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CORN FLOUR SUPPLEMENTATION

The Enrichment of Lime-Treated Corn Flour with Proteins, Lysine and Tryptophan, and Vitamins

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The results indicate that the nutritive value of lime-treated corn can be improved significantly both in quantity and quality by adding any one of the following quantities of protein-rich materials of either animal or vegetable origin: fish flour 3%, meat flour 5%, whole egg flour 3%, casein 5%, skim milk powder 8%, soybean protein 8%, soybean flour 8%, cottonseed flour 9%, pepitoria flour 9%, and torula yeast 3%. The improvement in the protein quality of lime-treated corn was higher with the animal products and is due to the contribution made by the supplements in providing lysine, tryptophan, and isoleucine, amino acids limiting in lime-treated corn. A highly significant correlation ($r = 0.874$) was found between PER and the lysine content of the supplement. It was estimated that the greatest improvement in PER of lime-treated corn can be obtained by adding a supplement providing around 0.6 gram of nitrogen, 0.25 gram of lysine, 0.045 gram of tryptophan, 0.20 gram of isoleucine, and 0.075 gram of methionine. The nutritive value of lime-treated corn is also improved by the addition of vitamins, particularly riboflavin.

The per capita average daily corn consumption in rural communities of the Central American countries has been reported to vary from 185 to 423 grams (*7, 8*). These quantities provide 15 to 34 grams of protein, which are equivalent to 32 to 49% of the daily protein intake which varies from 40 to 66 grams per adult. In several communities in Guatemala, corn contributes up to 74% of the daily protein intake. The average quantities of animal protein consumed are relatively low, varying from 7 to 20 grams per person daily (*8*).

The tortilla is the most important form in which corn is consumed, but its preparation involving alkaline cooking, washing with water, and baking reduces significantly the concentrations of thiamine and riboflavin (*4, 6*). Although the cooking improves the protein quality slightly (*3, 16*), this improvement is of relatively small practical significance.

Since the quality of corn protein is so extremely poor (*1, 10, 14, 17*), and since protein malnutrition frequently occurs in areas of the world where corn is the most important staple food, it would be highly desirable if corn could be cheaply enriched to provide more and better quality protein as well as other essential nutrients in short supply in local diets.

Because commercial corn flours are already being produced in Central America (*3*), their enrichment with protein and vitamins could be easily accomplished. This paper reports the results of studies designed to find the minimum quantity of protein-rich foods of animal and vegetable origin needed to obtain a maximum improvement in the quality of corn protein. The possibilities of adding synthetic vitamins are also explored.

Materials and Methods

A commercial lime-treated corn masa prepared as described previously (*6*) was brought to the laboratory still moist

Table I. Proximate Chemical Composition and Lysine, Tryptophan, Methionine, and Isoleucine Percentages of Materials Used in Study

Material	Moisture, %	Ether Extract, %	Crude Fiber, %	Ash, %	Nitrogen, %	Lysine, %	Tryptophan, %	Methionine, %	Isoleucine, %
Lime-treated corn flour	3.3	4.8	1.8	1.8	1.43	0.193	0.034	0.182	0.390
Fish flour	11.4	0.4	0.5	11.2	12.14	7.835	0.754	2.135	4.446
Meat flour	15.1	0.2	0.5	3.9	13.55	7.688	1.003	1.962	4.082
Whole egg flour	12.2	2.4	...	4.4	11.04	5.257	1.012	2.483	4.584
Vitamin-free casein	13.4	1.3	...	1.2	14.26	7.625	1.126	2.260	6.065
Skim milk powder	5.2	1.6	...	7.9	5.10	2.768	0.502	0.870	2.271
Soybean protein	10.8	1.1	0.5	2.4	13.33	5.521	0.990	1.082	4.734
Soybean flour	15.8	2.0	3.0	5.7	7.71	3.091	0.571	0.661	2.250
Cottonseed flour	13.2	3.8	6.3	7.8	7.42	2.190	0.572	0.782	1.200
Pepitoria flour	13.8	3.3	3.1	10.4	9.27	2.243	0.734	1.198	2.595
Torula yeast	10.6	2.3	4.9	6.6	7.98	3.648	0.636	0.778	2.980

Table II. Effect of Supplementing Lime-Treated Corn Flour with Several Amounts of Fish Flour

