/d	Serum Albumin gm/100 ml Change	Serum Albumin gm/100 ml Final
404[g]	C	6.4	9	4- 9	3920	1434	90	37	111	10.3	+7.0	+0.29	3.86
	WS	6.4	9	4- 9	3136	227	69	7	240	7.4	+2.2	+0.01	3.87
	C	6.4	6	1- 6	3136	843	86	27	72	13.5	+5.5	-0.22	3.65
	WS	12.0	6	1- 6	4998	1088	78	22	192	11.0	+0.9	+0.14	3.79
409[h]	C	6.4	9	4- 9	2600	906	81	35	96	9.2	+5.1	-0.25	3.84
	WS	6.4	9	4- 9	2599	575	68	22	153	6.2	+7.1	-0.09	3.75
	C	6.4	6	1- 6	2600	836	82	32	82	10.3	+4.5	-0.01	3.74

* Serum albumin at onset of first casein period.

	Hosp. Day	Age (mo)	Length (cm)	L. Age (mo)	Weight (kg)	Wt. Age (mo)
a	117	17.5	72.5	10.0	8.530	7.8
b	80	26.5	73.0	10.5	8.240	7.2
c	41	14.0	69.0	7.5	6.950	5.2
d	42	18.5	75.5	12.4	7.710	6.2
e	78	19.5	75.7	12.4	9.950	11.5
f	44	29.5	78.3	14.7	9.570	10.5
g	32	16.0	77.0	13.8	9.450	10.1
h	60	18.0	70.5	8.5	6.160	3.7

Table II

Prolonged feeding of whey soy to four infant girls. Evolution in height age (HA) and weight age (WA) in relation to chronologic (CA).

Patient No.	Initial Age (mo)			Diet Protein		# of Days (mos)	Progress (mo)			Serum Albumin	
	CA	HA	WA	gm/kg	% Kcal		CA	HA	WA	Change	Final
405	15	7.5	5.1	1.5-3.0	8	78 (2.6)	2.6	2.0	6.5	+0.07	3.70
407	17	9.25	5.5	1.0-3.75	8	76 (2.5)	2.5	1.25	2.74	-0.23	3.62
412	18.5	11.5	7.3	1.5-2.5	8	74 (2.5)	2.5	1.25	6.2	+0.07	4.02
413	7.5	2.4	1.6	2.5-3.0	8	69 (2.3)	2.3	1.3	3.2	+0.47	4.06

Table III

Nitrogen retentions during long-term studies

Patient No.	Protein Intake gm/kg	Protein Intake % Kcal	On Days	% N Retention
407	2.0	8	55-61	20
412	2.5	8	26-32	27
413	3.0	8	45-50	31.5