

LIME-HEAT EFFECTS ON CORN NUTRIENTS

Effect of Lime Treatment on in Vitro Availability of Essential Amino Acids and Solubility of Protein Fractions in Corn

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CORN consumed in the form of tortillas is the basic staple of diets in Mexico and Central America. In work carried out in Mexico, Tapia, Miranda, and Harris (30) reported that the protein of tortillas was inferior in quality to that of corn, and Massieu and associates (23) indicated that during tortilla preparation there are considerable losses of tryptophan, histidine, threonine, and arginine. Nevertheless, rats and pigs fed on tortillas or on alkali-treated corn grow better than when given raw corn (9, 12, 16, 18, 22, 27, 28). In view of the fairly high losses in niacin during the process of tortilla formation, Cravioto and coworkers (9) and Squibb and collaborators (28) postulated that this is because of changes in amino acid rather than niacin availability. Furthermore, Pearson and coworkers (27) cite evidence that the improved rat growth occurs when the corn is merely boiled, whether or not lime is added.

Evidence that cereal proteins may be altered by heat treatment has been reviewed and summarized by the National Research Council (25) and by Liener (19). Determinations of the solubility of the proteins and the in vitro release of essential amino acids provide information as to the nutritive value and amino acid availability of foods and help to supplement and explain the results of in vivo experiments. The present paper reports such studies after the combined lime and heat treatment on corn protein.

Materials and Methods

Samples of corn and tortillas were collected, during a nutritional survey in

Guatemala City, and air-dried before grinding to pass 40 mesh. Protein fractionation of corn and tortillas was carried out as indicated by Nagy, Weidlein, and Hixon (24). Protein nitrogen determinations were done by the micro-Kjeldahl method (7), α -amino nitrogen by the method of Van Slyke, MacFadyen, and Hamilton (37), and amino acid assays on acid, alkaline, and enzymatic hydrolyzates using media proposed by Steele *et al.* (29) and *Leuconostoc mesenteroides*, *Leuconostoc citrovorum*, and *Streptococcus faecalis*. The process of tortilla preparation as described by Bressani, Paz y Paz, and Scrimshaw (5) is summarized below.

