ALL-VEGETABLE PROTEIN MIXTURES FOR HUMAN FEEDING

XII. BIOCHEMICAL OBSERVATIONS ON RATS FED INCAP VEGETABLE MIXTURE 9 AND ANIMAL PROTEINS[1]

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Abstract

Biological tests were carried out to study the effect of INCAP (Institute of Nutrition of Central America and Panama) Vegetable Mixture 9, casein, and skim milk on the carcass, liver, blood, and bone composition of rats. At low levels of protein intake body and liver fat levels were higher in the animals fed the vegetable mixture. Higher values for total serum protein, albumin, globulin, and urea nitrogen concentration were obtained with skim milk, but the albumin/globulin ratio and the red and white blood cell count were similar for both protein foods. The fresh weights of the femur and tibia were found to be significantly lower for the rats fed Vegetable Mixture 9, while bone moisture and fat were significantly higher. Likewise, the percentages of ash, calcium, and phosphorus were also significantly lower in the animals fed the vegetable mixture.

At higher levels of protein intake, carcass and liver fat were again higher for the animals fed the vegetable mixture. The blood analyses revealed no differences between the two proteins tested, except for a higher urea content in the animals fed skim milk.

The differences in carcass, liver, and bone composition between animals fed the vegetable mixture and the animal proteins were probably due to minor essential amino acid deficiencies in the mixture, since supplementation with lysine, threonine, and methionine resulted in similar chemical composition values. Higher protein levels of intake also decreased the differences in carcass, liver, and bone composition of rats fed INCAP Vegetable Mixture 9, casein, or skim milk.

Introduction

During the last few years, the Institute of Nutrition of Central America and Panama (INCAP) has actively been engaged in developing all-vegetable protein mixtures for supplementary feeding of infants, young children, and adults as a partial solution to the protein malnutrition problem existing in the developing areas of Central America. One of the mixtures, INCAP Vegetable Mixture 9 (1), made of whole ground corn, 28%; whole ground sorghum grain, 28%; cottonseed flour, 38%; dehydrated leaf meal, 3%; and torula yeast, 3%, has been tested extensively for its nutritive value in chicks (2), rats (3), dogs, swine (4), and children (5). Subsequent tests were carried out to determine the effect on the body composition of rats when Mixture 9 was the sole source of protein and when it was fed at different levels of protein intake. The effects of the mixture were always compared with those obtained in control animals fed animal protein diets.

This paper will summarize observations regarding the effects of INCAP Vegetable Mixture 9 on the chemical composition of bodies and organs of rats.

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Materials and Methods

A description of the dietary ingredients used in INCAP Vegetable Mixture 9, as well as in the preparation of the diets, has already been given (6). Cornstarch was used in the experiments to decrease the concentration of the protein in Vegetable Mixture 9, in casein, and in skim milk. In one experiment both Mixture 9 and skim milk were fed at a 10% protein level in the diet, and in another the protein level was 20% of the diet for both foods. In two additional experiments, the protein in Vegetable Mixture 9 and vitamin-free casein was diluted to levels of 5, 10, 15, 20, and 25% protein in the diet. All diets were supplemented with 4% mineral mixture, 5% cottonseed oil, and 1% cod liver oil plus 5 ml/100 g of a complete vitamin mixture (7).

Weanling rats of the Wistar strain from the INCAP colony were distributed equally by weight and sex among the experimental groups. The number of animals per experimental group varied from 6 to 10, to be described in the respective tables. The animals were placed in all-wire screen cages with raised screen bottoms and were fed and given water ad libitum. Diet consumption and weight changes were measured every 7 days for 28 days. At the end of the experimental period, the animals were sacrificed by decapitation, and blood samples were obtained; the liver, femurs, and tibias were extracted and dried to constant weight in a hot-air oven (80° C) for later analysis. Nitrogen determinations were carried out by the Kjeldahl method, fat by ethyl ether extraction, and ash in an oven at 600° C. Calcium content of the ash was determined by the AOAC method (8), and phosphorus was determined by the method of Lowry and López (9). The bones were treated and analyzed as described by Braham *et al.* (10). The serum was analyzed for total protein by the method of Lowry and Hunter (11) and albumin by the method of Lowry *et al.* (12). Serum urea was determined by the method of Gentzkow and Mosen (13) and cholesterol by the method of Abell *et al.* (14). The hemoglobin was determined by the method of Cannan (15), and the hematocrit by the method of Wintrobe (16).

