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All-Vegetable Protein Mixtures for Human Feeding VIII. Biological Testing of INCAP Vegetable Mixture Nine in Rats[a,b]

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SUMMARY

The nutritive value of INCAP Vegetable Mixture 9 in young and protein-depleted adult rats was studied. This mixture contains 28% lime-treated corn, 28% sorghum grain, 38% cottonseed flour, 3% kikuyu leaf meal, and 3% torula yeast. When Mixture 9, skim milk, casein, or meat flour was fed combined with different amounts of starch to give a range of dietary protein levels, the nutritive value of the vegetable mixture for rats compared favorably with that of animal proteins at the higher levels of protein intake. Both young growing rats and protein-depleted rats were used. The growth response was generally less to the vegetable mixture at low protein levels than when animal proteins were fed at the same dietary level, presumably because of a lysine deficiency also observed in previous chick trials.

When the corn and sorghum combination was replaced in the mixture by processed corn, sorghum, rice, whole wheat, or oats, no significant changes were noted in the nutritive value. Furthermore, roasting, boiling, and lime-treating corn and sorghum did not affect the nutritive value of the mixture.

INCAP Vegetable Mixture 9 contains 27.5% protein, of which approximately 70% comes from cottonseed flour, 25% from corn-sorghum mixtures, and 5% from yeast protein (Bressani *et al.*, 1961b). Extensive studies in chicks (Bressani *et al.*, 1961a) have shown it to be free of toxic effects and relatively high in protein quality. Its nutritive value has also been confirmed by nitrogen-balance studies in children and by its use as the sole source of protein in the treatment of kwashiorkor (Scrimshaw *et al.*, 1961). Additional information on the nutritive value of INCAP Vegetable Mixture 9 was obtained from experiments with young and adult rats before the clinical studies were undertaken. This paper gives these results and compares Vegetable Mixture 9 with several animal proteins at various dietary levels of protein. It also describes the effects of various methods of processing the cereal grain employed in the mixture.

MATERIALS AND METHODS

Boiled corn and sorghum, grown and cultivated in the highlands of Guatemala, were prepared by cooking 1,000 g each of the two grains in the autoclave with 1,000 ml of water for 15 min, at 15 pounds pressure (131°C), and drying with hot air for 16 hr at 70°C. Germinated corn and sorghum were prepared by soaking 1,000 g each of corn and sorghum for 18 hr, germinating the seed between filter papers for 24 hr, and drying as with the boiled corn and sorghum. Roasted corn and sorghum were prepared by roasting equal weights of the two grains for 20 min in a small, electrically heated, rotary coffee roaster, with roasting temperatures varying from 180 to 220°F.

All grain preparations, when cooled and dried, were ground in a Wiley mill to pass 30 mesh and stored in bottles at 4°C until used. The source and description of the cottonseed flour, lime-treated corn, sorghum grain, and other ingredients of INCAP Vegetable Mixture 9 have already been

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given (Bressani *et al.*, 1961a, b; Scrimshaw *et al.*, 1957).

The animal proteins used were skim milk (furnished by UNICEF), casein (V.F., Nutritional Biochemical Corp., Cleveland, Ohio), and meat flour. The meat-flour protein was prepared in the laboratory from prime-quality fresh local beef by drying for 24 hr with hot air at 70°C, grinding in a meat grinder, and then extracting with petroleum ether until free of fat. After a second drying the meat was ground in a Wiley mill to pass 40 mesh and stored at 4°C. This material contained 13.55% nitrogen.

The experimental animals were young and adult rats of the Wistar strain of the INCAP colony, assigned randomly by weight to the different experimental groups, so that the average initial weight per group was the same. Unless otherwise specified, 3 female and 3 male rats were used per group. They were placed in individual wire-screen cages with raised screen bottoms. Water and feed were given *ad libitum.* The young rats were weighed every 7 days during a 28-day growing period.

Adult rats were protein-depleted by feeding on a protein-free diet of 91% cornstarch, 4% cottonseed oil, 4% minerals (Hegsted *et al.*, 1941), 1% cod liver oil, and a complete vitamin solution previously described (Manna and Hauge, 1953). After losing 22–25% of their initial weight, they were fed the experimental diets for 14 days, with weight changes recorded each 7 days. Diet consumption was recorded and the diets were analyzed for nitrogen using the Kjeldahl method.