Fish Flour Added, %	Av. Initial Weight, Grams	Av. Weight Gain, Grams	PER[a]	Corn Gluten Added, %	Av. Initial Weight, Grams	Av. Weight Gain, Grams	PER[a]
			EXPERIMENT No. 1[b]				
0	50	25	1.61	...	..	..	...
1	50	42	2.16	1.4	50	27	1.38
2	50	74	2.36	2.7	50	33	1.34
3	50	79	2.44	4.1	50	32	1.32
4	50	112	2.51	5.4	50	33	1.27
5	50	109	2.38	6.8	50	37	1.22
			EXPERIMENT No. 2[b]				
0	47	21	1.01				
2	47	61	1.95				
4	47	88	2.10				
6	47	104	2.18				
8	47	127	2.24				
10	47	125	2.06				
			EXPERIMENT No. 3[b]				
0	51	21	1.14				
2.7[c]	51	83	2.33				
2.7[d]	51	69	2.50				

[a] Protein efficiency ratio: weight gain/protein consumed.
[b] Percentage lime-treated corn flour in respective diets for experiments 1, 2, and 3: 83.0, 87, and 87%, respectively.
[c] Lime-treated corn flour with fish flour, not baked into tortillas.
[d] Percentage lime-treated corn flour: 89%.

(50% moisture) and used in all experiments. The masa was then spread on trays and dried with hot air (80° C.) for 16 to 20 hours. After drying, it was ground to pass 40 mesh and stored at 4° C. until ready to be used. The animal protein concentrates used were casein (Nutritional Biochemical Corp., Cleveland, Ohio), a commercial vitamin-free product; meat flour, prepared in the INCAP laboratory from fresh meat (The following method was used: the meat, cleaned of visible fat, was cut into thin strips and dried as described above. After grinding, it was subjected to exhaustive fat extraction with petroleum ether. The material was ground once more and stored as above); fish flour (VioBin), a commercial product; whole, defatted egg powder, prepared by drying slices of hard-boiled eggs in hot air, grinding them, and extracting the fat with petroleum ether and ethyl alcohol for 72 to 80 hours; skim milk powder (courtesy of UNICEF), a commercial product.

The vegetable protein concentrates used were: soybean protein (Drackett), torula yeast (Lake State Yeast Corp., Rhinelander, Wis.), cottonseed flour prepared in the laboratory from fresh kernels treated with acetone-water-hexane solvent to remove fat (the dried material was ground and stored as before); pepitoria (*Cucurbita farinosa*) nut flour (*2*), prepared by grinding the nuts, followed by fat extraction with hexane for 72 to 80 hours; corn gluten (supplied by E. L. Powell, American Maize Co., Roby, Ind.), a commercial product.

All the materials were analyzed for their proximate composition using the A.O.A.C. official methods of analysis and were also assayed microbiologically for lysine, tryptophan, methionine, and isoleucine, using methods described previously (*2*, *5*). The values for the other essential amino acids were obtained from Orr and Watt (*15*).

The biological trials were carried out with young Wistar rats of INCAP's colony. The animal groups, using three males and three females per group, were distributed so that each group had the same initial weight. The rats were placed in individual, all-wire screen cages with raised screen bottoms and were fed and given water *ad libitum* for a period of 28 days. Weight changes and food consumption records were recorded every 7 days. At the end of the experimental period, the animals were sacrificed, and blood was collected for total serum protein determination (*11*).

For the supplementation studies to determine the minimum quantity of protein needed for maximum protein efficiency ratio (PER), all trials were designed to be as similar as possible. The protein supplement was added in quantities not greater than 12% of the diet. In some experiments, a basal diet with corn gluten was included to serve as a protein-content control. All diets were supplemented with 4% cottonseed oil, 4% mineral mixture, 2% cod liver oil, and 5 ml. of a vitamin solution to give the vitamin levels recommended by Manna and Hauge (*12*), and were analyzed for total nitrogen by the Kjeldahl method. The design of each of eight experiments is discussed under Results.

