Effect of Lime Treatment on the Availability of Calcium in Diets of Tortillas and Beans: Rat Growth and Balance Studies

SERGIO O. SERNA-SALDIVAR,[1] LLOYD W. ROONEY,[1] and LAWRENCE W. GREENE[2]

ABSTRACT

Quality protein maize (QPM), regular corn, and sorghum were lime-cooked (with CaO) and processed into tortillas. Weanling rats were fed raw grain or tortilla-based diets supplemented with dry, cooked pinto beans and a Ca-free or Ca-rich mineral premix. Processing raw grains into tortillas increased the Ca content from 11 to 163 mg/100 g. The raw grain and tortillas from QPM contained 53% more lysine than did raw grains and tortillas from regular corn and sorghum. Rats consuming Ca-supplemented QPM products consumed more feed and gained more weight but had feed conversion ratios similar to those of their counterparts fed regular corn and sorghum. Rats fed raw grains not supplemented with Ca consumed less feed, gained less weight, and had poorer feed conversion ratios than did their counterparts fed Ca-supplemented diets. The negative effect of the lack of Ca was more noticeable as the experiment progressed. The Ca from tortillas was highly available because rats fed tortilla apparently absorbed and retained more Ca than did rats fed unsupplemented raw grains. Rats fed tortilla diets supplemented with Ca had the poorest apparent P absorption and retention values.

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Corn in the form of tortillas is a staple food for large groups of people in Latin America. Likewise, tortilla-based products (i.e., tortilla chips and corn chips) are steadily increasing in popularity in the United States and the rest of the world. The process to convert corn into tortillas, called nixtamalization, starts when the grain is cooked in lime (CaO) to produce nixtamal. The moist nixtamal is stone-ground into a dough, or masa, that is further shaped into disks and baked on a hot griddle or gas-fired oven to produce tortillas (Serna-Saldivar et al 1990).

White sorghums bred for food usage are currently used to manufacture tortillas in Central America and Southern Mexico. Sorghum is an alternative to corn for tortillas when corn is not available due to a prolonged drought or other unfavorable agronomic conditions. Quality protein maize (QPM) is a high-lysine, high-tryptophan cultivar derived from opaque-2 corn. QPM varieties and hybrids are becoming more popular because of their higher yields and superior nutritional value. Raw QPM and tortillas

[1]Cereal Quality Laboratory, Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843.
[2]Department of Animal Science, Texas A&M University, College Station, TX 77843.

made from QPM have better nutritional value than products made from regular corn (Bressani et al 1968, Sproule et al 1987, Sullivan et al 1989).

The requirement for dietary calcium is one of the most difficult to meet in developing countries. This is mainly due to the scarcity and high price of dairy products, the low Ca content of cereals, and the higher incidence of lactose intolerance among the populations. The problem is accentuated further in infants and pregnant and lactating women who require Ca for bone tissue development and milk secretion. Ca absorption is enhanced by 1α,25-dihydroxycholecalciferol (vitamin D-3) and negatively affected by phytic acid, oxalates, and insoluble dietary fiber. Calcium availability is also greatly affected by the relative amounts of phosphorus (Kies 1985).

The consumption of tortillas in some parts of Mexico and Central America is estimated at 120 kg per capita per year (Paredes-Lopez and Saharopulos-Paredes 1983). Adults consuming these amounts of tortillas can meet approximately 37% of their total Ca requirement. In contrast, people consuming the unlimed cooked grain (as is generally the case in Africa and other parts of the world) meet less than 1% of their requirement. Braham and Bressani (1966) found that the bioavailability of Ca in tortillas was high and that the addition of L-lysine to the tortilla resulted in increased Ca absorption and retention. Recently, Poneros and Erdman (1988) confirmed the high bioavailability of Ca in tortillas fed to rats.

The objectives of this research were to study the bioavailability of calcium in tortillas from regular corn, QPM, and sorghum and to determine the effect of calcium supplementation on the growth of weanling rats fed raw grain and tortilla-based diets.