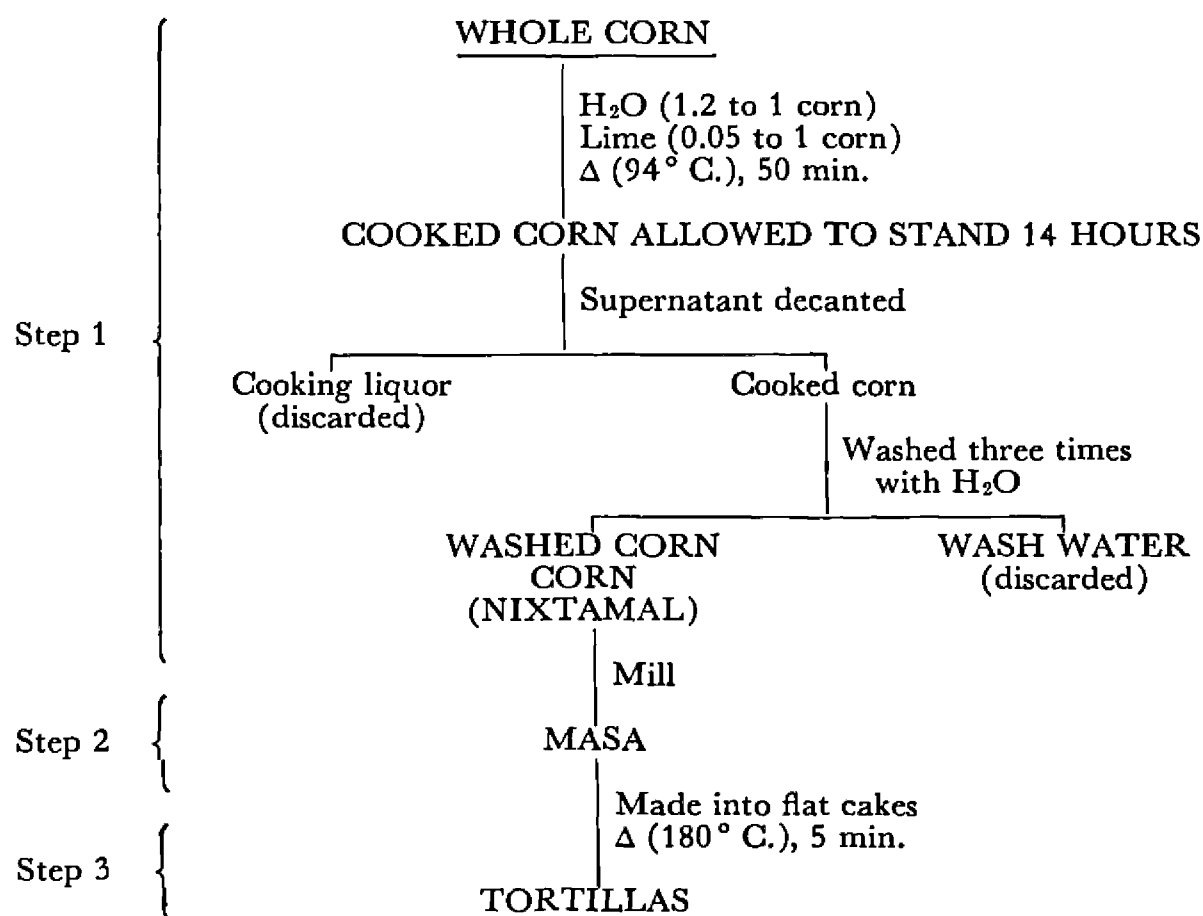
Results

Nitrogen Fractionation. The changes taking place in total nitrogen content during the process of preparing tortillas from corn are shown in Table I. There

Table I. Per Cent of Total Nitrogen at Each Stage of Tortilla Preparation^a

Type of Corn	Raw Corn	Masa	Tortilla	Cooking Liquor
Lowland	1.43	1.41	1.49	0.05
Highland	1.19	1.25	1.22	0.04

^a Values expressed on basis of raw corn moisture content of 12.85%.



Data show that the solubility of zein—alcohol-soluble fraction—is significantly decreased by the lime-heat treatment, which corn receives during the preparation of tortillas. Other nitrogen fractions also show a lower solubility but not to the same extent. The decreased solubility of zein should improve the biological value of the soluble proteins, as zein is the poorest of the corn proteins. The lime-heat treatment also increases the rate of release of most of the essential amino acids. These results suggest an explanation for the improved growth of rats fed tortillas as compared to corn.

Table II. Per Cent Protein Distribution of Corn and Tortillas

Solvent	Corn Number					Av.	Tortilla Number					Av.
	1	2	3	4	5		1	2	3	4	5	
Water	18.87	17.64	14.67	15.90	16.26	16.67	10.07	8.38	6.89	13.12	8.74	9.44
Sodium chloride	16.92	14.56	12.85	15.69	11.82	14.37	8.40	7.06	5.74	7.76	6.04	7.00
Alcohol	31.80	31.67	29.00	32.90	28.20	30.71	7.30	4.52	6.07	7.99	6.16	6.41
Sodium hydroxide	27.28	29.58	24.42	31.48	29.55	28.46	18.06	13.00	13.29	19.55	13.41	15.46
Insoluble	5.13	6.55	19.06	4.03	14.17	9.39	56.17	67.04	68.01	51.58	65.65	61.69
Total	100.00	100.00	100.00	100.00	100.00	...	100.00	100.00	100.00	100.00	100.00	...

is a slight increase in the percentage of nitrogen due to the loss of the seed coat of the grain. The loss of nitrogen in the cooking liquor is 3.4% of that originally present in the whole kernel and includes the nitrogen contained in the seed coat which is 2.9% of the total.

The results of the fractionation of the total nitrogen of corn and of tortillas are shown in Table II. The solubility of all nitrogen fractions of whole corn is significantly reduced during tortilla preparation. The reduction in solubility for the water-soluble fraction amounted to 43.4%, while that for the salt-soluble, alcohol-soluble, and sodium hydroxide-soluble fractions were 51.3, 79.1, and 45.7%, respectively.