The basal diet used for the vitamin enrichment experiments consisted of 90.0% lime-treated corn flour, 4% mineral mixture (*9*), 4% cottonseed oil, and 2% cod liver oil. In the first experiment, lasting 10 weeks, five groups of rats were used. The first group was fed the basal diet without a vitamin supplement; the second, the basal diet with 3.0 mg.% of riboflavin; the third

Table III. Effect on the Growth of Rats of Adding Several Amounts of Animal Protein to Lime-Treated Corn Flour[a,b]

Ingredients	1	2	3	4	5	6	7	8
Meat flour, %	...	1.0	3.0	5.0	7.0	9.0	11.0	...
Corn gluten, %	...	...	...	...	...	...	...	21.6
Av. weight gain, grams	6	21	65	102	115	120	142	46
PER[c]	0.42	0.98	2.30	2.45	2.47	2.29	2.14	1.08
Defatted whole egg flour, %	...	1.0	3.0	5.0	7.0	9.0	11.0	
Av. weight gain, grams	25	40	76	87	115	124	149	
PER[c]	1.22	1.76	2.28	2.24	2.19	2.24	2.34	
Vitamin-free casein, %	...	1.0	3.0	5.0	7.0	9.0	11.0	...
Corn gluten, %	...	...	...	...	...	...	...	11.0
Av. weight gain, grams	25	49	121	121	131	150	138	58
PER[c]	1.06	1.64	2.09	2.37	2.22	2.27	1.96	1.23
Skim milk, %	...	2.0	4.0	6.0	8.0	10.0	...	...
Av. weight gain, grams	26	47	66	85	104	125		
PER[c]	1.35	1.87	2.13	2.39	2.63	2.43		

[a] Lime-treated corn flour in meat flour, defatted whole egg flour, vitamin-free casein, and skim milk experiments: 79%. [b] Average initial weight of rats in meat flour, defatted whole egg flour, vitamin-free casein and skim milk experiments: 57, 42, 51, and 42 grams, respectively. [c] Protein efficiency ratio: weight gain/protein consumed.

Table IV. Effect on the Growth of Rats of Adding Several Amounts of Vegetable Protein to Lime-Treated Corn Flour[a,b]

Ingredients	1	2	3	4	5	6	7	8
Soybean protein, %	...	2.0	4.0	6.0	8.0	10.0	12.0	...
Corn gluten, %	...	...	...	...	...	...	...	19.5
Av. weight gain, grams	15	33	79	110	138	145	152	47
PER[c]	0.89	1.50	2.17	2.33	2.46	2.30	2.20	1.12
Soybean flour	...	1.0	3.0	5.0	7.0	9.0	11.0	...
Corn gluten	...	...	...	...	...	...	...	11.0
Av. weight gain, grams	12	22	36	64	79	113	125	35
PER[c]	0.82	1.19	1.54	1.96	2.09	2.37	2.42	0.73
Cottonseed flour, %	...	1.0	3.0	5.0	7.0	9.0	11.0	...
Corn gluten, %	...	...	...	...	...	...	...	11.0
Av. weight gain, grams	12	15	24	51	62	73	89	35
PER[c]	0.82	0.86	1.11	1.70	1.76	1.82	1.98	0.73
Pepitoria flour, %	...	1.0	3.0	5.0	7.0	9.0	11.0	
Av. weight gain, grams	14	19	30	48	51	70	88	
PER[c]	0.90	1.12	1.30	1.68	1.61	1.75	1.84	
Torula yeast, %	...	1.0	2.0	3.0	4.0	5.0	...	...
Corn gluten, %	...	...	...	...	...	...	2.7	4.4
Av. weight gain, grams	24	47	54	77	76	90	35	42
PER[c]	1.23	1.71	1.84	2.06	1.86	2.00	1.26	1.29

[a] Lime-treated corn flour in soybean protein, soybean flour, cottonseed flour, pepitoria flour, and torula yeast experiments: 79, 79, 79, 79, and 83%, respectively. [b] Average initial weight of rats in soybean protein, soybean flour, cottonseed flour, pepitoria flour, and torula yeast experiments: 53, 51, 51, 47, and 50 grams, respectively. [c] Protein efficiency ratio: weight gain/protein consumed.

group, the basal diet with 3.0 mg.% of riboflavin and 3.0 mg.% of thiamine; the fourth, the basal diet with the same quantities of riboflavin and thiamine as the third, plus 5.0 mg.% of niacin; and the fifth group, with 5 ml. of a complete vitamin solution (*12*) containing the same quantities of riboflavin, thiamine, and niacin as used in the previous diets. The animals were handled as described previously.