Results

Since no significant sex differences were observed, the data for males and females were pooled in all experiments, except for those presented in Table III where separate values for males and females are presented.

Table I describes the results obtained when the protein level of both Mixture 9 and skim milk was 10%. In this experiment skim milk induced better weight gain and feed and protein efficiency. Higher values in total serum protein, albumin, and globulin were obtained with skim milk, but the albumin/globulin (A/G) ratio, urea concentration, and the red and white blood cell counts were similar for both foods. Hemoglobin values were significantly different ($P<0.01$) between the two groups. Table II presents the biochemical information yielded by the first experiment. Femur moisture and femur and tibia fat were significantly higher in the rats fed Mixture 9, no difference in protein content was

TABLE I

Growth response, protein efficiency ratio, and blood composition of rats fed 10% protein diets of INCAP Vegetable Mixture 9 and of skim milk[a]

	Vegetable Mixture 9	S.D.	Skim milk	S.D.
Initial weight, g	43		42	
Weight gain, g	58	15.0	110	9.0
Feed efficiency[b]	5.5	1.3	3.2	0.2
Protein efficiency ratio[c]	1.49	0.31	2.64	0.16
Total serum protein, %	4.36**	0.20	5.02	0.29
Albumin, %	2.28*	0.18	2.70	0.31
Globulin, %	2.08*	0.15	2.31	0.10
Albumin/globulin	1.10	0.13	1.17	0.16
Serum urea N, mg/100 ml	19.5	0.20	18.9	0.20
Red blood cells (millions per cc)	5.93	0.57	5.33	0.69
White blood cells (per cc)	5041	514.0	5708	1563.0
Hemoglobin,%	13.0**	0.3	13.6	0.7

[a]Six rats per group, 3 females and 3 males. Experimental period, 28 days.
[b]Average feed, g/average weight gain.
[c]Average weight gain/average protein consumed.
*Significantly different than value for skim milk ($P < 0.05$).
**Significantly different than value for skim milk ($P < 0.01$).

TABLE II

Bone composition of rats fed 10% protein diets from Vegetable Mixture 9 and from skim milk[a]

	Vegetable Mixture 9				Skim milk			
	Femur	Tibia	All	S.D.	Femur	Tibia	All	S.D.
Moisture, %	51.2*	44.6	47.9**	7.8	48.5*	43.6	46.0	7.1
Fat, %	3.6*	6.0	4.8**	2.9	1.5*	3.6	2.6	2.7
Protein, %	18.5	19.0	18.8	0.6	19.0	19.3	19.1	1.4
Ash, %	25.2	25.8	25.5**	3.2	26.4	27.1	26.8	1.1
Calcium, % of ash	35.0*	38.4	36.7**	3.9	38.3	40.7	39.5	4.1
Phosphorus, % of ash	17.3*	18.3	17.8**	1.5	20.0	21.0	20.5	2.1

[a]Six animals per group (3 females and 3 males).
*Significant difference between femur and tibia within diet at the 5% level.
**Significant difference between all bones between diets at the 1% level.

noticed. The percentages of ash, calcium, and phosphorus were significantly lower in the animals fed the vegetable mixture.

