Effects of Fortification and Enrichment of Maize Tortillas on Growth and Brain Development of Rats Throughout Two Generations

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ABSTRACT

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The growth and brain development of laboratory rats fed typical indigenous tortilla-based diets were determined throughout two generations. The experiment compared three different types of tortilla-based diets: regular tortillas produced from dry masa flour (RDMF), tortillas obtained from fresh masa (FM), and tortillas produced from dry masa flour fortified with 6% defatted soybean and enriched with vitamins B_1, B_2, niacin, and folic acid and microminerals iron and zinc (FEDMF). Female rats were mated 58 days postweaning with males belonging to the same treatment with the objective of obtaining second generation pups that were further subjected to regular lactation and 28 day postweaned growth. A comparison between growth of first and second generation rats was determined. In addition, representative animals of each physiological stage were first exsanguineted for hematocrit determination and then slaughtered with the aim of obtaining femur and brain tissues. Cerebral DNA and number of neurons were determined in each of the brains sampled. Growth of rats fed FEDMF was significantly higher ($P < 0.05$) in both generations than counterparts fed RDMF or FM. The difference among treatments was more evident in second generation rats. Pregnancy rate, number of newborns per litter, litter weight, and pup's survival rate was higher for the control and FEDMF treatments. Femur growth was also higher ($P < 0.05$) for first-generation male adult rats fed control and FEDMF. The concentration and total content of cerebral DNA and number of neurons in males and females belonging to the first generation was similar. However, for second generation rats, these values were lower for animals fed regular tortilla diets. This data clearly demonstrates that the negative effects of malnutrition on brain development of pups occurred during gestation and lactation.

Hunger and malnutrition in developing countries are the most severe problems facing mankind (WHO 1998). *Kwashiorkor*, *Marasmus* and other malnutrition problems inhibit growth and weaken human resources and, in some way, affects at least 1 billion of the 5.98 billion inhabitants of the world (Onuma Okezie 1998; FAO 2001). The most severe and widely spread forms of malnutrition are protein and energy followed by lack of some micronutrients. Malnutrition during the perinatal and postnatal periods causes an important loss in growth and development that affects other physiological stages during the life cycle. In addition, these people are more prone to infectious diseases and death at an early age (Clydesdale and Weimer 1985). Pregnant and lactating females are also strongly affected due to lack of nutrients. During these physiological stages, protein, vitamin and mineral requirements increased significantly. Newborns of these affected mothers have low birth weight and higher incidences of disease and death (ACC/SCN 1992).

Even though world food production has increased at a faster rate than population, the malnutrition problem persists mainly in developing countries. More than 800 million people, including 200 million children, are subchronically malnourished. Furthermore, malnutrition causes the death of 6.6 million preschool children per year (WHO 1998). Malnutrition is a problem that affects inhabitants of rural and marginal areas of Mexico. Data provided by UNICEF indicates that ≈43% of the Mexicans suffer from malnutrition. The most deficient nutrients are protein, iron, vitamin A, and some B vitamins (Chavez et al 1993). Data from the Secretaría de Salud (personal communication) indicate that 2.4 million Mexican children have severe growth retardation, with 85% of these children living in the central and southern regions of the country.

Mexico, with an estimated population of 97.4 million people in 1999 (FAO 2001) has the highest world per capita consumption of maize (*Zea mays* L). The main food product from maize is tortilla. Tortillas are produced from fresh masa or from nixtamalized dry masa flour. More than 2.7 million tons per year of dry masa flour are produced industrially in Mexico. This output produces 4.4 million tons of tortillas (46.7 kg of tortillas produced from dry masa flour per capita) which represents ≈25% of the tortillas consumed throughout Mexico. The lower the socioeconomic status, the greater the dependence on tortillas.

Tortilla per capita consumption in some groups is higher than 120 kg per year. In rural areas, maize provides ≈70% of the calories and 50% of the daily protein intake (Serna Saldivar et al 1988). Tortillas are considered an excellent source of calories due to their high starch content. In addition, they are a good source of calcium due to the lime added for cooking. Studies conducted with laboratory rats indicate that the calcium provided by the lime is highly available (Serna Saldivar et al 1991, 1992). Lime cooking also increases the bioavailability of niacin, one of the most important B vitamins (Serna Saldivar et al 1987, 1988, 1990). Unfortunately, tortillas are not a perfect food because they lack good quality protein and adequate levels of iron, zinc and vitamins A, D, E and B_{12}. From a practical viewpoint, the consumption of tortillas without high quality protein foods produces *Kwashiorkor* in infants. This is due to the lack of two essential amino acids, lysine and tryptophan. Supplementation of maize tortillas with beans or other legumes or animal products is the best alternative to alleviate protein malnutrition. Unfortunately, some groups of people do not have funds to purchase these products.