MATERIALS AND METHODS

Sources of Grain and Production of Tortillas

Two contrasting corns, a food-grade commercial hybrid (Asgrow 405 White) and a high-lysine, quality protein maize (Yellow QPM) were processed into tortillas in a pilot plant. Regular corn and QPM (22 kg) were cooked at boiling temperature in a steam kettle containing 66 L of water and 220 g of lime (CaO reagent powder). Cooking times were 40 and 30 min, respectively. Then, grains were steeped for 12 hr, washed with tap water to remove pericarp tissue, and stone-ground (stone grinder model CC, Casa Herrera, Los Angeles) into a moist dough, or masa (52% moisture). Masa was continuously sheeted, shaped into disks (36 g) and baked into tortillas in a triple-pass gas-fired oven (Superior model CO440). A white food-grade sorghum (Sureno) from Central America was processed similarly into tortillas. In that process, the cooking liquor contained 2.66 g of lime per liter, and optimum cooking and steeping times were 2 min and 5 hr, respectively.

Pinto beans were processed by soaking 12 kg of beans in 52 L of tap water, cooking for 13.5 min at 98°C with 240 g of salt, and steeping in the hot cooking solution for 6 hr.

The cooked beans and tortillas, with 68 and 42% moisture, respectively, were placed on trays and dried for 36 hr in a rotating oven set at 60°C. Products were hammer-milled to pass through a 2-mm screen. After a time for the moisture to equilibrate, samples were packaged in polyethylene bags and stored in a freezer (−10°C).

Experimental Treatments and Diet Formulation

The experiment consisted of 12 different treatments in a 3 × 2 × 2 factorial arrangement. Dietary treatments were formulated to contain the same amount of supplemented beans, vitamins, and corn oil (Table I). The sources of variation were types of cereal (regular corn, QPM, or sorghum), type of product (raw grain or tortilla), and mineral supplement (with or without Ca). The Ca-rich mineral supplement contained enough Ca to provide the amount required (600 mg/100 g of diet) for a growing rat (National Research Council 1972). Dry, cooked pinto beans were included in the diets to improve the nutritional status of the rats due to the upgraded protein quality of the beans and to assure some Ca intake for those rats fed the negative control diets of raw grains. Moreover, pinto beans were chosen because they are usually served with tortillas in Latin America and are considered one of the main protein sources.

Animals and Feeding Experiments

A total of 84 Sprague Dawley, male, albino, weanling rats (average initial weight of 64.6 g) were blocked by initial weight and randomly assigned to eight blocks of 12 animals each. Upon arrival, rats had a three-day cage-adaptation period in which a lab chow diet was fed. Rats were individually housed in stainless steel wire-bottomed cages under controlled environmental conditions (temperature of 21°C and alternating 12-hr periods of arti-

TABLE I
Chemical Composition of Experimental Diets[a,b]

Grain Type	Ca[c] Supplement	Moisture (%)	Protein (N × 6.25)	Fat (%)	Ash (%)	Lysine (mg/100 g)	Minerals (mg/100 g) Ca	P	Mg	Ca-P Ratio
Corn										
Grain	−	11.0	12.5	8.2	5.6	0.49	23	718	141	0.03
	+	11.2	12.6	8.1	4.9	0.49	581	664	154	0.88
Tortilla	−	11.5	12.7	6.1	5.3	0.48	124	677	141	0.18
	+	10.7	12.6	6.1	4.6	0.48	674	593	151	1.14
Quality protein maize										
Grain	−	11.4	12.5	7.8	5.8	0.59	32	733	151	0.04
	+	10.9	12.4	7.6	4.9	0.59	590	696	147	0.85
Tortilla	−	11.4	12.3	6.5	5.5	0.59	170	722	138	0.23
	+	11.4	12.3	6.4	4.9	0.59	729	620	149	1.18
Sorghum										
Grain	−	10.8	13.1	6.0	5.7	0.48	37	684	160	0.05
	+	10.9	12.4	7.6	4.9	0.59	597	649	175	0.92
Tortilla	−	11.4	12.3	6.5	5.5	0.59	144	710	168	0.20
	+	11.4	12.3	6.4	4.9	0.59	704	642	177	1.10

[a] Each diet consisted of 75% raw grain or tortilla (14.3% moisture basis), 17.5% dry cooked pinto beans, 3.5% Ca-rich or -free mineral supplement, 3.0% corn oil, and 1% vitamin premix. Vitamin premix (per kg of mixture): 600 mg thiamin hydrochloride, 600 mg riboflavin, 700 mg pyridoxine hydrochloride, 3 g nicotinic acid, 1.6 g D-calcium pantothenate, 200 mg folic acid, 20 mg D-biotin, 1 mg cyanocobalamin, 1.6 g retinyl palmitate, 20 mg DL-α-tocopherol acetate, 250 mg cholecalciferol, 5 mg menaguinone, and 972.9 g sucrose.