The base in all experiments was INCAP Vegetable Mixture 9 (Bressani *et al.*, 1961b), containing 28% corn, 28% sorghum, 38% cottonseed flour, 3% torula yeast, and 3% dehydrated leaf meal. Unless otherwise specified, the diets as fed contained 10% protein, obtained by diluting the 27.5% protein content of the mixture, and were supplemented with 5% cottonseed oil, 4% Hegsted mineral mixture, 1% cod liver oil, and a complete vitamin solution (Manna and Hauge, 1953).

RESULTS

Comparison with casein, skim milk, and meat flour. The data of Table 1 indicate the growth-promoting value of INCAP Vegetable Mixture 9 as compared to that of casein, skim milk, and meat fed at four different levels of protein in the diet. The diets were prepared by diluting the protein in the vegetable mixture (27.5% protein), in casein (89.5%), in skim milk (33.0%), and in meat flour (84.7%), to a calculated protein level of 5, 10, 15, 20, or 25%. Growth at all protein levels was slightly less for the vegetable mixture

Table 1. Effect of different corn-sorghum combinations on the nutritive value of Vegetable Mixture 9[a] (3 males, 3 females/group).

Variation in corn-sorghum combination[b]	Av. initial weight (g)	Av. weight gain (g)	F.E.[c]	P.E.R.[d]
I. Growth trial[e]				
100% corn	57	121	4.47	1.54
75% corn & 25% sorghum	57	112	4.70	1.46
50% corn & 50% sorghum	57	124	4.30	1.60
25% corn & 75% sorghum	57	120	4.53	1.52
100% sorghum	57	120	4.65	1.48
Casein & 0.3% cystine	59	103	3.27	1.91
II. Depletion-repletion trial[f]				
100% corn	131	63	4.21	1.63
75% corn & 25% sorghum	128	63	4.21	1.63
50% corn & 50% sorghum	139	60	4.45	1.54
25% corn & 75% sorghum	136	69	4.01	1.71
100% sorghum	136	70	3.95	1.74
Skim milk & 0.3% cystine	155	66	3.21	1.94

[a] Vegetable Mixture 9: 28% lime-treated corn, 28% sorghum, 38% cottonseed, 3% kikuyu leaf meal, and 3% torula yeast.
[b] All diets contained approximately 14.55% protein and were supplemented with: 5% NBCo No. 2 mineral mixture, 2% cod liver oil, 5% cottonseed oil, 2% alphacel, cornstarch to adjust to 100%, and 4 ml/100 g of a vitamin solution (Manna and Hauge, 1953).
[c] Feed efficiency: food consumed/weight gain.
[d] Protein efficiency ratio: weight gain/protein consumed.
[e] Experimental period lasted 28 days.
[f] Experimental period lasted 14 days.

than for casein. Feed efficiencies were similar. Protein efficiency ratios were higher with casein at low protein levels, but similar at the 10 to 25% levels for the two protein sources. With both Vegetable Mixture 9 and skim milk, growth increased in direct proportion to the increase in their protein levels, and feed efficiencies improved proportionately. Protein efficiency ratios were higher for skim milk than for casein or for Vegetable Mixture 9 at low protein levels, and similar at higher protein levels.

Only four protein levels were compared for Vegetable Mixture 9 and meat flour. Again, higher levels of protein in the diet resulted in higher weight gains from both protein sources, and at all protein levels the weight gains were the same for both the vegetable mixture and the meat flour. As protein levels in both foods rose, feed efficiencies also improved. Protein efficiencies were again slightly higher with meat flour than with Vegetable Mixture 9, particularly when the protein levels were low.

Effect of different corn-sorghum combinations. In these experiments, the corn and sorghum percentage distribution varied from 100% corn and 0% sorghum to 0% corn and 100% sorghum. The other ingredients of the mixture remained at 38% cottonseed flour, 3% torula yeast, and 3% dehydrated leaf meal, before adjustment at 10% protein. Table 2 gives the results of two trials. In the first, weight gains and feed and protein efficiencies were similar in both the 100% corn and 100% sorghum diets to those of the other groups. In the second, a protein-repletion experiment, the casein control group had less weight gain but the feed and protein efficiencies were similar to those of the other groups. The repletion weight gain and feed and protein efficiency for the skim milk control group were similar to those of any of the combinations of corn and sorghum in the basic formula, and none of these variations appeared to alter the protein value of the mixture.

