All-Vegetable Protein Mixtures for Human Feeding

X. Effect of Torula Yeast on the Protein Quality of INCAP Vegetable Mixture 9

N. S. Scrimshaw, PH.D., M.D., M.P.H.,* R. Bressani, P.H.D.,† D. Wilson, M.D.‡ and M. Béhar, M.D., M.P.H.§

Several investigators[1–5] have shown that when small amounts of torula yeast are used as a supplement to cereal grains, they not only provide B-complex vitamins, but also improve the nutritive value of the protein. Torula yeast is used in INCAP Vegetable Mixture 9 as a source of B-complex vitamins.[6] Because it also contains 50 per cent protein of relatively good lysine content, it was considered important to learn if the addition of 3 per cent torula yeast to INCAP Vegetable Mixture 9 would measurably improve its protein value for children, as suggested by previous experiments with baby chicks.[7] This paper presents the results of feeding INCAP Vegetable Mixture 9 with and without torula yeast on the growth of rats and on the nitrogen balance of children.

MATERIAL AND METHODS

The ingredients of INCAP Vegetable Mixture 9 have already been described.[6] The basic formula, consisting of 28 per cent lime-treated corn, 28 per cent ground sorghum grain, 38 per cent cottonseed flour, 3 per cent kikuyu leaf meal, and 3 per cent torula yeast, was fed uncooked in the rat experiments. Formula 9B, containing 29 per cent ground corn, 29 per cent ground sorghum, 38 per cent cottonseed flour, 1 per cent calcium carbonate and 3 per cent torula yeast, was cooked as a cereal gruel for the nitrogen balance experiments in children.

Experiments with Rats

Weanling white rats of the INCAP colony's Wistar strain, distributed so that the average initial weight was the same in all groups, were used. The animals were put in individual, all wire cages with raised screen bottoms. Food and water were provided *ad libitum*, and food intake and weight gains were recorded every seven days for an experimental period of twenty-eight days.

At each protein level in the diet, in all three experiments, one group of rats was fed the vegetable mixture with and without the torula supplement at three protein levels. The protein of the mixture was diluted with cornstarch from 27.5 to 10, 15 and 20 per cent. In the third experiment, additional groups received the mixture with either 0.15 per cent lysine or 3 per cent cottonseed flour substituted for the yeast. All diets were supplemented with 4 per cent Hegsted mineral mixture,[8] 1 per cent cod liver oil, 5 per cent cottonseed oil and 4 ml. of a complete vitamin solution.[9]

Nitrogen Balance Studies with Children

The subjects, preschool children hospitalized in the metabolism unit of INCAP, had recovered from kwashiorkor. Vegetable Mixture 9B diets, containing 2 gm. of protein and 90 calories per kg. of body weight per day, were given to eight children. Five

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* Consultant Director, INCAP, and Head, Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Boston, Massachusetts; † Chief, Division of Agricultural and Food Chemistry; ‡ Medical Assistant, Division of Clinical Investigations; § Director, INCAP.

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TABLE I

Effect of Torula Yeast on the Nutritive Value of Vegetable Mixture 9 at Two Levels of Protein in the Diet of Rats

Variation on Vegetable Mixture 9*	Protein % in Diet	Average Initial Weight (gm.)	Average Weight Gain (gm.)	Feed Efficiency†	Protein Efficiency Ratio‡
Experiment No. 1§					
+ Torula yeast	10.0	49	81 ± 25	5.0	1.98
− Torula yeast	10.0	50	79 ± 8	4.9	2.04
+ Torula yeast	20.0	49	130 ± 36	3.4	1.48
− Torula yeast	20.0	49	131 ± 26	3.4	1.48
Experiment No. 2‖					
+ Torula yeast	10.0	56	88 ± 20	4.7	2.14
− Torula yeast	9.5	56	54 ± 15	6.3	1.68
+ Torula yeast	17.7	56	177 ± 10	3.0	1.90
− Torula yeast	16.7	57	169 ± 22	3.2	1.90
Experiment No. 3¶					
+ Torula yeast	11.8	45	66 ± 8	5.0	1.68
− Torula yeast	10.8	45	54 ± 8	5.8	1.59
− Torula yeast + L-lysine HCl	11.4	45	72 ± 9	4.7	1.86
− Torula yeast − L-lysine HCl + Cottonseed flour	11.2	45	59 ± 8	5.6	1.59
+ Torula yeast	16.6	45	120 ± 26	3.5	1.73
− Torula yeast	15.3	45	101 ± 26	3.9	1.70
− Torula yeast + L-lysine HCl	15.6	45	120 ± 27	3.5	1.82
− Torula yeast − L-lysine HCl + Cottonseed flour	15.6	45	111 ± 21	3.8	1.70