[b] All values are expressed on a dry matter basis.

[c] Plus = AIN mineral mixture 76 (g/kg of mixture): 500 dibasic calcium phosphate, 74 sodium chloride, 220 potassium citrate, 52 potassium sulfate, 24 magnesium oxide, 3.5 manganous carbonate, 6 ferric citrate, 1.6 zinc carbonate, 0.3 cupric carbonate, 0.01 potassium iodate, 0.01 sodium selenite, 0.55 chromium potassium sulfate, and 118 sucrose. Minus = calcium-free salt mixture (g/kg of mixture): 528.7 dibasic potassium phosphate, 231.3 sodium chloride, 103.1 monosodium phosphate, 81.9 magnesium sulfate, 45 ferric citrate, 7.4 manganese sulfate, 1.3 potassium iodide, 0.8 zinc chloride, 0.55 chromium potassium sulfate, 0.5 copper sulfate, and 0.01 sodium selenite.

ficial light and dark). Feed and water were provided ad libitum. Growth and balance experiments were conducted simultaneously. Cages for the balance study had the same dimensions as the growth cages but contained funnels to separately collect feces and urine. The growth trial was conducted for eight weeks, during which feed consumption and rat weight gains were monitored weekly. The balance experiment was divided into four biweekly periods in which the feed consumption and fecal and urine output of 24 rats (two rats per treatment) were obtained. Therefore, a total of eight observations per treatment were recorded. During the collection periods, feces and urine were collected daily for 10 consecutive days and stored in a freezer. One milliliter of HCl (1:1) was added daily to the urine collection containers to prevent nitrogen loss and microbial contamination.

Chemical Analyses

Samples of raw grains, tortillas, pinto beans, and formulated diets were analyzed for moisture, ash (AOAC 1984), ether extract (AOAC 1984), nitrogen (Technicon 1978), amino acids, and several minerals. The conversion factor of N × 6.25 was used to calculate crude protein values. Lysine and tryptophan levels were determined after acid (Spackman et al 1958) and alkaline (LaRue 1985) hydrolysis. Hydrolysates were analyzed via ion exchange chromatography in a Beckman 121M amino acid analyzer. Ca, P, Mg, Fe, Zn, and Cu were analyzed after digestion with nitric and perchloric acids. Minerals excluding P were quantified by atomic absorption spectrometry (Sandel 1950). For Ca and Mg analysis, samples were diluted with 2% lanthanum chloride solution. Phosphorus was determined after reaction with molybdate and 1-amino-2-napthol-4-sulfonic acid and quantified with a spectrophotometer (Fiske and Subarrow 1925).

Rat feces were lyophilized, weighed, ground, and analyzed for moisture and protein. The minerals Ca, P, and Mg were analyzed as explained above. Urine samples were filtered through ashless filter paper, diluted to 125 ml with deionized water, and analyzed for nitrogen, Ca, and P. Ca was estimated directly in an aliquot of the urine diluted with a lanthanum chloride solution. For urinary P analysis, an aliquot of the urine was first digested with hydrogen peroxide and perchloric acid and then was quantified as explained above.

Statistical Analyses

Growth and performance data were analyzed as a randomized complete block design using analysis of variance procedures with the SAS computer software (Freund and Littell 1986). Duncan's test was applied to determine statistical differences ($P < 0.05$) among treatment and block means.