The results presented in Table III are from the second experiment in which the level of protein in the diet was 20%. Here, weight gain was higher for Mixture 9, although both feed and protein efficiencies were equal. The carcass analyses showed no differences in moisture but protein and fat content were significantly different in the animals fed Vegetable Mixture 9. The liver analyses also showed higher fat values for the animals fed vegetable protein and showed no differences in moisture and nitrogen content. The blood analyses revealed significant differences between the two groups of animals; urea, cholesterol, albumin, and globulin content were significantly different between Vegetable Mixture 9 and skim milk.

TABLE III

Weight gain, protein efficiency ratio, carcass and liver chemical composition and blood composition of rats fed 20% protein diets from INCAP Vegetable Mixture 9 and from skim milk[a]

	Vegetable Mixture 9				Skim milk			
	Female	Male	All	S.D.	Female	Male	All	S.D.
			Growth data					
Initial weight, g	46	51.	49		46	51	49	
Weight gain, g	114	178	146	35	106	137	121	20
Feed efficiency	3.3	2.8	3.0	0.28	3.2	2.8	3.0	0.31
PER	1.59	1.89	1.75	0.17	1.65	1.86	1.75	0.17
			Carcass analysis					
Moisture, %	68.3	66.0	67.1	4.3	67.9	68.1	68.0	2.1
Fat,[b] %	25.7	29.6	27.9**	4.3	19.2	18.7	18.9	3.4
Nitrogen,[b] %	11.6	11.1	11.4**	0.4	11.9	11.9	11.9	0.2
			Liver analysis					
Moisture, %	67.9	67.3	67.7	0.5	67.8	67.8	67.8	0.3
Fat,[c] %	3.68	3.02	3.35*	1.01	2.99	2.99	2.99	0.21
Nitrogen,[b] %	10.37	9.44	9.90	0.65	9.87	9.98	9.92	0.42
			Blood serum chemistry					
Protein, %	5.58	5.05	5.32	0.27	5.11	5.25	5.18	0.51
Albumin, %	3.10	2.83	2.96**	0.39	2.83	2.43	2.63	0.34
Globulin, %	2.48	2.23	2.35*	0.30	2.27	2.81	2.54	0.47
Albumin/globulin	1.27	1.29	1.28	0.22	1.30	0.87	1.09	0.31
Blood urea N, mg/100 ml	17.3	19.5	18.5**	0.20	26.0	24.0	25.0	0.36
Cholesterol, mg/100 ml	87.0	99.0	94.0*	12.1	74.0	77.0	76.0	18.0
			Total blood constituents					
Hemoglobin, %	14.8	14.0	14.4	0.71	14.1	14.3	14.2	0.6
Red blood cells (millions per cc)	5.05	5.63	5.34	0.45	4.45	5.05	4.75	0.68
White blood cells (per cc)	5420	7220	6320	1466	4930	5800	5360	1787

[a]Ten animals per group (5 males and 5 females).
[b]Moisture-free basis.
[c]Fresh weight basis.
*Significantly different than value for skim milk ($P < 0.05$).
**Significantly different than value for skim milk ($P < 0.01$).

Table IV presents the growth records and the carcass and liver analyses resulting from the third study in which a group of rats were fed various levels of protein from Vegetable Mixture 9 and casein. Weight gains were lower for Mixture 9 than for casein at low levels of protein in the diet, as has already been described (3). An additional group of rats received Vegetable Mixture 9 supplemented with lysine, threonine, and isoleucine at a 10% level of protein in the diet, while still another group was fed casein supplemented with methionine. The amino acid supplements improved the protein efficiency ratio (PER) significantly for both proteins. The moisture and protein content of the carcass and liver of the animals fed the vegetable mixture were similar to the values found for casein; however, fat content was significantly higher in the carcasses of the animals fed Vegetable Mixture 9. Except for the group fed 15% of protein, fat content in the carcass and liver decreased as the protein in the diet increased. The liver weight was slightly lower for the group fed the vegetable protein. Addition of amino acids to both protein foods resulted in livers and carcasses with similar compositions.