The second experiment with eight groups of six rats each studied a possible interaction between lysine and tryptophan addition and vitamin supplementation. Groups 1, 3, 5, and 7 were fed the diets with the vitamin supplements as described above and did not receive the amino acid supplements, while groups 2, 4, 6, and 8 received the diets with the same distribution of vitamins as used formerly plus 0.41% L-lysine HCl and 0.10% DL-tryptophan. The animals were handled as described previously, and at the end of 8 weeks they were sacrificed, and their serum protein concentration was determined (*11*).

Results

Chemical and Amino Acid Composition. Table I presents the chemical composition and the lysine, tryptophan, methionine, and isoleucine content of the principal ingredients of the diets. All the protein supplements were found to contain relatively large quantities of both protein and the four amino acids.

Enrichment of Lime-Treated Corn Flour with Animal Proteins. FISH FLOUR. This experiment employed 11 diets, five with a fish flour supplement, five containing various levels of corn gluten adjusted to provide the same quantity of protein as the fish flour supplements, and one served as the negative control. Table II describes in part the composition of the diets and presents the results of this experiment.

The average weight gain of the rats increased as the amount of fish flour increased in the diet; on the other hand, the weight gain of the rats fed the corn gluten supplements remained essentially the same, even though the total protein of the diet was increased. The addition of around 3% fish flour to the basal, lime-treated corn diet gave maximum protein efficiency ratio, while PER decreased with the addition of larger amounts of corn gluten.

The results of a second experiment with larger additions of fish flour are also shown in Table II. The findings are the same as described above. In a third experiment, tortillas were made with 3% fish flour and fed in a basal diet to rats. Baking fish flour-enriched, lime-treated corn did not reduce the nutritive value of the tortilla.

MEAT FLOUR. Table III describes the supplementation of lime-treated corn flour with meat flour. Of the eight

Table V. Essential Amino Acid Content of Lime-Treated Corn Supplemented with Optimum Quantities of Animal and Vegetable Protein Concentrates (Mg./Gram of N)

Amino Acid	Lime-Treated Corn	Fish Flour	Meat Flour	Whole Egg Flour	Casein	Skim Milk	Soybean Protein	Soybean Flour	Cottonseed Flour	Pepitoria Flour	Torula Yeast
Arginine	258	300	313	293	257	257	435	327	423	530	290
Histidine	154	142	178	153	168	162	174	152	158	152	156
Isoleucine	272	295	283	304	331	318	313	279	231	275	289
Leucine	608	584	572	595	617	628	586	563	519	558	589
Lysine	134	259	297	212	289	242	195	228	194	180	188
Methionine	127	139	134	149	139	139	105	113	119	128	122
Cystine	56	62	64	76	43	57	50	75	73	58	56
Phenylalanine	238	237	245	265	277	265	305	263	271	251	250
Tyrosine	244	226	232	250	291	262	249	228	217	220	258
Threonine	195	236	226	222	224	229	243	213	205	206	215
Tryptophan	24	33	43	39	45	43	48	42	43	47	33
Valine	301	306	318	337	364	348	355	310	304	391	316
Nitrogen, grams	1.130	1.494	1.807	1.461	1.843	1.538	2.196	1.747	1.798	1.964	1.429
PER	1.0	2.44	2.45	2.28	2.37	2.48	2.46	2.40	1.90	1.84	2.06
Optimum level, %	...	3	5	3	5	8	8	8	9	9	3

diets prepared, six received 1, 3, 5, 7, 9, and 11% of meat flour; one served as control, and the eighth was supplemented with corn gluten to provide the same quantity of protein as the 11% meat flour. The results, also shown in Table III, indicate that the weight gain of the rats increased with larger additions of meat flour. The addition of corn gluten increased growth, when compared with the growth resulting from the basal diet, but the weight gain was far below that resulting from the addition of 11% meat flour. The PER also increased with an increase in meat flour in the diet; and for practical purposes, maximum PER occurred from the addition of 5% meat flour.

WHOLE EGG FLOUR. As described in Table III, seven diets were prepared for this experiment, supplemented with 0, 1, 3, 5, 7, 9, and 11% of whole egg flour. The results, also shown in Table III, indicate that growth of the rats increased as the egg flour was increased. The PER also increased with maximum values occurring from the addition of 3% whole egg flour to the diet.