Effect of treatment of the corn and sorghum. Because corn and sorghum are important in Latin-American diets (Bressani *et al.*, 1958, 1959; Bressani and Ríos, 1961), tests were made to determine the effect of various methods of preparation of these grains on the nutritive value of Vegetable Mixture 9. Five diets (partially described in Table 3), each containing approximately 15% protein, were fed for 28 days to 5 groups of rats. Weight gain was highest in the groups fed the vegetable mixture containing corn and sorghum, either raw, boiled in water, or cooked with lime. The other two groups fed the vegetable mixture with germinated or roasted corn and sorghum showed lower weight gains and feed protein efficiencies.

Substitution of other cereal grains. The same cereal grains or combinations, other than corn and sorghum, that were tested in chicks (Bressani *et al.*, 1959, 1961a) were also tested in rats. The experiment consisted of feeding variations of Vegetable Mixture 9 and a control diet of skim milk to 8 groups of rats. Table 3 gives the results and a partial description of the diets.

The oat cereal-based Vegetable Mixture 9 resulted in the best growth, slightly lower than that of the skim-milk control group. The descending order of weight gain for rats fed the other cereal grains was as follows: whole ground wheat, rice, wheat flour, and whole ground corn. The lime-treated corn-oat mixture and the lime-treated corn-rice mixture induced growth and feed efficiencies similar to that observed with the rice and wheat-flour diet, but lower than those of oats and higher than those for the mixtures containing only raw corn. For rats receiving either skim milk or any of the cereal diets, protein efficiencies were very similar except for the lower value for the group receiving wheat flour.

Comparative repletion of protein-depleted rats. Table 4 shows the results of two trials indicating that the vegetable mixture with 15% protein produced repletion weight gains comparable to those observed when the skim milk and casein or meat-flour diets were fed at the 10% protein level. Even at the 10% protein level the casein and vegetable mixtures gave similar repletion weight gains.

DISCUSSION

Previous (Bressani *et al.*, 1961a) and present testing of INCAP Vegetable Mixture 9 showed that the 56% of cereal in the corn and sorghum formula could be supplied equally by corn, sorghum, or any combination of the two. They also showed that ground rice, oat, and wheat or wheat flour may be substituted for the ground corn and sorghum in the basic formula without affecting nutritive value significantly.

The comparison of Vegetable Mixture 9 with various animal proteins further indicates that the mixture is of good nutritive value, particularly at higher protein levels. It is well known that amino acid deficiencies in proteins become more evident at lower levels of dietary protein than at higher levels, and an amino acid deficiency can often be corrected either by increasing the protein level in the diet or the protein intake (Harper, 1959). At levels lower than 10% of protein in the diet, the animal proteins induced better growth than the

Table 2. Twenty-eight-day comparisons between the nutritive value of Vegetable Mixture 9 and casein, skim milk and meat flour at different protein levels in the diet (3 male and 3 female rats/group).

Treatment	% test protein in diet	% protein content in diet	Av. initial weight (g)	Av. weight gain (g)	F.E.[a]	P.E.R.[b]
Vegetable Mixture 9[c,d]	17.0	5.82	47	22	13.9	1.23
Vegetable Mixture 9[c,d]	34.0	10.28	44	84	4.8	2.03
Vegetable Mixture 9[c,d]	51.0	14.78	45	133	3.4	2.00
Vegetable Mixture 9[c,d]	68.0	18.48	44	149	3.2	1.71
Vegetable Mixture 9[c,d]	85.0	22.61	45	156	2.9	1.51
Casein[e]	5.0	5.73	47	30	9.5	1.83
Casein[e]	10.0	10.25	44	96	4.2	2.29
Casein[e]	15.0	15.31	45	142	3.0	2.14
Casein[e]	20.0	20.10	44	154	2.7	1.83
Casein[e]	25.0	24.91	44	184	2.3	1.73
Vegetable Mixture 9[f]	18.5	5.16	50	17	17.6	1.14
Vegetable Mixture 9[f]	37.0	10.58	50	89	4.6	2.04
Vegetable Mixture 9[f]	55.6	15.48	50	125	3.5	1.85
Vegetable Mixture 9[f]	74.1	19.60	50	129	3.1	1.67
Vegetable Mixture 9[f]	90.0	24.50	50	147	2.9	1.42
Skim milk[g]	15.6	5.70	50	36	7.5	2.34
Skim milk[g]	31.2	10.26	50	117	3.4	2.88
Skim milk[g]	46.8	14.31	51	127	3.1	2.24
Skim milk[g]	62.4	19.62	50	148	2.6	1.94
Skim milk[g]	78.0	23.53	50	133	2.9	1.48
Vegetable Mixture 9[h]	37.0	11.64	52	74	6.5	1.33
Vegetable Mixture 9[h]	55.6	14.48	51	113	4.1	1.70
Vegetable Mixture 9[h]	74.1	21.09	51	138	3.1	1.51
Vegetable Mixture 9[h]	90.0	24.36	51	156	2.8	1.48
Meat flour[h]	11.1	11.21	51	54	6.0	1.49
Meat flour[h]	16.7	14.33	51	123	3.1	2.21
Meat flour[h]	22.3	19.81	51	130	3.2	1.60
Meat flour[h]	27.8	25.36	51	172	2.6	1.52