Table V summarizes the results of the blood composition determinations as well as the chemical composition of the bones of all the rats. Total serum protein was slightly higher for the animals fed the vegetable protein. The values increased as the dietary protein level increased up to the 20% protein level, higher levels of protein in the diet had no effect on serum proteins. Albumin, globulin, and urea nitrogen content were significantly different between the group fed the vegetable protein mixture and those fed casein, while no major differences were found in the hemoglobin concentration or the red and white blood cell count.

Similar values for moisture, fat, ash, and protein in the bone of both groups of animals were found. Similar bone fat values were found with protein contents of 15–24% for the vegetable protein mixture and 10.6–24.4% for casein. A decrease in bone fat content was observed with both proteins, when the protein content of the diet increases from the lowest level to 10%. Further increases in dietary protein did not alter bone fat content. The calcium and phosphorus content of the ash were similar in all groups for both proteins.

Discussion

The results of the present investigation agree with previous reports (1, 3, 5) which showed that the nutritive value of Vegetable Mixture 9 is not as good at low levels of dietary protein as it is at higher levels of protein intake. This is due to the relative deficiencies of the amino acids when protein levels are low, as reported in other publications (17), and as indicated in the present study. In the first experiment reported, the values for the rats fed the vegetable mixture, particularly PER, are generally lower than those found in previous experiments (1, 3, 17). More representative results were obtained from the group of rats fed 11.11% protein from the vegetable mixture in the experiments where it was compared to casein. The chemical composition of the

TABLE

Weight gain, protein efficiency ratio, carcass of rats fed different levels of protein

	Vegetable Mixture 9 protein in diet, %											
	6.44	S.D.	11.11	S.D.	15.23	S.D.	19.00	S.D.	24.24	S.D.	11.53[b]	S.D.
Average initial weight, g	54	4	54	6	55	6	55	6	55	6	54	3
Weight gain, g	18	12	65	17	114	8	147	23	141	49	117	16
Feed efficiency	13.4	10.5	5.2	0.7	3.6	0.2	3.1	0.3	3.0	0.6	3.5	0.2
PER	1.16	0.59	1.73	0.24	1.80	0.10	1.71	0.15	1.38	0.24	2.47	0.17
												Carcass
Moisture, %	67.1	1.1	66.3	2.3	66.7	1.5	65.0	1.6	67.7	1.3	67.1	2.8
Fat,[d] %	30.8	4.0	30.0	4.6	26.7	4.6	28.9	2.0	24.9	8.1	30.1	2.6
Nitrogen,[d] %	8.41	0.54	7.96	0.67	8.80	0.89	8.10	0.39	8.85	0.49	8.28	0.38
												Liver
Fresh weight, g	3.54	0.47	5.28	1.02	8.36	1.23	9.89	1.72	9.80	2.30	8.42	1.05
Moisture, %	67.0	1.4	69.0	0.7	68.9	1.0	64.5	0.7	67.7	0.6	71.6	0.5
Fat,[e] %	6.80	2.94	4.48	0.81	2.90	0.58	3.22	0.66	2.96	0.24	4.06	0.59
Protein,[f] %	65.5	4.4	65.1	6.0	65.7	5.5	66.5	4.8	72.3	5.7	68.5	4.9
Ash, %	4.06	0.38	4.07	0.33	4.14	0.09	4.19	0.39	4.31	0.10	4.12	0.11
Iron, mg %	75.31	21.80	49.41	18.37	49.22	29.29	29.50	7.48	50.87	43.70	29.67	6.14

[a]Six animals per group (3 males and 3 females).
[b]Supplemented with 0.25 g % lysine, 0.20 g threonine, and 0.20 g % isoleucine.
[c]Supplemented with 0.10 g % methionine.
[d]Moisture-free carcass.
[e]Fresh weight basis.
[f]Moisture-free basis.