The changes in the solubility characteristics of the nitrogen of corn in the

Table III. Changes in Solubility of the Nitrogen of Corn during Tortilla Preparation

Solvent	Soluble Nitrogen, %			Percentage Change	
	Raw corn 1 ^a	Masa 2 ^a	Tortilla 3 ^a	1-2 ^a	2-3 ^a
HIGHLAND CORN					
Water	19.42	7.28	11.56	62.5	...
Salt	17.43	5.17	5.78	70.3	...
Alcohol	30.03	17.14	9.86	42.9	42.5
Sodium hydroxide	31.36	29.55	27.88	5.8	5.7
Insoluble	1.76	40.86	44.92
LOWLAND CORN					
Water	15.26	6.47	5.34	57.6	17.5
Salt	11.30	8.16	8.09	27.8	0.9
Alcohol	22.02	16.12	10.23	26.8	36.5
Sodium hydroxide	27.91	23.80	23.87	14.7	...
Insoluble	23.51	45.45	52.47

^a Step in process of tortilla preparation.

Table IV. Amino Acid Content of Corn and Tortillas and Per Cent Change of Amino Acids

Amino Acid	Corn Number					Tortilla Number					Corn ^a	Tortilla ^b	Amino Acid, G./N.G.		Change, %
	1	2	3	4	5	1	2	3	4	5			Corn	Tortilla	
	G./100 G.														
Arginine	0.40	0.44	0.41	0.38	0.59	0.37	0.38	0.38	0.34	0.39	0.44	0.37	0.32	0.26	18.7
Histidine	0.20	0.23	0.21	0.22	0.28	0.20	0.20	0.21	0.21	0.28	0.23	0.22	0.17	0.15	11.7
Isoleucine	0.33	0.36	0.30	0.35	0.46	0.32	0.35	0.37	0.36	0.59	0.36	0.40	0.26	0.28	...
Leucine	1.09	1.18	0.94	1.16	0.88	0.79	0.80	0.89	0.90	0.98	1.05	0.87	0.76	0.60	21.0
Lysine	0.24	0.28	0.21	0.24	0.32	0.23	0.23	0.24	0.27	0.32	0.26	0.26	0.19	0.18	5.3
Methionine	0.16	0.18	0.16	0.16	0.17	0.16	0.18	0.18	0.16	0.17	0.17	0.17	0.12	0.12	...
Cystine	0.08	0.10	0.10	0.10	0.05	0.08	0.08	0.10	0.11	0.05	0.09	0.08	0.064	0.056	12.5
Phenylalanine	0.31	0.33	0.32	0.34	0.55	0.29	0.32	0.38	0.39	0.58	0.32	0.34	0.23	0.24	...
Tyrosine	0.34	0.39	0.30	0.34	0.16	0.33	0.32	0.39	0.37	0.17	0.34	0.35	0.24	0.24	...
Threonine	0.24	0.26	0.24	0.26	0.29	0.29	0.29	0.27	0.27	0.29	0.26	0.28	0.19	0.19	...
Tryptophan	0.042	0.053	0.040	0.044	0.035	0.050	0.051	0.049	0.052	0.038	0.043	0.048	0.031	0.033	...
Valine	0.36	0.38	0.34	0.38	0.49	0.37	0.41	0.41	0.46	0.52	0.39	0.43	0.28	0.30	...
Glutamic acid	...	1.74	1.56	1.92	1.86	...	1.66	1.53	1.86	1.82	1.77	1.72	1.27	1.19	6.3
Aspartic acid	...	0.58	0.44	0.56	0.62	...	0.52	0.54	0.55	0.61	0.55	0.56	0.39	0.39	...
Glycine	...	0.44	0.38	0.42	0.49	...	0.42	0.45	0.38	0.48	0.43	0.43	0.30	0.30	...
Alanine	...	0.73	0.67	0.82	0.80	...	0.77	0.75	0.85	0.81	0.76	0.80	0.55	0.55	...
Serine	...	0.42	0.32	0.44	0.38	...	0.38	0.34	0.41	0.39	0.39	0.38	0.28	0.26	7.1
Proline	...	0.94	0.88	1.07	0.96	...	0.87	0.92	0.94	0.92	0.96	0.91	0.69	0.63	8.7
Nitrogen, %	1.36	1.49	1.30	1.40	1.42	1.31	1.44	1.43	1.47	1.53	1.39	1.44
Moisture, %	12.0	9.0	13.7	13.6	12.9	14.7	14.6	16.8	14.3	11.5	12.2	14.4

^a Average values of 5 corns. ^b Average values of 5 tortillas.

process of tortilla preparation are shown in Table III and take place mainly in the preparation of the masa. The changes which result from preparation of the tortillas from the masa are small, except for the alcohol-soluble fraction, where 42.9% is lost in preparation of the masa, and 42.5% in the preparation of tortillas from the masa.

