AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

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1. SUBJECT CLASSIFICATION

A. PRIMARY: Public Health

B. SECONDARY: Nutrition

2. TITLE AND SUBTITLE

Dietary protein quality in infants and children: V. A. wheat flour-wheat concentrate mixture

3. AUTHOR(S)

Graham, G.G.; Cordano, Angel; Morales, Enrique, Acevedo, Gladys; Placko, R.P.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1970	5 p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

British-American Hospital, Department of Research, Lima, Peru

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

(In Plant foods for Human Nutrition, r. 2, p. 23-27)

9. ABSTRACT

Evaluation of a mixture of unbleached wheat flour and protein concentrate from wheat shorts. The protein content of the mixture was 18.8% and it retained most of the characteristics of wheat flour. Since approximately two-thirds of its protein came from the shorts, with a higher content of lysine than the flour, its biological value theoretically should be higher than that of wheat flour. A mixture of equal parts of unbleached wheat flour and protein concentrate from shorts has been shown to have a protein value for infants and small children of approximately 60% of that of casein. Lysine fortification produced a 50% enhancement in protein value. Further improvement probably was limited by moderately inferior digestibility.

10. CONTROL NUMBER

PN-AAC-587

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Children
Flours
Food mixes
Infants
Lysine
Protein metabolism
Wheat

13. PROJECT NUMBER

14. CONTRACT NUMBER

CSD-1433 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

· csd-1433 Rec.
PN-AAC-587
612.398.G739n

Pl. Fds. hum. Nutr. Vol. 2, pp. 23-27, 1970. Pergamon Press. Printed in Northern Ireland

DIETARY PROTEIN QUALITY IN INFANTS AND CHILDREN V. A WHEAT FLOUR-WHEAT CONCENTRATE MIXTURE*

GEORGE G. GRAHAM, ANGEL CORDANO, ENRIQUE MORALES, GLADYS ACEVEDO and ROBERT P. PLACKO

Departments of Pediatrics, School of Medicine, The Johns Hopkins University, and Baltimore City Hospitals, Baltimore, Maryland
and
Grace Department of Research, British American Hospital, Lima, Peru

(*Received* 7 *November* 1969)

Summary—A mixture of equal parts of unbleached wheat flour and protein concentrate from shorts has been shown to have a protein value for infants and small children of approximately 60 per cent of that of casein. Lysine fortification produced a 50 per cent enhancement in protein value. Further improvement was probably limited by moderately inferior digestibility.

THE RELATIVELY low protein content of most common cereals, coupled with their known deficiencies of specific essential amino acids, makes them only marginal in adequacy as the main source of protein for the growing human being. Their fortification with protein concentrates, particularly if these are rich in the deficient amino acids, will correct these shortcomings but will at the same time alter their organoleptic characteristics, creating problems of acceptability which are not easily overcome. The addition of the missing amino acids in pure form will significantly enhance the biological value (GRAHAM *et al.*, 1969) without creating problems of acceptability. Despite the resultant increase in "available" protein, however, total nitrogen intake will not be increased.

The present report covers our experience in the evaluation of a mixture of unbleached wheat flour and protein concentrate from wheat shorts. The protein content of the mixture was 18·8 per cent and it retained most of the characteristics of wheat flour. Since approximately two thirds of its protein came from the shorts, with a higher content of lysine than the flour, its biological value should theoretically be higher than that of wheat flour.

MATERIALS AND METHODS

The mixture used was submitted to us by the Food Conservation Division of UNICEF and identified by their code #Ms-15. It was produced at the Western Utilization Research and Development Division of the Agricultural Research Service of the U.S. Department of Agriculture. A dry mix was prepared with the following composition:

120 lb unbleached flour
120 lb Protein Concentrate from shorts
2·45 lb $CaCO_3$
2·45 lb $CaHPO_4$
0·61 lb NaCl
0·25 lb of Rhozyme H-39.

* Supported by Research Contract csd/1433 from the Agency for International Development, U.S. Department of State and Research Grant AM-09980 from the National Institutes of health, U.S.P.H.S.

This was thoroughly mixed in the dry form and added to water in the ratio of 1 weight of dry mix to 1·8 weights of water, heated to 75°C, held 1 hr, cooled, and spray dried. Its proximate composition is as follows:

Moisture	5·9 per cent
Protein (N × 6)	18·8 per cent
Crude fat	0·9 per cent
Crude fibre	1·6 per cent
Ash	4·6 per cent

The PER as obtained was 1·73 ± 0·041 (standardized 1·31), that of casein was 3·27 ± 0·044. The NPU was reported as 47. These values are superior to those commonly reported for wheat flour.