* In all experiments the basal formula for Vegetable Mixture 9 used consisted of whole ground yellow corn 28 per cent, whole ground sorghum 28 per cent, cottonseed flour 38 per cent, torula yeast 3 per cent, and Kikuyu leaf meal 3 per cent. In experiments 1 and 2 the 3 per cent of torula yeast was replaced by 3 per cent cornstarch. In experiment 3, the 3 per cent yeast was replaced by (1) 3 per cent cornstarch, (2) 2.85 per cent cornstarch, 0.15 L-lysine HCl and (3) by 3 per cent cottonseed flour.

† Average in grams of food consumed per average weight gained in grams.

‡ Average weight gain in grams per average protein consumed in grams.

§ Two female and two male rats per group.

‖ Five male rats per group.

¶ Three female and three male rats per group.

of the eight children received first the mixture with torula yeast for two consecutive three-day periods; they were then placed on the mixture without torula yeast for a one and a half day adaptation period, and then for two three-day balance periods. The order of diets was reversed for the other three children. Three additional children received similar diets with only 1 gm. of protein per kg. of body weight per day.

Sugar was used to adjust caloric intake and a multivitamin and mineral capsule* was given daily.

* Gevral,® courtesy of Lederle Laboratories, American Cyanamid Co., New York.

In those formulas in which torula yeast was omitted, it was replaced by cornstarch. Carmine was fed at the start of each period to facilitate separation of fecal samples. Aliquots of the food and each three-day collection of feces and urine were analyzed for total nitrogen by the Kjeldahl method.

RESULTS

Rat Studies

As shown in Table I, when rats were fed Vegetable Mixture 9 with 3 per cent torula yeast at the 10 per cent protein level, there

TABLE II

Nitrogen Balance Data of Children Fed Approximately 2 gm. of Protein/kg./day from INCAP Vegetable Mixture 9 (VM9) with and without Torula Yeast (TY)*

Treatment	mg. nitrogen/kg./day					% Absorbed	% Retained†	% Retained‡	Average Weight (kg.)
	Nitrogen Intake	Fecal Nitrogen	Urinary Nitrogen	Nitrogen Absorbed	Nitrogen Retained				
PC-91 (3 yr., 10 mo.; 17.5 kg.)									
VM9 + TY	307	75	152	232	80	75.6	26.0	34.5	17.52
VM9 − TY	306	93	151	213	62	69.6	20.3	29.1	17.86
PC-92 (3 yr., 5 mo.; 13.6 kg.)									
VM9 + TY	306	111	153	195	42	63.7	13.7	21.5	13.52
VM9 − TY	299	100	152	199	47	66.5	15.7	23.6	13.62
PC-95 (3 yr., 8 mo.; 13.5 kg.)									
VM9 + TY	336	78	165	258	93	76.8	27.7	36.0	13.48
VM9 − TY	321	73	158	248	90	77.2	28.0	36.3	13.84
PC-98 (2 yr., 7 mo.; 10.4 kg.)									
VM9 + TY	298	96	147	202	55	67.8	18.4	27.2	10.40
VM9 − TY	292	88	160	204	44	69.9	15.1	21.6	10.40
PC-99 (4 yr., 3 mo.; 14.1 kg.)									
VM9 + TY	326	126	151	200	49	61.3	15.0	24.5	14.15
VM9 − TY	274	110	123	164	41	59.8	15.0	25.0	14.29
PC-105 (5 yr., 3 mo.; 23.2 kg.)									
VM9 − TY	294	78	160	216	56	73.5	19.0	25.9	23.33
VM9 + TY	323	85	171	238	67	73.7	20.7	28.1	23.40
IN-4 (4 yr., 3 mo.; 14.1 kg.)									
VM9 − TY	301	85	150	216	66	71.8	21.9	30.5	14.26
VM9 + TY	308	81	155	227	83	73.7	26.9	36.6	14.48
IN-3 (5 yr.; 13.7 kg.)									
VM9 − TY	311	68	136	243	107	78.1	34.4	44.0	13.80
VM9 + TY	308	74	162	234	72	76.0	23.4	30.8	14.09
Over-all Average									
VM9 + TY	314	91	157	223	66	71.0	21.0	29.6	...
VM9 − TY	300	87	149	213	74	71.0	21.3	30.0	...