TABLE

Composition of blood and bone protein from INCAP Vegetable

	Vegetable Mixture 9 protein in diet, %											
	6.44	S.D.	11.11	S.D.	15.23	S.D.	19.00	S.D.	24.24	S.D.	11.53[b]	S.D.
Total serum protein, %	4.97	0.72	5.33	0.29	6.15	0.60	6.23	0.49	6.10	0.19	5.48	0.52
Albumin, %	3.30	0.42	3.19	0.38	3.60	0.44	3.62	0.49	3.21	0.20	3.13	0.20
Globulin, %	1.67	0.79	2.14	0.31	2.55	0.78	2.62	0.49	2.90	0.09	2.35	0.45
A/G ratio	1.98	0.20	1.54	0.41	1.56	0.61	1.44	0.40	1.11	0.09	1.37	0.27
Urea N, mg/100 ml	—	—	9.2	3.0	12.2	3.1	13.7	2.1	20.0	5.1	12.9	2.3
Hemoglobin, %	12.9	0.7	13.1	0.6	13.6	0.2	14.6	0.9	14.3	0.4	13.7	0.7
Red blood cell, 10^6/cc	7.30	1.13	6.78	1.46	5.59	0.36	6.63	1.38	5.96	0.83	5.30	0.50
White blood cell, per cc	5183	25	7100	58	6367	76	6108	172	5858	274	5250	52
												Bone
Moisture, %	44.2	2.4	42.1	3.1	44.6	2.3	44.2	2.6	43.5	3.5	46.3	2.1
Fat, %	6.3	2.8	2.0	3.3	0.7	0.6	0.6	1.0	1.0	0.5	2.0	1.0
Protein, %	20.2	1.1	21.4	1.0	21.0	1.6	20.6	0.5	21.4	0.5	20.3	0.6
Ash, %	25.8	1.0	28.1	1.3	27.5	0.6	27.4	0.6	30.0	1.0	28.2	0.7
Ca, % of ash	42.5	2.7	41.5	2.1	40.0	1.8	40.0	2.2	38.8	3.3	36.5	1.1
P, % of ash	19.3	2.6	20.8	2.3	20.1	0.8	19.6	1.4	18.8	1.3	19.0	0.6

[a]Six animals per group (3 males and 3 females).
[b]Supplemented with 0.25 g % lysine, 0.20 g % threonine, and 0.20 g % isoleucine.
[c]Supplemented with 0.10 g % methionine.

IV
and liver chemical composition
from INCAP Vegetable Mixture 9 and from casein[a]

Casein Protein in diet, %											
6.64	S.D.	10.59	S.D.	15.92	S.D.	20.07	S.D.	24.42	S.D.	11.04[c]	S.D.
55	3	55	4	55	2	55	2	54	3	55	2
25	12	92	8	159	36	151	40	149	30	138	38
11.0	4.5	3.8	0.4	2.7	0.2	2.5	0.4	2.3	0.3	2.9	0.3
1.36	0.45	2.52	0.25	2.37	0.22	1.97	0.26	1.79	0.20	2.87	0.36
analysis											
68.0	1.0	66.7	4.2	65.0	2.4	66.2	1.6	66.3	11.5	65.4	1.8
25.8	3.3	28.2	5.5	26.7	8.3	19.6	5.6	18.4	3.9	29.0	3.3
8.65	0.40	8.01	0.64	8.02	1.01	8.73	0.85	8.74	0.58	8.04	1.02
analysis											
3.81	1.08	6.98	1.10	10.08	2.21	9.39	1.46	10.08	1.93	8.37	2.09
67.1	1.6	66.5	0.5	67.1	1.0	68.4	0.8	66.6	1.6	68.5	1.1
6.20	1.28	3.48	0.28	2.90	0.31	3.44	0.32	3.09	0.34	3.94	0.74
60.0	3.1	70.5	9.2	72.2	2.7	71.9	1.1	70.6	3.2	65.4	5.0
4.71	0.43	4.61	0.63	4.70	0.26	4.25	0.21	4.29	0.20	4.58	0.15
86.98	32.74	30.52	29.90	27.90	8.83	29.41	9.72	28.75	8.82	29.48	11.35