RESULTS AND DISCUSSION

Chemical Composition of Raw Grains, Tortillas, and Beans

QPM products contained approximately 53% more lysine than regular corn and sorghum (Table II) and 68% more tryptophan than regular corn products. The increased lysine and tryptophan content is due to the opaque-2 gene that significantly increases albumins and globulins and decreases prolamins (Ortega et al 1986). As a result of these changes, the opaque-2 gene improves the essential amino acid composition, overall protein quality, and growth-promoting abilities (Bressani et al 1968; Graham et al 1980a,b; Sullivan et al 1989; Sproule et al 1987). Raw grains contained more fat than their corresponding tortillas but similar amounts of protein, lysine, and ash. This is in agreement with previous investigations (Serna-Saldivar et al 1987, 1988). As expected, raw grains contained very small quantities of Ca. Regular corn and sorghum contained the lowest and highest amounts of Ca, respectively (Table II). Commercial cereal grains are known to be low in Ca, and availability of the mineral is questionable due to the presence of phytic acid, oxalates, and fiber (Kies 1985). Processing raw grains into tortillas significantly increased the amounts of Ca, improved the Ca-P ratio, but did not significantly affect the amounts of Mg, Fe, Zn, and Cu. The regular corn tortilla contained the least amount of Ca, probably because the raw kernel had the hardest endosperm texture, which retarded CaO uptake during cooking and steeping. This occurred despite the fact that the cooking time of regular corn was prolonged to compensate for its harder endosperm texture. The amount of lime remaining in the grain is mainly the result of the concentration used during processing and the length of cooking and steeping. Grain hardness and degree of nixtamal washing are negatively related to the final amount of Ca in the nixtamal (Serna-Saldivar et al 1990). According to Gomez (1988), most of the Ca in the cooked nixtamal is retained by the germ.

Pinto beans contained an amount of Ca similar to that in the tortilla products. In addition, the cooked pinto beans contained at least twice as much protein and 2.7 and 1.7 times as much lysine as the QPM and the other two grains, respectively. Pinto beans also contained more ash, Fe, Zn, Cu, and P than the raw grains and their tortillas. Thus, pinto beans are an adequate vehicle to upgrade both the essential amino acid profile and the mineral composition of cereal-based diets.

Effect of Dietary Calcium, Tortilla Processing, and QPM on Rat Growth

Rats fed raw grains without supplemented Ca had the poorest performance (Table III) because their diet contained only 5% of the recommended Ca level (National Research Council 1972), which was most apparent during the second month of the experiment. Throughout this period, animals consumed less food, gained less weight, and had the worst feed efficiency ratios. The poor performance was a direct consequence of the significantly lower feed intake caused by the lack of dietary Ca. Animals fed tortillas without supplemented Ca performed better ($P < 0.05$) than those fed unsupplemented raw grain.

Rats fed QPM and sorghum tortillas without supplemented

TABLE II
Chemical Composition of Grains, Tortillas, and Pinto Beans[a]

Product Type	Protein (N × 6.25)	Fat (%)	Ash (%)	Amino Acids (g/16 g N) Lys	Trp	Ca[b]	P[b]	Ca-P Ratio	Mg[b]	Fe[c]	Zn[c]	Cu[c]
Corn												
Grain	10.86	5.63	1.47	2.76	0.53	1	263	0.00	116	43.9	31.8	15.3
Tortilla	10.88	3.23	1.49	2.57	0.55	138	272	0.51	125	39.4	35.9	14.1
Quality protein maize												
Grain	10.75	5.36	1.78	4.19	0.91	15	325	0.05	126	41.2	43.4	13.0
Tortilla	10.72	3.64	1.72	4.01	0.90	195	288	0.68	116	53.2	45.4	14.4
Sorghum												
Grain	11.70	2.78	1.58	2.31	0.84	18	260	0.07	150	43.0	24.9	11.3
Tortilla	11.79	1.85	1.81	2.29	0.84	157	284	0.55	165	64.5	28.4	14.2
Pinto beans												
Cooked	25.23	1.64	4.15	7.29	0.85	165	397	0.42	129	80.4	39.7	17.1

[a] Values are expressed on a dry matter basis.
[b] Milligrams per 100 g.
[c] Milligrams per kilogram.

Ca had feed consumption, weight gain, and feed conversion levels similar to those of their counterparts fed Ca-supplemented tortillas (Table III). This occurred despite the fact that the unsupplemented tortilla diets provided only 25% of the Ca required (National Research Council 1972) for a growing rat. Ca supplementation of regular corn tortillas improved weight gain, feed intake, and feed conversion because this tortilla contained the lowest amount of Ca.