The casein used in our human studies was Casec, kindly supplied by Mead Johnson International.

All children received daily two vitamin mixtures: one provided 1500 units of vitamin A, 400 units of vitamin D and 25 mg of vitamin C and the other 0·38 mg of thiamine, 2·0 mg of riboflavin, 8·3 mg of nicotinamide, 3·5 mg of Ca pantothenate, 0·67 mg of pyridoxine, 180 mg of inositol, 0·5 mg of para-aminobenzoic acid, 0·05 mg of folic acid, 147 mg of choline, 0·03 mg of biotin and 0·015 mg of cyanocobalamin. They received 1 g/kg of body wt/day of a mineral mixture with the following composition: NaCl, 17·452 per cent; $MgSO_4 \cdot 7H_2O$, 12·853 per cent; $CaHPO_4$, 23·453 per cent; $KHCO_3$, 40·997 per cent; KCl, 2·659 per cent; ferric citrate, 2·041 per cent; $CuSO_4 \cdot 5H_2O$, 0·347 per cent; $MnSO_4 \cdot H_2O$; 0·156 per cent; KI, 0·014 per cent; and NaF, 0·028 per cent. These mixtures were made for us by the Roerig division of Chas. Pfizer & Co. The children also received 2·2 mg of $ZnSO_4 \cdot 7H_2O$/ kg/day.

These studies were carried out in infants and children who had been admitted to the British American Hospital suffering from severe malnutrition and were well on the way to recovery, with steady weight gain, satisfactory nitrogen retention and normal serum proteins. They were also presumably free of infection.

For each child the approximate minimum intake of milk protein and total calories which would support continued accelerated growth and normal serum proteins was determined. At this point comparisons were made at isonitrogenous and isocaloric levels between casein and the test product. Rates of weight gain, nitrogen (N) absorptions and retentions, changes in serum albumin and the fasting plasma free amino acid concentrations were used in evaluating dietary protein adequacy. Casein periods of nine or more days preceded and followed all the Ms-15 periods, which were of 15 or 30 days.

Cottonseed oil and cane sugar were added to the Ms-15 and the same plus corn starch to the casein in order to achieve the desired protein and calorie intakes. In all diets 53 per cent of non-protein calories came from fat, 47 per cent from carbohydrate. Water was added to the blend to achieve intakes of 100–120 ml/kg/day and appropriate amounts of Na and K to maintain their intakes constant at 2·0 and 3·3 mEq/kg/day respectively.

Nitrogen in food, feces and urine was determined by the micro-Kjeldahl method, serum total protein by the Biuret method, serum albumin by paper electrophoresis, and free amino acids in plasma by liquid column chromatography (SPACKMAN *et al.*, 1958). For technical reasons (GRAHAM *et al.*, 1969) tryptophan and cystine have been excluded from the value for "essential" amino acids, leaving valine, leucine, isoleucine, phenylalanine, tyrosine, lysine, threonine and methionine. For similar reasons taurine, aspartic acid and

glutamic acid were excluded from the value for non-essentials, leaving alanine, glycine, proline, serine, glutamine, asparagine, arginine, histidine and ornithine. These plus the eight essentials gave us the figure for "total" amino acids.

TABLE 1. NITROGEN ABSORPTIONS (abs) AND RETENTIONS (ret), RATES OF WEIGHT GAIN (Δ wt), AND SERUM ALBUMIN (SA) LEVELS OF THREE CONVALESCENT MALNOURISHED INFANTS RECEIVING A WHEAT FLOUR-WHEAT CONCENTRATE MIXTURE (Ms-15) AS THE SOLE SOURCE OF DIETARY PROTEIN, COMPARISONS WITH CASEIN (C) AT ISOCALORIC AND ISONITROGENOUS LEVELS

Subject (age)	Protein	Per cent of kcal	No. days	Per cent N intake abs	Per cent N intake ret.	mg N/kg/day in.	mg N/kg/day ret	Δ wt (g/kg/day)	SA g/100 ml Δ	SA g/100 ml Final
#197	C	5·3	12	91	41	320	130	8·8	0·22	4·28
(12 months)	Ms-15	5·3	30	80	27	319	86			
				83	29	320	95	6·5	−1·22	3·06
	C	5·3	9	84	45	320	145	6·8	0·45	3·51
#207	C	6·7	9	88	33	396	131	5·1	0·01	4·10
(10 months)	Ms-15	6·7	15	72	21	380	80	2·6	−0·87	3·23
	C	6·7	9	87	39	400	155	4·4	0·39	3·62
#212	C	6·7	10	78	30	320	96	5·4	0·44	4·44
(41 months)	Ms-15	6·7	15	80	22	320	71	3·7	−0·44	4·00
	C	6·7	9	79	28	320	88	4·4	0·25	4·25