[a] Feed efficiency: food consumed/average weight gained.

[b] Protein efficiency ratio: average weight gain/average protein consumed.

[c] Lime-treated corn 28%, ground sorghum 28%, cottonseed flour 38%, kikuyu leaf meal 3%, and torula yeast 3%.

[d] All diets were supplemented with 4% NBCo No. 2 mineral mixture, 0.3% cod liver oil, (4.3, 3.6, 2.9, 1.4)% cottonseed oil, (1.9, 1.4, 1.0, 0.5)% alphacel, enough cornstarch to adjust to 100%, and 4 ml per 100 g of a vitamin supplement (Manna and Hauge, 1953).

[e] All diets were supplemented with 4% NBCo No. 2 mineral mixture, 0.3% cod liver oil, 5% cottonseed oil, 2.4% alphacel, enough cornstarch to adjust to 100%, and 4 ml per 100 g of a vitamin supplement (Manna and Hauge, 1953).

[f] All diets were supplemented with 4% Hegsted mineral mixture, 1.0% cod liver oil, 5% cottonseed oil, 10% cornstarch, sugar to adjust to 100%, and 4 ml of a complete vitamin supplement (Manna and Hauge, 1953).

[g] All diets were supplemented as in *f*, plus 2% celluflour.

[h] All diets were supplemented with 4% salmina mineral mixture (Bressani *et al.*, 1961b), 1% cod liver oil, 5% cottonseed oil, enough cornstarch to adjust to 100%, and 4 ml of a complete vitamin supplement (Manna and Hauge, 1953).

vegetable mixture, presumably because of amino acid deficiencies in the vegetable protein mixture. Previous studies showed that lysine in Vegetable Mixture 9 was limiting for the chick (Bressani *et al.*, 1961a), and the same appears to be true for rats when this mixture is fed at low protein levels.

The protein-efficiency ratio of Vegetable Mixture 9 at higher protein levels in the diet was similar to that of the animal proteins tested, suggesting that the nutritive value of the vegetable mixture is slightly lower than that of the animal proteins as tested in the young rat. The biological determination of nutritive value of Vegetable Mixture 9, however, showed it to be very similar to animal proteins in protein quality. Certainly it is possible to develop vegetable mixtures closely approaching the nutritive value of good-quality animal proteins such as eggs, milk, and meat. Such a conclusion is supported by the results obtained with the adult protein-depleted rats.

Since corn and sorghum are prepared in different ways for consumption in Central America, it was considered important to determine the extent to which the nutritive value of Vegetable Mixture 9 would be affected by the treatment. Most of the treatments tested are commercially available and

Table 3. Effect of the substitution of lime-treated corn and sorghum grain in Vegetable Mixture 9 by other corn and sorghum preparations and other cereal grains (3 male and 3 female rats/group, 28 days).

Variation in Vegetable Mixture 9 [a]	% protein in diet	Av. initial weight (g)	Av. weight gain (g)	F.E.[b]	P.E.R.[c]
Whole ground corn and sorghum grain [d, e]	13.50	61	132	3.5	2.10
Lime-treated corn and boiled sorghum [d, e]	14.10	61	140	3.5	2.04
Boiled corn and boiled sorghum [d, e]	14.20	61	128	3.5	2.02
Germinated corn and germinated sorghum [d, e]	14.40	61	120	3.8	1.81
Roasted corn and roasted sorghum [d, e]	13.70	61	126	3.7	1.99
Whole ground corn [f, g]	13.81	49	128	3.5	2.05
Whole ground wheat [f, g]	14.74	48	143	3.4	1.97
Oats [f, g]	15.58	50	151	3.3	1.93
White rice [f, g]	13.97	50	134	3.6	2.01
Wheat flour [f, g]	15.05	50	135	3.6	1.85
Lime-treated corn and oats [f, g]	15.07	49	133	3.4	1.95
Lime-treated corn and rice [f, g]	13.99	49	136	3.6	1.98
Skim milk [h]	16.28	49	158	3.0	2.07

[a] Vegetable Mixture 9: 28% lime-treated corn, 28% sorghum grain, 38% cottonseed flour, 3% kikuyu leaf meal, 3% torula yeast.