* The figures are the average of two three-day balance periods.
† Percentage nitrogen retention calculated on the basis of the nitrogen intake.
‡ Percentage nitrogen retention calculated on the basis of the nitrogen absorbed.

TABLE III

Nitrogen Balance Data of Children Fed Approximately 1 gm. of protein/kg./day from INCAP Vegetable Mixture 9 with and without Torula Yeast*

Treatment	mg. nitrogen/kg./day					% Absorbed	% Retained†	% Retained‡	Average Weight (kg.)
	Nitrogen Intake	Fecal Nitrogen	Urinary Nitrogen	Nitrogen Absorbed	Nitrogen Retained				
				VV-7 (6 yr.; 16.3 kg.)					
VM9 − TY	175	49	128	126	−2	72.0	−1.1	−1.6	16.21
VM9 + TY	166	43	107	123	16	74.1	9.6	13.0	16.25
				VV-8 (5 yr., 3 mo.; 15.2 kg.)					
VM9 − TY	171	46	130	125	−5	73.1	−2.9	−4.0	15.27
VM9 + TY	163	45	121	118	−3	72.4	−1.8	−2.5	15.23
				VV-9 (5 yr., 7 mo.; 15.8 kg.)					
VM9 − TY	177	59	110	118	+8	66.7	4.5	7.3	15.76
VM9 + TY	179	56	123	123	0	68.7	0	0	15.65
				Over-all Average					
VM9 − TY	174	51	123	123	0	70.7	0	0	...
VM9 + TY	169	48	117	121	4	71.6	2.3	3.3	...

* Average of three three-day balance periods.
† Percentage of the nitrogen intake.
‡ Percentage of the nitrogen absorbed.

was better growth and feed efficiency than when the yeast was omitted. At 15 and 20 per cent protein levels, the addition of torula yeast induced only a slightly better growth and feed efficiency, and protein efficiency was the same for both groups. When yeast-free diets, with and without the amount of lysine that the 3 per cent torula yeast provided, were compared, the results were essentially the same. When the torula yeast was replaced by 3 per cent cottonseed flour, the results were also similar except that the response at the 10 per cent protein level was poorer.

Nitrogen Balance with Children

Table II summarizes the nitrogen balance results in children when Vegetable Mixture 9 diets were fed with and without 3 per cent torula yeast. In the first five cases the diet with yeast was fed first and in the remaining three, the order was reversed. On the average, the mixture with torula yeast produced a slightly higher nitrogen intake than that without yeast; however, nitrogen absorption as percentage of intake was essentially the same in both treatments. Nitrogen retention was slightly higher with the mixture containing yeast than for the one without it, but the difference was not statistically significant.

When the protein intake was adjusted to 1 gm. per kg. of body weight per day, nitrogen retention, as shown in Table III, was very low in all three children, yet was higher in one child when the mixture contained torula yeast and slightly lower in another. In the third case, nitrogen balance, with and without yeast, was equally negative. When over-all averages were compared, they were not significantly different.

COMMENTS

Torula yeast was added to INCAP Vege-

table Mixture 9, as in previous formulas,[6,7,10] as a source of B-complex vitamins. The results of the experiments with weanling rats clearly indicate that, as previously demonstrated in chick experiments,[7] torula yeast also contributes to the protein value of Vegetable Mixture 9 for rats. The positive effect may be attributed to the fact that torula yeast contains 50 per cent protein and 5 per cent lysine; thus, 3 per cent of this ingredient in formula 9 contributed 1.5 per cent protein and 0.15 gm. lysine. Experiments carried out with chicks[7,11] and rats[12,13] have indicated that Vegetable Mixture 9 is deficient in lysine, but the addition of torula yeast partially corrects this deficiency. As in chick studies[7,11] the results reported here indicate that synthetic L-lysine is at least as active physiologically as the lysine in yeast. Tsien et al.[14] found the lysine of yeast slightly less available.

The effect of torula yeast is more evident when the protein of the mixture is diluted to 10 per cent in the diet than at higher levels because essential amino acid deficiencies become more significant. It is obvious from the results that the yeast cannot be replaced by 3 per cent cottonseed flour, even though both contain the same amount of total protein. The poor results with cottonseed flour are probably due to the fact that its lysine content is not only slightly lower, but is also not completely available physiologically to the animal because it is bound to gossypol during the processing of cottonseed for oil.[15]

In the nitrogen balance studies with children fed Vegetable Mixture 9, with and without the addition of torula yeast, no difference in nitrogen retention could be detected even when the level of protein intake was lowered from 2 to 1 gm. of protein per kg. body weight per day. The lack of effect may be due to the use of the nitrogen balance technic and to the smallness of the differences in essential amino acid content. Another possibility is that the protein quality of the mixture is somewhat higher for man than for chicks and rats. Even though no effect on protein quality for the child could be detected by the method used, torula yeast should be included as an ingredient of INCAP Vegetable Mixture 9 because of its contribution to the B-vitamin content of the formula.