In developing countries, enrichment and fortification of staple foods are the most effective ways to upgrade the nutritional status of the population (PAHO 1997). Due to high consumption, maize tortillas can be utilized as a vehicle to diminish protein malnutrition and deficiency of other important nutrients. Their nutritional quality is improved by protein fortification, utilizing quality protein maize (Sproule et al 1988; Serna Saldivar et al 1990) or adding soybean products and enriching with selected vitamins and minerals (Muñoz de Chavez and Chavez 1997).

In Mexico, the dry masa flour and wheat milling industries signed a federal agreement to enrich flours with 5 mg/kg of B_1, 3.0 mg/kg of B_2, 35 mg/kg of niacin, and 0.5 mg/kg of folic acid, 30 mg/kg of iron, and 20 mg/kg of zinc starting in 1999. The dry masa flour industry also lunched an enriched flour fortified with soybean meal to reduce protein malnutrition. This flour is being targeted to marginal and low economic groups by some state governments.

The objective of this research was to evaluate growth, physiological development and cerebral development throughout two generations of laboratory rats fed with tortillas produced from a commercial enriched and soybean fortified nixtamalized flour.

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MATERIALS AND METHODS

Diet Preparation

Rats were fed a typical diet consumed by Otomi Indians, an indigenous group that inhabits the central part of Mexico. Types and quantities of foods consumed by this indigenous group were provided by Instituto Nacional de Nutrición Salvador Zubirán of Mexico (Muñoz de Chavez and Chavez 1997). Rat diets were elaborated using the same foods, but ingredients were dehydrated and ground before diet mixing and added according to average dry matter composition (Table I). The source of variation among experimental diets was the type of tortilla that constituted 73.6% of the total diet. Tortillas from dry masa flours were made using conventional procedures in which the flour was hydrated with 1.2 L of water to form a dough that was formed into disks for baking in a three-tier gas-fired oven. Tortillas were baked for ≈50 sec at an average temperature of 290°C. For the production of fresh masa tortillas, 30 kg of masa were purchased from a commercial tortilla factory. The masa was processed through the same equipment used to produce the other tortillas. Resulting tortillas were dried in a convection oven set at 50°C for 8 hr, then ground in a Wiley mill equipped with a 2-mm screen. Dry ingredients of the diets were first blended for 3 min in a Hobart mixer equipped with a paddle attachment. The cooking oil and melted shortening were then added, and the resulting mixture was blended for 5 min. Powdered diets were fed to animals during growth; pelleted diets were fed during mating, pregnancy, and lactation periods. Pellets were manufactured by mixing 10 g of bentonite and 142 mL of water per kg of powdered diet. The mixture was extruded through a 2-cm diameter die using a meat stuffer (Vemag, model 500, type128/90, Verden Germany). The extrudate was manually cut into pieces ≈3 cm long and dried at 50–60°C for 8 hr.

TABLE I
Composition of Experimental Diets Used During Rat Bioassays[a,b]

Foods	(%)
Maize tortillas	73.60
Beans	3.46
Cactus	2.09
Pasta	1.10
Bread	1.36
Potatoes	0.24
Rice	0.08
Milk	1.12
Meat	1.13
Egg	0.61
Tomato	0.26
Pepper	0.39
Other vegetables[c]	0.14
Fruits[d]	0.11
Oil and lard[e]	2.86
Sugar	11.45
Total	100.0

[a] All products were dehydrated before diet mixing. Quantities are expressed on dry matter basis.
[b] Typical Otomi diet. Data provided by Muñoz de Chavez and Chavez (1997). Source of variation among diets was the type of tortilla.
[c] Onion and carrot (1:1).
[d] Banana and squash (1:1).
[e] Vegetable oil and lard (2:1).

The control casein based diet supplemented with vitamin and mineral mixes was formulated to contain 10% (w/w) crude protein according to standard PER procedures (AOAC 1990).

Treatments

Four different diets were tested, three consisted of the experimental diets based on tortillas from regular dry masa flour (RDMF), fresh masa (FM), and fortified and enriched dry masa flour (FEDMF). The other treatment consisted of the control group fed a balanced casein based diet (AOAC 1990) that contained all necessary nutrients for optimum rat growth.

Chemical Composition

The proximate composition of formulated diets were determined according to established procedures (AOAC 1990). Nitrogen-free extract was determined as NFE_{db} (%) = 100 – % protein – % ether extract – % ash – % crude fiber, expressed on dry matter basis. Digestible energy was calculated based on the amounts of NFE, protein, and ether extract using the equation DE = (% NFE × 4 kcal/g) + (% protein × 4 kcal/g) + (% ether extract × 9 kcal/g).