RESULTS

Table 1 summarizes the comparisons between casein and Ms-15 in three children. In the first of these, #197, N balances were determined for 9 days at the beginning and 9 days at the end of a 30-day period on Ms-15 at a critical level of protein calories, 5·3 per cent of the total, a figure which is usually not adequate for milk protein. Absorption of N from Ms-15 was over 80 per cent of intake, better than that of most vegetable proteins (65–75 per cent in our experience) but inferior to that from white wheat flour (85–90 per cent), which is exceptionally high. Apparent N retention was approximately 65 per cent of that observed during the preceding and following casein periods. Weight gain was only moderately lower but there was a sharp drop in serum albumin level.

In the second child absorption of N from Ms-15 was inferior to that from casein, or from Ms-15 in the other two patients. Apparent N retention was only 56 per cent of that from casein, weight gain was significantly lower, and there was again a sharp fall in serum albumin.

In the third child, much older than the others, absorption of N equalled that from casein, retention was 77 per cent of that from casein, weight gain was moderately less, and the drop in serum albumin was considerably less important.

Table 2 summarizes additional comparisons with casein in two infants, including the effect of lysine supplementation. In the first of these, without lysine, N retention was 50 per cent of that from casein, weight gain was slightly less and there was a moderate fall in serum albumin. With supplementary lysine N retention was 79 per cent of that from casein, weight gain was significantly greater, and the fall in serum albumin was less. In the second infant, the youngest in this study, without lysine N retention was 52 per cent of that from casein, weight gain was moderately lower, and there was a sharp drop in serum albumin. When lysine was added to the Ms-15, N retention was 74 per cent of that from casein, weight gain was greater, and the fall in serum albumin much less important.

TABLE 2. SUPPLEMENTATION WITH L-LYSINE OF A WHEAT FLOUR-WHEAT CONCENTRATE MIXTURE (Ms-15). CASEIN (C) AS A CONTROL. EFFECT ON NITROGEN RETENTION, WEIGHT GAIN, AND SERUM ALBUMIN LEVELS

Subject (age)	Protein	Per cent kcal	Lysine mg/kg/day	No. days	mg N/kg/day in.	ret.	wt g/kg/day	Ser. albumin Δ	Final
#221	C	6·7	–	9	400	130	7·7	−0·07	4·20
(9 months)	Ms-15	6·7	–	15	385	80	6·0	−0·35	3·85
	C	6·7	–	9	372	191	6·5	0·48	4·33
	Ms-15	6·7	48	15	400	157	8·0	−0·24	4·09
	C	6·7	–	12	400	208	5·7	0·17	4·26
#222	C	6·7	–	9	400	178	5·8	0·27	4·28
(5 months)	Ms-15	6·7	–	15	400	89	4·2	−0·98	3·30
	C	6·7	–	9	400	164	5·7	0·11	3·41
	Ms-15	6·7	48	15	400	125	6·5	−0·34	3·07
	C	6·7	–	12	400	172	4·4	0·44	3·51

TABLE 3. FASTING PLASMA FREE AMINO ACIDS OF INFANTS RECEIVING A WHEAT FLOUR-WHEAT CONCENTRATE MIXTURE (Ms-15) AS THE SOLE SOURCE OF DIETARY PROTEIN. TOTAL AMINO ACIDS (AA), TOTAL ESSENTIAL AMINO ACIDS (EAA), THE RATIO OF ESSENTIALS TO TOTAL (EAA/AA), AND LYSINE AS THE MOLAR FRACTION OF ESSENTIALS (lys/EAA). CONTROL VALUES ARE FROM 6 INFANTS RECEIVING UNENRICHED WHITE FLOUR AND THE SAME ENRICHED WITH THE EQUIVALENT OF 0·4% LYSINE, AND FROM 39 INFANTS RECEIVING MILK PROTEIN AT 6·4–8·0% OF CALORIES. NONE OF THE CONTROLS RECEIVED MORE THAN 2·0 g PROTEIN/kg BODY WEIGHT/DAY