V
of rats fed different level of
Mixture 9 and from casein[a]

Casein protein in diet, %											
6.64	S.D.	10.59	S.D.	15.92	S.D.	20.07	S.D.	24.42	S.D.	11.04[c]	S.D.
5.19	0.22	5.46	0.38	5.74	0.74	5.94	0.28	5.91	0.21	5.82	0.29
2.92	0.19	2.92	0.25	3.07	0.39	2.99	0.16	2.87	0.13	3.13	0.30
2.27	0.27	2.55	0.29	2.67	0.50	2.95	0.25	3.04	0.20	2.69	0 28
1.31	0.21	1.16	0.18	1.18	0.21	1.02	0.01	0.95	0.06	1.18	0.21
17.3	3.6	14.8	3.5	16.5	5.0	20.6	4.2	25.4	2.1	9.8	1.9
13.8	1.1	13.6	0.5	14.3	0.7	14.6	0.8	15.2	0.6	14.6	0.1
6.21	0.36	5.82	0.90	5.21	0.30	5.22	0.71	5.17	0.93	5.35	0.55
4983	39	5558	194	5630	154	5600	152	5780	138	4450	89
composition											
44.0	1.4	45.4	1.9	44.7	2.1	45.2	2.1	43.1	1.4	44.9	2.4
5.6	2.6	0.7	0.8	0.6	0.6	1.3	0.3	1.5	0.2	1.8	0.2
20.9	0.4	20.8	0.7	19.9	1.0	20.7	0.5	21.3	0.7	20.3	0.5
27.6	1.1	28.2	0.7	28.3	0.9	28.0	0.4	29.5	5.3	28.1	0.6
41.3	2.6	37.1	1.6	39.4	1.4	40.1	2.0	38.1	1.1	39.0	1.6
16.8	1.4	19.8	1.8	19.5	0.9	19.6	2.0	19.6	0.6	19.4	0.5

animals fed Vegetable Mixture 9 was, in most components, similar to that of the animals fed animal protein, except in the amounts of body and liver fat which were higher with the mixture than with animal protein, particularly at low levels of protein intake in the diet. This is further indication of essential amino acid deficiencies. Since animals tend to accumulate fat rather than protein at low levels of protein intake, as shown by these results, it is advisable in evaluating proteins by animal weight gain to test them at several levels of protein in the diet, as suggested by Allison and Fitzpatrick (18) and Guzmán *et al.* (19).

From the results it is evident that proteins in diets can alter body as well as bone composition, particularly with respect to fat. More differences in bone composition were found when the vegetable mixture was compared to skim milk than when it was compared to casein. No explanation can be advanced for this finding, unless the minerals are better utilized with a protein such as that of milk than with a vitamin-free casein, a product which has been chemically treated.

The fat content of bones from rats fed the vegetable mixture was significantly higher than the fat content of bones from rats fed skim milk. This finding also reflects relative deficiencies of amino acids, particularly at low levels of protein intake. Braham *et al.* (10) reported that the bones of chicks fed lysine-deficient diets contained more fat than did those of the controls. As protein in the diet increases or the amino acid deficiencies are corrected, fat content in the bone of the animal fed the vegetable protein is essentially the same as that of rats fed animal protein.

The levels of blood urea were usually higher with the animal proteins, probably indicating both a higher absorption and a higher metabolic breakdown of amino acids. The findings in general are similar to those reported by Allison *et al.* (20) in their study of the nutritive value of cottonseed protein.

In general, Vegetable Mixture 9 is of good protein quality, particularly at high levels of protein intakes; and it does not produce adverse effects, either in growth of the animals or in the chemical composition of their organs. Furthermore, the results show that proper combinations of different vegetable protein sources can supply to the animal the essential amino acids to meet body needs for growth.

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