TABLE II
Chemical Composition and Digestible Energy of Tortilla-Based Diets[a]

Diet[b]	Moisture (%)	Ash (%)	Protein (%)	Ether Extract (%)	Crude Fiber (%)	NFE[c] (%)	DE[d] (kcal/100g)
RDMF	6.26	1.71	8.94	2.80	1.24	85.31	377.2
FM	6.72	2.22	9.24	2.85	1.48	84.21	372.6
FEDMF	7.19	1.96	10.56	3.90	1.22	82.36	378.0
Control[e]	9.85	4.09	10.06	5.23	0.21	80.41	369.6

[a] Values are means of two observations and expressed on dry matter basis.
[b] RDMF = tortilla-based diet from regular dry masa flour; FM = tortilla-based diet from fresh masa; FEDMF = tortilla-based diet from protein fortified and enriched dry masa flour; control = casein-based diet.
[c] NFE = nitrogen free extract.
[d] DE (digestible energy) = (%NFE × 4 kcal/g) + (%protein × 4 kcal/g) + (%ether extract × 9 kcal/g).
[e] Control diet consisted of 13.26% casein (AIN Purified High Nitrogen), 1% cellulose (Alphacel, ICN Biomedicals), 1% vitamin mixture (AIN 76, ICN Biomedicals), 4% mineral mixture (AIN 76, ICN Biomedicals), 52.3% corn starch, 10% sucrose, 14.75% vegetable oil and 3.71% water.

TABLE III
Essential Amino Acid Composition of Tortilla-Based Diets[a]

	His	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Trp	Val	EAA Score[b] (%)
Standard[c]	1.9	2.8	6.6	5.8	2.5	6.3	3.4	1.1	3.5	100.0
Diets[d]										
RDMF	2.55	3.47	10.82	3.27	3.47	6.94	3.27	0.71	4.69	56.3
FM	2.12	3.01	9.55	3.10	2.92	6.28	2.92	0.62	3.98	53.4
FEDMF	2.65	3.88	10.98	4.36	3.69	7.86	3.60	0.85	4.92	75.1
Control	2.74	4.68	9.76	7.98	2.98	8.95	3.55	1.13	6.37	100.0

[a] Amino acids values expressed as g of AA/100 g of protein (dry basis).
[b] Requirements for a 2-year-old child (FAO/WHO 1990).
[c] EAA (essential amino acid) score = limiting EAA / requirement FAO/WHO.
[d] RDMF = tortilla-based diet from regular dry masa flour; FM = tortilla-based diet from fresh masa; FEDMF = tortilla-based diet from protein fortified and enriched dry masa flour; control = casein-based diet.

Amino Acid, Vitamins and Mineral Composition

Representative samples were sent for amino acid and vitamin B_1, B_2, niacin, folic acid, iron, and zinc analysis (Ralston Analytical Laboratories, St. Louis, MO).

Rat Bioassays

For the experiments, 32 Winstar rats (ITESM, Monterrey N.L., Mexico), 24 females and 8 males, 25 days old with an average initial body weight of 50.8 g, were blocked by initial weight and randomly assigned to eight blocks. Six blocks housed female rats and the remaining two males. During growth, rats were housed in individual stainless steel cages at controlled environmental conditions (20–22°C with alternating 12-hr periods of light and darkness). Food and water were provided ad libitum. The first stage of the experiment was a growth study conducted for 58 days. Weight gains and feed conversion were calculated after 28 and 58 days. At the end of the growth study, three females with one male belonging to the same treatment were housed in collective cages and fed pellets. The rats were identified with ear notches. Female animals were allowed to mate with the first male for nine days and nine additional days with the second male. Females were separated from the males, who were immediately anesthetized in a closed container containing chloroform. Blood was sampled by intracardiac puncture for determination of EDTA capillary hematocrit and to obtain serum. For serum, blood was centrifuged for 5–7 min at 3,500 rpm. Immediately afterward, the rats were terminated; brain and femurs were surgically removed. Brains were weighed and sagittaly cut to separate both cerebral hemispheres.

Pregnant rats were individually housed in maternal acrylic cages and fed pellets ad libitum. The number of pups born alive and total weight of the litters were registered after parturition. Newborns were allowed to lactate for 28 ± 1 days. At the end of lactation, the number of males and females were determined and rats were individually weighed. Two rats (one male and one female) with the average litter weight were fed powdered diets for 28 days. The rest of the rats were anesthetized, sampled, and terminated.

Cerebral DNA Determination

Cerebral DNA was extracted, purified, and quantified according to the procedure suggested by Persing et al (1993). DNA was quantified in a UV spectrophotometer calibrated at 260 nm. DNA concentration (µg/µL) was calculated as $\text{DNA} = (A_{260})\times(200)\times(50)/1{,}000 = (A_{260})\times 10$. Number of neuron cells was estimated after determining DNA concentration as number of neuron cells = µg of $\text{DNA}/6.4 \times 10^{-12}$.