[b] Feed efficiency: food consumed/average weight gained.

[c] Protein efficiency ratio: average weight gain/average protein consumed.

[d] The percentages of cereal grains in *a* were replaced by the same percentage of the treated grains as indicated.

[e] All diets were supplemented with 1% cod liver oil, 4% Hegsted mineral mixture, 4% cottonseed oil, cornstarch to adjust to 100%, and 3 ml/100 g of a complete vitamin solution (Manna and Hauge, 1953).

[f] The percentages of cereal grains in *a* were replaced totally by the cereal indicated.

[g] All diets were supplemented with 1% cod liver oil, 4% Hegsted mineral mixture, 4% cottonseed oil, 20% sugar, 20% cornstarch and 3 ml/100 g of a complete vitamin solution (Manna and Hauge, 1953).

[h] This diet contained 46.8% skim milk, 2% celluflour, 21.2% sugar, 20% cornstarch, 1% cod liver oil, 4% Hegsted mineral mixture, 5% cottonseed oil, and 3 ml/100 g of a complete vitamin solution (Manna and Hauge, 1953).

Table 4. Repletion of protein depleted rats with Vegetable Mixture 9, skim milk, casein, whole egg, and meat flour.[a]

Treatment	% test protein in diet	% protein content in diet	Av. initial weight (g)	Av. weight gain (g)	F.E.[b]	P.E.R.[c]
Vegetable Mixture 9	37.0	10.19	158	48 [d]	5.6	1.74
Vegetable Mixture 9	55.0	15.03	158	59 [d]	4.2	1.58
Skim milk	31.3	10.48	158	71 [d]	3.5	2.72
Casein	11.0	9.70	158	69 [d]	3.8	2.70
Whole egg	15.5	10.04	158	76 [d]	3.3	3.03
Meat flour	12.0	10.60	158	75 [d]	3.5	2.69
Vegetable Mixture 9	37.0	10.58	175	41 [e]	3.2	3.00
Vegetable Mixture 9	55.0	14.09	174	55 [e]	2.4	2.91
Vegetable Mixture 9	74.0	19.29	175	66 [e]	2.0	2.57
Skim milk	31.3	10.05	175	51 [e]	2.3	4.30
Casein	11.0	8.77	175	42 [e]	2.9	3.96
Meat flour	12.0	9.94	175	52 [e]	2.6	3.93

[a] All diets were supplemented with 4% Hegsted mineral mixture, 5% cottonseed oil, 1% cod liver oil, cornstarch to adjust to 100%, and 4 ml of a complete vitamin solution (Manna and Hauge, 1953).
[b] Feed efficiency: food consumed/weight gain.
[c] Protein efficiency ratio: weight gain/protein consumed.
[d] 3 male and 3 female rats/group; experimental period, 14 days.
[e] 6 male rats/group; experimental period, 7 days.

could be used in the industrial production of vegetable mixtures.

Roasting and vapor-heating treatments have been shown most likely to decrease the already low nutritive quality of cereal proteins (Liener, 1950). Nevertheless, the experiments carried out with raw, lime-treated, boiled, roasted, or germinated corn and sorghum in the mixture showed that these treated cereals did not alter Mixture 9's nutritive value significantly. Since some of these treated cereals keep better than raw cereals, it would be advantageous to use them; the treatments destroy enzymatic activity, and the final product can thus be stored for longer periods.

The experiments also showed that a variety of other cereal grains can be substituted for corn or sorghum in the vegetable mixture without altering its nutritive value, providing the proportion of the protein from cottonseed flour is not altered. These make the Vegetable Mixture 9 formula more adaptable to parts of the world where corn and sorghum are not grown. Oats, whole wheat, and rice, actually improved the nutritive value of the mixture further, since corn, as is well known, has perhaps the lowest protein quantity among the cereals.

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