In all treatments, the best feed conversion and growth rates were observed during the first month of the study. The food consumption and weight gains of rats fed Ca-supplemented QPM diets were similar to those of their counterparts fed the other Ca-supplemented diets. In previous investigations, QPM diets produced better weight gains, feed conversions, and protein efficiency ratios than did regular diets (Bressani et al 1968, Sproule et al 1987). In this experiment, supplementation of the diets with 17.5% pinto beans obscured the effect of the enhanced protein quality of QPM. This is because the effect of a higher lysine content in the diet diminished as the level approached the amount required by the animal. Bressani et al (1968) reported better nitrogen retentions and rat growth rates when regular and opaque-2 tortillas were supplemented with beans. Opaque-2 products required reduced levels of beans to reach the optimum dietary lysine level and to achieve the same rat growth performance.

Rats consuming Ca-supplemented corn grains had feed conversion ratios similar to those of rats fed the corresponding Ca-supplemented tortillas (Table III). Rats fed sorghum tortillas had a significantly lower feed conversion ratio than their counterparts fed raw grains. Previous research (Chu et al 1976, Sanderson et al 1978, Serna-Saldivar et al 1987, 1988) has demonstrated that lime or alkali cooking for tortilla production slightly lowers protein digestibility, nitrogen retention, and lysine availability and, thus, the rate of growth. Rats fed sorghum grain and tortillas supplemented with Ca consumed more feed and gained more weight than rats fed regular corn products but had similar feed conversion ratios. In other experiments (Serna-Saldivar et al 1987, 1988), rats fed regular corn grain and tortillas digested the protein better and gained slightly more weight than rats fed sorghum. Sorghum grain and tortillas contained more Ca than the respective corn products (Table II). This could explain the higher feed intake and weight gains in rats consuming sorghum diets (Table III)

TABLE III
Effect of Tortilla Processing and Calcium Supplementation on Growth of Weanling Rats Fed Regular Corn, Quality Protein Maize, and Sorghum for 56 Days[a]

Diet	Ca[b] Supplement	Weight Gain[c] (g)	Feed Consumed (g)	Feed Conversion (g gain/100 g of food)
Corn				
Grain	−	135 f	812 f	16.6 e
	+	212 ab	942 cd	22.6 ab
Tortilla	−	167 de	826 f	20.5 cd
	+	211 ab	894 de	23.6 a
Quality protein maize				
Grain	−	149 ef	845 ef	17.6 e
	+	232 a	1,043 a	22.1 abc
Tortilla	−	207 abc	948 cd	21.8 bc
	+	217 a	976 bc	22.2 abc
Sorghum				
Grain	−	166 de	911 de	18.1 e
	+	224 a	1,023 ab	21.9 abc
Tortilla	−	188 cde	942 cd	20.0 d
	+	184 cde	929 de	19.8 d

[a] Means with different letters within a column are statistically different at $P < 0.05$ level. Average of seven observations.
[b] Plus = calcium-rich supplement; minus = calcium-free mineral supplement.
[c] The average initial rat weight was 64.6 g.

Effect of Tortilla Processing and Ca Supplementation on Ca and P Balance

Rats consuming tortillas without supplemented Ca absorbed and retained at least three times more Ca than rats fed unsupplemented raw grains (Table IV). Animals fed QPM tortillas apparently absorbed and retained more Ca, followed by rats fed sorghum and regular corn tortillas. The better apparent Ca retention of rats fed QPM tortillas was mainly due to their significantly higher feed intake. Rats fed Ca-supplemented regular corn, QPM grain, and sorghum grain diets absorbed 7, 12.9, and 11 times as much Ca as rats fed the unsupplemented diets, respectively (data calculated from Table IV). The effect of Ca supplementation on regular corn, QPM, and sorghum tortilla diets was less pronounced because rats absorbed 2.6, 1.9, and 2.0 times as much Ca as their unsupplemented counterparts, respectively (data calculated from Table IV). Interestingly, a negative relationship was found between dietary Ca intake and the percent of Ca absorbed as a percent of intake (Table IV). Around 70% of the Ca present in the unsupplemented raw grain diets was absorbed, whereas rats fed the Ca-supplemented diets absorbed approximately 43% of the total Ca. A similar effect was observed when Ca-supplemented and -unsupplemented tortilla diets were compared. These data confirm that the Ca from tortillas is highly absorbable (around 85%), as previously indicated by Braham and Bressani (1966), and that its absorption greatly depends on dietary levels (Boyle et al 1972, Rivovich and DeLuca 1978, Isselbacher 1981).