Amino Acid Composition. Five samples each of corn and tortilla were analyzed for 18 amino acids (Table IV). Differences among the five samples were not significant except for a higher value for phenylalanine and a lower one for tyrosine in corn 5.

The average amino acid content of corn and tortillas, expressed on the basis of grams of amino acid per gram of nitrogen, is shown in Table IV. In calculating the averages, the phenylalanine and tyrosine values of the samples from corn 5 were omitted. From these figures the average amino acid changes were then calculated. There was a 21.0% decrease for leucine, 18.7% for arginine, 12.5% for cystine, 11.7% for histidine, and 5.3% for lysine. Of the nonessential amino acids, changes were found in proline, serine, and glutamic acid, amounting respectively to a decrease of 8.7, 7.1, and 6.3%.

The amino acid pattern of both corn and tortillas, compared with the pattern of the provisional protein proposed by FAO (13) is shown in Figure 1. Despite the large losses of certain amino acids, the differences between corn and tortillas in milligrams of amino acid per gram of nitrogen are not significant, except for a slightly lower content of leucine in tortillas. The comparison does not provide an explanation of the biological differences between corn and tortillas encountered in in vivo experiments.

In Vitro Enzymatic Studies. Corn 2 and its corresponding tortillas were used for in vitro enzymatic studies. (Pepsin and the pancreatin were obtained from Merck & Co., Inc., and trypsin from the General Biochemicals, Inc.) A 4-gram sample of each material was hydrolyzed at pH 1 to 2 with 15 mg. of pepsin (potency 1 to 10,000) for 12 hours at 37° C. followed by the addition of 1.3 grams of potassium phosphate, adjustment of the pH to 8.4 with 1*N* sodium hydroxide, and subsequent hydrolysis with 15 mg. of trypsin (potency 1 to 300) for 24 hours. The pH was adjusted to 7.6 with 1*N* sodium hydroxide and the material was further hydrolyzed with 150 mg. of pancreatin for another 24 hours. Samples were obtained at 12, 24, 36, 48, and 60 hours of hydrolysis and assayed for total and α -amino nitrogen and the essential amino acids.

The total nitrogen and the α -amino nitrogen released during enzymatic digestion are shown in Table V. The results show that total nitrogen and α -amino

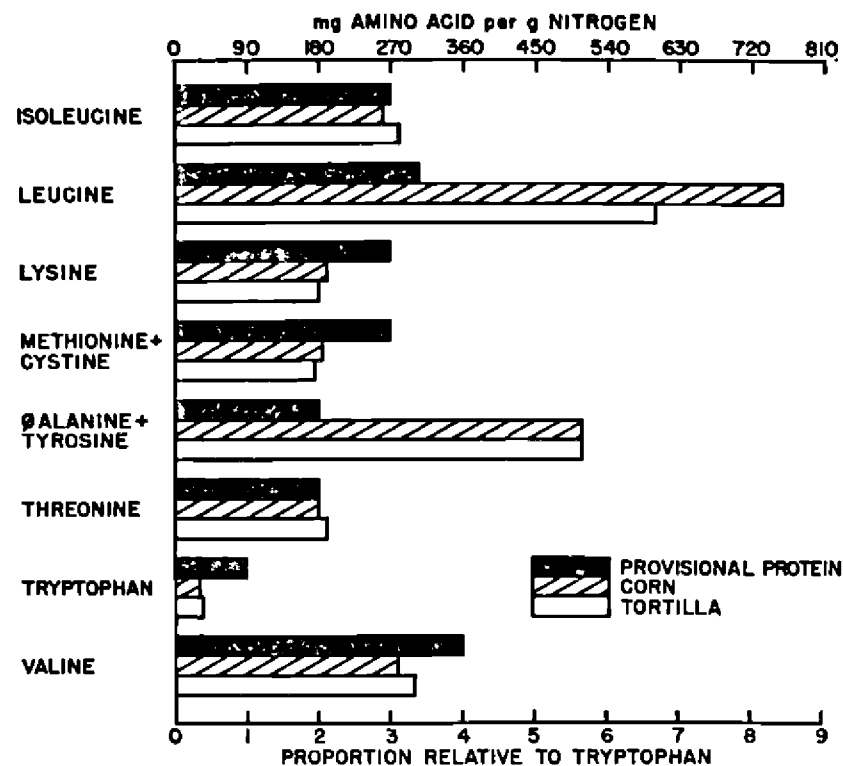


Figure 1. Amino acid pattern of provisional protein, corn, and tortilla

Table V. Total and α -Amino Nitrogen of Enzymatic Hydrolyzates of Corn and Tortillas