Case No.	Protein g/kg/day	per cent of Kcal	No. of days	Plasma amino acids AA (μM/ml)	EAA (μM/ml)	EAA/AA	Lys/EAA
197	2·0	5·3	30	1·523	0·305	0·200	0·118
207	2·5	6·7	15	1·781	0·330	0·185	0·109
212	2·0	6·7	15	2·353	0·563	0·239	0·123
221	2·5	6·7	15	3·208	0·898	0·280	0·158
221	2·5*	6·7	15	3·489	1·391	0·399	0·172
222	2·5	6·7	15	3·390	1·081	0·319	0·158
Controls—Wheat flour				2·220	0·582	0·258	0·098
Controls—Same + 0·4 per cent lysine				2·335	0·576	0·237	0·191
Controls—Milk protein				2·298	0·691	0·296	0·157

* Lysine 48 mg/kg body wt/day added to diet.

In Table 3 we have summarized the plasma amino acid values taken in the fasting state at the end of each of the Ms-15 periods presented in Tables 1 and 2. The sample after lysine supplementation in the last infant, #222, was unfortunatley lost. In the first two children the low values for total amino acids probably are the result of the very low protein to calorie ratio in #197 and of the poor absorption in #207. The low EAA/AA ratios in the same two children probably reflect the same low ratio in the dietary protein plus inadequacy of the protein intake in each case. In the third child the value for total amino acids was very similar to that of the controls and was probably the result of efficient absorption. The EAA/AA ratio was similar to that of the wheat controls, suggesting that the diet was more nearly adequate. The low lysine/EAA ratios in all three children point to this as the limiting amino acid in their diet.

In both samples from #221 and in that from #222 the high total and essential amino acid concentrations, the high EAA/AA ratios, and the relatively high lysine/EAA ratios are all most probably a reflection of higher protein intakes and satisfactory absorptions,

80–81 per cent of N intake in #221, and 76 per cent in #222. The improvement in the EAA/AA and lysine/EAA ratios of #221 may be reflecting the enhanced adequacy of dietary protein brought about by lysine supplementation.

DISCUSSION

HANSEN and WATERLOW (1964) have rightfully pointed out that in short-term studies of protein value N retentions at low levels of intake are our most sensitive indicator of adequacy. In these studies, as in all others we have carried out, weight gain, even over much longer periods of time, has been consistently deceiving. If serum albumin is measured repeatedly, however, the nature of the weight gain can be correctly interpreted.

In the five Ms-15 periods without lysine supplementation N retention was 65, 56, 77, 50 and 52 per cent of that from the preceding and following casein periods, with a mean of 60 per cent, very close to the 63 per cent found in previous studies with wheat flour (GRAHAM *et al.*, 1969). Rates of weight gain for the same periods, however, were 83, 55, 76, 84 and 73 per cent of those from casein, with a mean of 74 per cent, higher than the 67 per cent found in the wheat flour studies. While in the same wheat flour studies there was a mean fall of 0·08 and a final level of 4·02 g of albumin/100 ml of serum, in the present studies there was a mean fall of 0·77 and a final level of 3·49 g/100 ml, a striking difference.

The fact that this mixture, despite its theoretically higher available lysine content and its demonstrated higher PER and NPU in the rat, had a biological value, relative to casein, in the human infant which was if anything lower than that of wheat flour is almost certainly explained by its inferior digestibility. This was generally most apparent in the younger children and was confirmed by the lower plasma amino acid concentration and the important fall in serum albumin levels. It is also conceivable that the flour in the mixture, with a lower lysine content, was better digested than the concentrate.

Lysine fortification though producing a 50 per cent improvement in the biological value of the protein mixture, still left it significantly below casein. With wheat flour alone, lysine fortification brought the relative biological value very close to that of casein. The flour was more efficiently digested than casein, while the mixture was moderately but significantly less well digested. The moderately higher lysine/EAA ratios in plasma after Ms-15, when compared to wheat flour alone, also suggest that inferior absorption of N, along with lysine deficiency, account for the inferior biological value, while in wheat flour lysine deficiency is almost the only factor of importance.

The mixture of wheat flour and wheat concentrate offers the possibility of significantly increasing the protein content of wheat products without major changes in their acceptable characteristics. A theoretical improvement in the lysine content is offset, at least in infants and small children, by inferior digestibility. The improvement in biological value brought about by appropriate lysine enrichment, at levels previously shown to produce maximal improvement in wheat flour, is of such an important degree, that such enrichment should be mandatory if the mixture is to be used as the major source of protein in the diets of small children, which it could then very well be.

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