Hypocalcemic rats absorbed more P (net amount and as percent absorbed per intake) than rats fed optimum Ca levels (Table V). This occurred despite the fact that all treatments contained similar amounts of P, which exceeded the National Research Council's

TABLE IV
Effect of Tortilla Processing and Calcium Supplementation on Calcium Balance[a]

Diet Type	Ca[b] Supplement	Calcium Intake (mg/day)	Absorbed (Percent of Intake)	Retained (Percent of Intake)	Retained (Percent of Absorbed)
Corn					
Grain	−	1.73 e	63.2 d	59.0 d	93.8 f
	+	47.53 c	42.0 e	40.1 e	95.5 c–f
Tortilla	−	9.56 ed	86.7 a	86.7 a	97.7 ab
	+	60.83 b	42.6 e	41.2 e	96.8 a–d
Quality protein maize					
Grain	−	2.72 e	75.0 bc	75.0 bc	97.3 a–c
	+	62.45 b	44.3 e	42.5 e	96.0 b–e
Tortilla	−	16.08 d	91.9 a	89.4 a	98.3 a
	+	71.29 a	38.7 e	36.3 e	94.8 d–f
Sorghum					
Grain	−	3.43 e	72.2 cd	69.4 cd	96.8 a–d
	+	62.55 b	43.4 e	41.4 e	96.0 b–e
Tortilla	−	13.57 d	84.3 ab	81.3 ab	97.3 a–c
	+	63.64 ab	35.1 e	33.0 e	94.1 ef

[a] Means with different letters within a column are statistically different at $P < 0.05$ level.
[b] Plus = calcium-rich supplement; minus = calcium-free mineral supplement.

TABLE V
Effect of Tortilla Processing and Calcium Supplementation on Phosphorus Balance[a]

Diet Type	Ca[b] Supplement	Phosphorus Intake (mg/day)	Absorbed (Percent of Intake)	Retained (Percent of Intake)	Retained (Percent of Absorbed)
Corn					
Grain	−	63.81 a	80.9 ab	21.9 bc	27.0 c
	+	62.06 a	53.3 c	16.9 c	31.6 bc
Tortilla	−	64.22 a	79.1 ab	32.0 a	40.4 a
	+	53.60 a	38.1 de	5.5 d	14.4 g
Quality protein maize					
Grain	−	63.51 a	87.6 a	16.1 c	18.4 f
	+	72.70 a	55.1 c	18.7 c	34.1 b
Tortilla	−	64.80 a	76.7 b	22.3 bc	29.3 c
	+	63.00 a	35.2 e	6.9 d	19.7 ef
Sorghum					
Grain	−	66.60 a	85.2 ab	21.3 b	26.3 cd
	+	67.90 a	46.1 cd	10.4 d	22.9 de
Tortilla	−	66.80 a	81.9 ab	25.4 b	31.2 bc
	+	58.10 a	35.0 e	6.7 d	20.4 ef

[a] Means with different letters within a column are statistically different at $P < 0.05$ level.
[b] Plus = calcium-rich supplement; minus = calcium-free mineral supplement.

(1972) recommended level. P absorption was affected by the relative amounts of dietary Ca. Peterlik et al (1981) explains that inorganic P absorption is highly dependent on vitamin D-3 but proceeds independently of Ca absorption. The sterol hormone raises the V_{max} approximately 2.5-fold, with the corresponding stimulation of P transport across epithelial cells. Ca-deficient rats fed raw grains or tortillas also retained more P than counterparts fed adequate Ca levels. The difference was more accentuated when tortilla diets were compared. Interestingly, rats fed Ca-supplemented tortilla diets retained the least amounts of P.

Results of this study suggest that the Ca from tortillas is highly available to the growing rat and that dietary Ca levels greatly affect Ca and P absorption. The lime cooking process for tortilla production considerably upgrades the Ca content of the product. Tortillas could be regarded as a good, inexpensive source of Ca, especially for people who depend on this product as their main food source.

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