VITAMIN-FREE CASEIN. For this experiment, eight diets were prepared as described in Table III. Seven received 0, 1, 3, 5, 7, 9, and 11% casein, and the eighth was supplemented with 11% corn gluten providing the same quantity of protein as 7% casein.

Table III shows that increasing casein induced increased weight gain, except in the group fed the diet supplemented with 11% casein. The addition of 11% corn gluten equivalent to the 7% casein, resulted in weight gains a little more than double that of the control and not quite two-fifths that for the group fed the diet supplemented with 7% casein. The addition of 5% casein induced maximum PER, while higher additions tended to decrease it.

SKIM MILK POWDER. Table III also describes both the design and growth response of this experiment, which used six groups of rats fed diets supplemented with 0, 2, 4, 6, 8, and 10% skim milk powder. As in previous experiments, the average weight gain of the rats increased as larger quantities of skim milk powder were added to the diet. Protein efficiency also increased with maximum values resulting from the 8% skim milk supplementation.

Enrichment of Lime-Treated Corn Flour with Vegetable Proteins. SOYBEAN PROTEIN. Table IV describes the partial composition and the results obtained from the eight diets used. Seven were supplemented with 0, 2, 4, 6, 8, 10 and 12% soybean protein, and one was supplemented with 19.5% of corn gluten to provide the same amount of protein as 12% soybean. Weight gain of the rats increased as soybean protein increased in the diet. The growth obtained with 19.5% corn gluten was three times that of animals receiving the basal diet, and was slightly less than one-third that of the group fed 12% soybean protein. For this material maximum PER occurred from the addition of 8% soybean protein.

SOYBEAN FLOUR. As with soybean protein, eight groups of animals were employed to carry out this experiment. Table IV shows, too, the partial composition of the diets and the results obtained. Seven of the diets were supplemented with 0, 1, 3, 5, 7, 9, and 11% of soybean flour; the last contained 11% corn gluten. Weight gain of the rats increased with increases of soybean flour. The rats fed the corn gluten supplement gained about 3 times as much as the group fed the basal diet, but they gained about 3.5 times less than the animals fed the basal diet with 11% soybean flour. PER also increased in proportion to larger amounts of soybean flour, but the PER of the corn gluten-supplemented diet was lower than that of the basal diet.

COTTONSEED FLOUR. The supplemental concentrations of cottonseed flour added in this exepriment were 0, 1, 3, 5, 7, 9, and 11% of the diet as described in Table IV. As in previous cases, the last diet was supplemented with 11% corn gluten, contributing an amount of protein similar to that in 11% cottonseed flour. The results, shown in Table IV, indicate again that greater cottonseed flour additions gave larger weight gains. The responses to the corn gluten supplement were, as before, slightly higher when compared to those from the basal diet, and significantly lower than those from the cottonseed flour protein. The protein efficiency ratios also increased with increases in cottonseed flour, the maximum PER falling between 9 and 11%, as was the case with soybean flour; the weight gains, however, were slightly lower for cottonseed flour.

PEPITORIA (*Cucurbita farinosa*) FLOUR. Table IV also presents the partial composition of the diet and the results of the experiment. The concentrations of pepitoria flour were 0, 1, 3, 5, 7, 9, and 11%. Both weight and PER increased as the quantity of the supplement became greater. The maximum PER for this vegetable protein concentrate was close to 11%, but for practical purposes, the amount to be added to enrich lime-treated corn flour is around 9% of the diet. PER values for this experiment were lower than for those of the previous experiments.

TORULA YEAST. The lower section of Table IV describes the partial composition of the diets and the results obtained in this experiment with eight groups of rats. Six were fed the basal diet supplemented with 2.7 and 4.4% corn gluten, which provided protein equal to 3 and 5% of torula yeast. The best weight gains were obtained with 3% yeast, while larger additions did not result in greater weight gains. Corn gluten induced only a small weight increase when compared to that of the basal diet and less than that resulting

Table VI. Effect of Vitamin, Lysine, and Tryptophan Supplementation on the Nutritive Value of Lime-Treated Corn Flour