SUMMARY

The effect of 3 per cent torula yeast as a protein supplement to INCAP Vegetable Mixture 9 was studied by growth experiments in rats and nitrogen balance studies in children. For the rats fed at a 10 per cent level of protein, torula yeast augmented growth, feed efficiency and protein efficiency. This effect could be duplicated by adding an amount of lysine equivalent to that contained in the yeast. At 15 and 20 per cent protein levels this effect is no longer observed. The nitrogen balance results in children failed to show any significant effect of 3 per cent torula yeast in INCAP Vegetable Mixture 9 fed at levels of 2 and 1 gm. of protein per kg. per day.

REFERENCES

1. Gilbert, C. and Gillman, J. Some methodological problems affecting the supplementation of maize assessed biologically with special reference to the value of skimmed milk powder, soybean, food yeast and casein. *South African J. M. Sc.*, 24: 41, 1959.
2. Groyco, J. A. (with the technical assistance of Santiago, C. L. and Rivera E.) Nitrogen balance of young adults consuming a deficient diet supplemented with Torula yeast and other nitrogenous products. *J. Nutrition*, 69: 49, 1959.
3. Sure, B. Relative supplementary values of dried food yeast, soybean flour, peanut meal, dried non-fat milk solids, and dried buttermilk to the proteins in milled white corn meal and milled enriched wheat flour. *J. Nutrition*, 36: 65, 1948.
4. Sure, B. Further studies on nutritional improvement of cereal flours and cereal grains with yeast. *J. Am. Dietet. A.*, 23: 113, 1947.
5. Bressani, R., Marenco, E. and Valiente, A. T. Enrichment of lime-treated corn flour, with animal and vegetable proteins and with amino acids. (Abstract.) In: Program of 5th International Congress of Nutrition, September 1–7, 1960, Washington, D. C., p. 59.
6. Scrimshaw, N. S, Squibb, R. L., Bressani, R., Béhar, M., Viteri, F. and Arroyave, G. Vegetable protein mixtures for the feeding of infants and young children. In: Amino Acid Malnutrition, p. 28. Edited by Cole, W. H., New Brunswick, 1957. Rutgers University Press.
7. Bressani, R., Aguirre, A., Elias, L. G., Arroyave, R., Jarquin, R. and Scrimshaw, N. S.

All-vegetable protein mixtures for human feeding. IV. Biological testing of INCAP Vegetable Mixture 9 in chicks. *J. Nutrition*, 74: 209, 1961.

8. HEGSTED, D. M , MILLS, R. C., ELVEHJEM, C. A. and HART, E. B. Choline in the nutrition of chicks. *J. Biol. Chem.*, 138: 459, 1941.
9. MANNA, L. and HAUGE, S. M. A possible relationship of vitamin B_{13}, to orotic acid. *J. Biol. Chem.*, 202: 91, 1953
10. SQUIBB, R. L., WYLD, M. K., SCRIMSHAW, N. S. and BRESSANI, R. All-vegetable protein mixtures for human feeding. I. Use of rats and baby chicks for evaluating corn-based vegetable mixtures. *J. Nutrition*, 69: 343, 1959.
11. BRESSANI, R., ELIAS, L. G., AGUIRRE, A. and SCRIMSHAW, N. S. All-vegetable protein mixtures for human feeding. III. The development of INCAP Vegetable Mixture 9. *J. Nutrition* 74: 201, 1961.
12. BRESSANI, R., ELIAS, L. G. and SCRIMSHAW, N. S. All-vegetable protein mixtures for human feeding. VIII. Biological testing of INCAP Vegetable Mixture 9 in rats. *J. Food Sc.*, 27: 203, 1962.
13. BRESSANI, R. and ELIAS, L. G. All-vegetable protein mixtures for human feeding. Amino acid supplementation and effect of the addition of small amounts of animal and vegetable protein concentrates to INCAP Vegetable Mixture 9. (In preparation).
14. TSIEN, W. S., JOHNSON, E. L. and LIENER, I. E. The availability of lysine from torula yeast. *Arch. Biochem. et Biophys.*, 71: 414, 1957.
15. ALTSCHUL, A. (ed.) Processed Plant Protein Foodstuffs. New York, 1958. Academic Press, Inc.

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