Time of Hydrolysis, Hr.	Mg. Total N/100 Ml. Extract		Mg. α -NH ₂ N/100 Ml. Extract		% α -NH ₂ N of Total	
	Corn	Tortilla	Corn	Tortilla	Corn	Tortilla
12	23.38	8.58	5.00	3.70	21.4	43.1
24	30.61	17.54	7.10	5.25	23.2	29.9
36	32.24	20.25	7.15	5.84	22.2	28.8
48	34.64	30.20	11.85	7.16	34.2	23.7
60	36.86	33.21	12.80	11.26	34.7	33.9

nitrogen were released at a faster rate from corn than from tortillas. However, total nitrogen released from the tortilla reached comparable levels to that of corn after 48 hours of hydrolysis. For α -amino nitrogen, the corn hydrolyzate showed consistently higher values, although the differences at the end of 60 hours are smaller. When the α -amino nitrogen is expressed as a per cent of the total nitrogen released, at the end of 12 hours, 43.1% of the nitrogen in the tortilla hydrolyzate is in the α -amino form compared with 21.4% for corn, but at subsequent periods the corresponding percentages are similar.

The percentage of each amino acid released during in vitro enzymatic digestion at 12, 36, and 60 hours of hydrolysis is shown in Table VI. The percentage of amino acid increased with time of hydrolysis. The rate of release of most of the amino acids assayed was higher in corn than in tortillas, except for histidine, isoleucine, lysine, and tryptophan, which were released at the same rate from both corn and tortilla. At the end of 60 hours of hydrolysis, there were comparable amounts of histidine, lysine, phenylalanine, tryptophan, and valine; only threonine was found in a significantly higher concentration in the tortilla hydrolyzate than in corn.

The amino acid release expressed as grams of amino acid per gram of nitrogen in the hydrolyzate is shown in Table VI. Expressed in this way, most of the amino acids were still released at a faster

rate from tortillas than from corn up to 36 hours of hydrolysis, with the exception of leucine, phenylalanine, tryptophan, and valine, which were released at about the same rate. At 60 hours of hydrolysis, the amino acid concentration between the two hydrolyzates reached comparable levels with the notable exception of methionine, which was released to a much greater extent from corn than from tortillas.

Discussion

Solubility characteristics have been used by several investigators to obtain a rapid evaluation of the nutritive value of cottonseed protein (6-8, 15, 20, 21, 26). In these studies, the authors found in most cases a fair, although incomplete correlation between protein quality and the solubility of the proteins in 0.02*N* sodium hydroxide solution. In the present study, zein, the principal protein of corn, proved to be proportionately much less soluble in 75% alcohol after heat and lime treatment, although all of the other fractions were affected in this way to a much lesser degree. Zein contains amino acid complexes, which are resistant to enzymatic hydrolysis (14), and it is a protein of very poor quality. Both the presence of resistant amino acid complexes and the decreased solubility of zein would be expected to result in the digestive enzymes in vivo acting preferentially on the remaining proteins of tortillas. Any decrease in