Ingredient	Av. Initial Weight, Grams	Av. Weight Gain, Grams	PER[a]	Av. Serum Protein, Grams/100 Ml.
Experiment No. 1[b]				
None	52	38	0.91	5.43
+ Riboflavin, 3 mg./100 grams	51	53	1.09	4.76
+ Riboflavin, 3 mg./100 grams, + thiamine, 3 mg./100 grams	52	50	1.00	5.12
+ Riboflavin, 3 mg./100 grams, + thiamine, 3 mg./100 grams, + niacin, 5 mg./100 grams	51	54	1.05	5.12
+ All B-complex[c]	51	65	1.13	4.96
Experiment No. 2[d]				
None	47	35	0.77	4.96
+ L-Lysine HCl (0.41%) + DL-tryptophan (0.10) (A)	46	70	1.62	5.58
+ Riboflavin, 3 mg./100 grams, + thiamine, 3 mg./100 grams (B)	46	51	1.30	4.62
+ A + B	46	136	2.28	5.62
+ Riboflavin, 3 mg./100 grams, + thiamine, 3 mg./100 grams, + niacin, 5 mg./100 grams (C)	46	40	1.09	4.90
+ A + C	46	173	2.41	6.24
+ All B-complex[c] (D)	46	54	1.16	5.04
+ A + D	46	162	2.37	5.93
Experiment No. 3				
None	52	78	...	5.48
+ A	53	124	...	5.98
+ Vitamin A	53	80	...	5.54
+ A + Vitamin A	52	149	...	6.17

[a] Protein efficiency ratio: weight gain/protein consumed.
[b] Percentage lime-treated corn flour: 90%.
[c] Reference No. 12.
[d] Percentage lime-treated corn flour: 89%.

when the 1% torula yeast supplement was fed. Protein efficiency ratios also increased with increase in torula yeast, with maximum values resulting from the addition of 3% yeast to the basal lime-treated corn diet.

Table V shows the essential amino acid composition of lime-treated corn supplemented with the optimum quantities found of both the animal and vegetable protein concentrates. The small additions of the supplements brought about a relatively large increase in the essential amino acid content of lime-treated corn.

Enrichment with Lysine, Tryptophan, and Vitamins. The upper part of Table VI describes the experimental design used to study the effect of vitamin supplementation of lime-treated corn and presents the results obtained. Judging from the weight gain of the rats, the riboflavin supplement improved the nutritive value of lime-treated corn most. Adding thiamine alone or thiamine and niacin combined to the riboflavin-enriched diet did not induce better growth; only the addition of all the vitamins produced a better response than did riboflavin alone. PER values showed that the vitamin supplements produced better protein utilization than the unsupplemented lime-treated corn flour. Serum protein values decreased with addition of all the vitamin supplements. Table VI shows the partial composition of the diets and the results of a second vitamin supplementation experiment in which the interaction between vitamin and lysine and tryptophan supplementation was studied. Addition of lysine and tryptophan, with and without vitamins, resulted, in every case, in greater growth and PER. The diet with thiamine and riboflavin gave better growth than the unsupplemented diet and growth equal to that of the diet with the complete vitamin supplement. The diet producing the best results was supplemented both with amino acids and the three vitamins. The vitamins and amino acids, added singly or together, improved the utilization of the protein based on the PER value, and tryptophan and lysine increased the total serum protein, which was still further increased by the addition of the vitamins.

Studies of supplementation with vitamin A, in the presence or absence of lysine and tryptophan, were also carried out. Partial composition of the diets and results after a 10-week period are shown in Table VI. Vitamin A added to lysine and tryptophan supplementation showed greater weight gain and increased total serum proteins.

Discussion

Although such nutrients as riboflavin and vitamin A are deficient, in many populations the most difficult of the nutritional deficiencies to solve is protein, because of both its quantitative and qualitative aspects. Since protein malnutrition is widespread and serious, it is important to find practical ways to improve the protein quality and quantity of the most largely consumed food which, in Central America, is corn (*7, 8*).

The results presented in this study indicate that the nutritive value of lime-treated corn can be improved significantly both in quantity and quality by adding small amounts of protein-rich materials of either animal or vegetable origin. In some instances, vegetable proteins were as effective as animal proteins, although in general the improvement in the protein quality of corn was higher with the animal products. The difference between the two types of protein supplements may be due to a lack of amino acid availability from the vegetable protein, either because its protein is less digestible or because the amino acids are bound and cannot be liberated by the digestive enzymes. Besides supplying the limiting amino acids in lime-treated corn, some of the supplements provide other nutrients. Among these, torula yeast is a good source of vitamins of the B-complex.