Table VI. Release of Amino Acids during Enzymatic Hydrolysis

Amino Acid	Sample	Hours			Enzyme 60-Hr. Hydrolysis	Hours			Acid Hydroly.	Enzyme 60-Hr. Hydrolysis
		12 ^a	36 ^b	60 ^c	Chem. Hydrolysis	12 ^a	36 ^b	60 ^c		Chem.
		G./100 g.			Amino acid, mg./N, g.			Hydrolysis		
Histidine	Corn	0.007	0.038	0.071	31	0.012	0.047	0.077	0.154	50
	Tortilla	0.006	0.027	0.066	33	0.028	0.053	0.079	0.139	57
Isoleucine	Corn	0.043	0.102	0.192	53	0.074	0.127	0.208	0.242	86
	Tortilla	0.034	0.100	0.149	43	0.158	0.198	0.180	0.243	74
Leucine	Corn	0.181	0.400	0.632	54	0.309	0.496	0.685	0.793	86
	Tortilla	0.077	0.264	0.533	67	0.358	0.522	0.642	0.556	115
Lysine	Corn	0.026	0.127	0.175	62	0.045	0.158	0.190	0.188	101
	Tortilla	0.027	0.136	0.171	74	0.126	0.268	0.206	0.160	129
Methionine	Corn	0.007	0.078	0.109	60	0.011	0.096	0.118	0.121	98
	Tortilla	0.005	0.025	0.045	25	0.023	0.050	0.055	0.125	44
Phenylalanine	Corn	0.043	0.250	0.280	85	0.074	0.310	0.304	0.221	138
	Tortilla	0.018	0.171	0.274	86	0.084	0.338	0.330	0.222	149
Threonine	Corn	0.085	0.153	0.188	72	0.145	0.189	0.204	0.175	117
	Tortilla	0.082	0.129	0.231	80	0.381	0.255	0.278	0.201	138
Tryptophan	Corn	0.004	0.029	0.038	72	0.007	0.036	0.041	0.036	114
	Tortilla	0.002	0.020	0.040	78	0.009	0.040	0.048	0.035	137
Valine	Corn	0.029	0.140	0.236	62	0.050	0.174	0.256	0.255	100
	Tortilla	0.011	0.085	0.207	50	0.051	0.168	0.249	0.285	87

^a Pepsin hydrolysis. ^b Trypsin hydrolysis. ^c Pancreatin hydrolysis.

the availability of the amino acids from the poorest of the corn proteins could in turn have the practical effect of improving the biological value of those actually released from corn by enzyme action.

The findings differed from those of Massieu *et al.* (23) in the lower losses of histidine and arginine and the lack of change with lime treatment in the amounts of tryptophan and threonine. On the other hand, an average of 21% of leucine was lost in the process, a change which should improve the biological value of tortilla protein by partially correcting the isoleucine to leucine disproportion (10, 11). In nitrogen balance measurements in children of the effect of amino acid supplementation of corn masa, correction of this disproportion is of major importance in improving nitrogen retention (4), along with restoring the deficiency of tryptophan and lysine. Zein is high in leucine (3, 11) and the more zein in the sample the greater the loss of this amino acid with lime treatment. The differences between the amino acid losses reported by Massieu *et al.* (23) and those encountered in this study are probably due to the minor differences in the method of tortilla preparation as well as to biochemical and physical differences among corn varieties.

The *in vitro* enzymatic digestion carried out with pepsin, trypsin, and pancreatin in sequence differs considerably from that *in vivo*, and the digestion periods are longer than those normally encountered in the intestine. Nevertheless, the clear indication that the release of amino acids is significantly

influenced by the lime treatment is of importance to populations which obtain up to three quarters of their total dietary protein from this source alone.

As shown in Table V, the corn contained considerably more extractable total nitrogen after 12 hours of digestion than did the tortilla, but twice as high a percentage of this nitrogen was in the form of α -amino nitrogen. These differences in total and α -amino nitrogen diminished with time and at the end of 60 hours of hydrolysis were nearly the same. As pepsin was the only enzyme applied before 12 hours, the protein of tortillas is probably more easily broken down to peptides than is that of corn.

More total nitrogen is released from corn than from tortillas, even though it appears to be incorporated in amino acid patterns, which are less favorable. The faster release of certain amino acids from tortillas than from corn gives a probable explanation of the better growth of tortilla-fed animals, although it has not been possible thus far to identify the specific amino acids responsible.

Isoleucine, a limiting amino acid for the growth of rats fed corn diets [Benton, Harper, and Elvehjem (2)] shows twice as fast an *in vitro* enzymatic release per gram of nitrogen from tortillas in the first 12 hours and 1.5 times after 36 hours as compared to corn. This more rapid release of isoleucine could also be of significance to *in vivo* studies by giving a more favorable isoleucine to leucine ratio.

Threonine, which in too high a proportion has been reported to interfere with the utilization of tryptophan and niacin

(17), is present in significantly higher proportion in tortillas at the stage of hydrolysis. Thus, although changes in threonine might have provided an explanation of the improved growth of rats fed tortillas, the present data do not support this possibility. Furthermore, Pearson (27) was unable to confirm it in rat growth studies in which various amounts of threonine were added to corn and tortilla diets.

Acknowledgment

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