Improvement in the protein quality of lime-treated corn is secured by supplementing its limiting amino acids, lysine, tryptophan, and isoleucine (*1*). When fish flour was added, the resulting improvement was due to its contribution to the limiting amino acids in lime-treated corn, rather than to greater amounts of protein. The results from the corn gluten experiments clearly demonstrate the same point since the quality of corn gluten protein is similar to that of corn and also has the same amino acid deficiencies. Correlation coefficients were calculated between the amounts of amino acids, expressed as grams amino acid per gram of nitrogen and the PER at the point of maximum effect. These were for PER, lysine, tryptophan, isoleucine, and methionine 0.874, −0.044, 0.596, and 0.243, respectively. Figure 1 shows the relationships for lysine, indicating that the responses obtained from the supplementation are due mainly to this amino acid. Similar observations have been reported for the protein enrichment of bread (*13*). Although lime-treated corn is also limiting in tryptophan, the correlation between this amino acid and PER was negative, probably because all the protein supplements are relatively rich sources of tryptophan and the amount required for improvement for the level of protein is probably very small. When the PER values found in

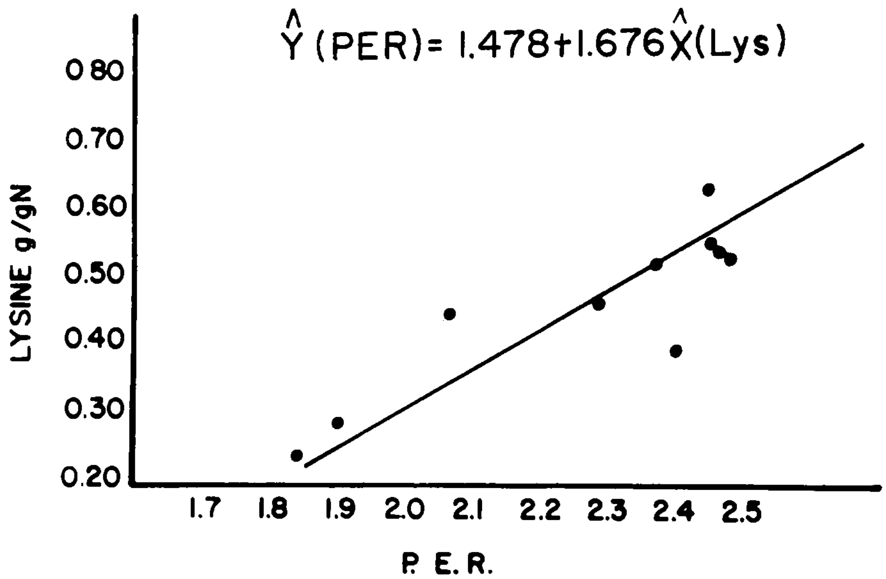

Figure 1. Relationship between PER and lysine

each experiment were plotted against the amounts of nitrogen, lysine, tryptophan, isoleucine, and methionine contributed by the supplement, curves were obtained which increase to a certain point and stay constant or decrease slightly with larger additions of supplement. From these curves, it was calculated that the greatest improvement in the efficiency of protein utilization of lime-treated corn can be obtained by adding a supplement providing approximately 0.6 gram of nitrogen, 0.25 gram of lysine, 0.045 gram of tryptophan, 0.20 gram of isoleucine, and 0.075 gram of methionine. This does not mean that an individual would not benefit from a greater proportion of supplement, but only that maximum quality of lime-treated corn protein is obtained from the addition of the above amino acid supplements.

The findings of vitamin enrichment are of interest. Of the vitamins tested, riboflavin gave the largest improvement in the nutritive value of lime-treated corn. These results corroborate the findings of Bressani *et al.* (*4*), who reported large losses of riboflavin during tortilla preparation. The addition of riboflavin in the presence of lysine and tryptophan improved the nutritive value of lime-treated corn significantly.

It is concluded from these results that all the supplements significantly improved the nutritive value of lime-treated corn. The results suggest that vitamins, at least riboflavin, must be added. It is suggested that the best supplement to achieve both protein and vitamin enrichment is torula yeast. Other protein supplements can also be used, provided the material is properly enriched with vitamins.

Acknowledgment

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