Femur Analysis

Femurs were cleaned to remove muscle and related tissues before measuring their weight, length, and thickness. The force required to break one of the femurs was determined with a texture analyzer (TA.XT2, Texture Technologies Corp., Scarsdale, NY). A cylindrical attachment was used to break the medial part of the bone, which

TABLE IV
Vitamin and Mineral Composition of Tortilla-Based Diets[a]

	Ca (mg)	Fe (mg)	Zn (mg)	Thiamin or B_1 (mg)	Riboflavin or B_2 (mg)	Niacin (mg)	Folic Acid (µg)
Requirement[b]	800	15	10.0	0.7	0.8	7.0	38.0
Diets[c]							
RDMF	122	10.2	6.4	0.95	0.33	5.17	9.2
FM	168	9.4	5.0	1.33	0.33	7.01	10.0
FEDMF	137	16.6	9.0	2.82	0.75	14.87	29.0
Control	465	21.6	16.2	1.73	3.96	10.07	74.5

[a] Values for 100 g expressed on dry matter basis.
[b] Requirements for 1–3 year old child (Recommended Dietary Allowances 1989).
[c] RDMF = tortilla-based diet from regular dry masa flour; FM = tortilla-based diet from fresh masa; FEDMF = tortilla-based diet from protein fortified and enriched dry masa flour; control = casein-based diet.

TABLE V
Effect of Tortilla Protein Fortification and Enrichment on Growth of First Generation Postweaned Rats[a]

Diets[b]	Initial Wt[c] (g)	Final Wt[d] (g)	ΔWt[e] (g)	FI[f] (g)	PI[g] (g)	ΔWt/PI
RDMF	50.75a	78.88b	28.13b	259.04a	23.16b	1.46b
FM	50.25a	81.38b	31.13b	261.74a	24.18ab	1.59b
FEDMF	51.25a	105.75a	54.50a	276.00a	29.15ab	2.05ab
Control	51.13a	112.38a	61.25a	298.26a	30.01a	2.34a

[a] Means with different letters within column were statistically different ($P < 0.05$).
[b] RDMF = tortilla-based diet from regular dry masa flour; FM = tortilla-based diet from fresh masa; FEDMF = tortilla-based diet from protein fortified and enriched dry masa flour; control = casein-based diet.
[c] Initial wt = initial rat weight.
[d] Final wt= final rat weight after 28 days of growth.
[e] ΔWT = weight gain.
[f] FI = food intake (28 days).
[g] PI = protein intake (28 days).

TABLE VI
Effect of Tortilla Protein Fortification and Enrichment on Pregnancy Rate, Litter Weight, and Growth Performance of Lactating Pups[a]

Diet[b]	Pregnancy Rate (%)	Number Pups per Litter	Birth Weight (g) Pup	Birth Weight (g) Litter	Weaning Weight (g) Pup	Weaning Weight (g) Litter	Survival Rate (%)	Males (%)	Females (%)
RDMF	40.0	4b	5.2ab	21.1b	13.2	92.3	50.0	57.1a	42.9a
FM	33.3	7b	4.4b	31.0ab	16.1	64.3	25.0	50.0a	50.0a
FEDMF	100	9ab	4.8ab	43.6a	21.0	101.7	54.3	69.5a	30.5a
Control	100	10a	5.4a	50.2a	30.6	263.1	74.9	58.9a	41.1a

[a] Means with different letters within column were statistically different ($P < 0.05$).
[b] RDMF = tortilla-based diet from regular dry masa flour; FM = tortilla-based diet from fresh masa; FEDMF = tortilla-based diet from protein fortified and enriched dry masa flour; control = casein-based diet.

was placed on a perforated base. A 2.5 mm/sec headspace speed was used; force was registered in Newtons. After collecting the broken femur, it was immediately analyzed for moisture and ash using established procedures (AOAC 1990).

Statistical Analyses

Data was analyzed as a randomized complete block design using analysis of variance procedures and computer software (SAS Institute, Cary, NC). Duncan's test was applied to determine statistical differences ($P < 0.05$).

RESULTS AND DISCUSSION

Diet Composition

The control casein diet contained 10.1% protein (Table II). Compared with the other experimental diets, the protein content of the FEDMF diet was 1.5% higher. The digestible energy content of the different diets were similar. The FM diet had slightly higher fiber content than the rest of the experimental diets probably due to the higher retention of the fiber-rich pericarp tissue after nixtamalization. Generally, tortillas produced from fresh masa contained higher levels of ash and calcium than counterparts produced from dry masa flour because processors use higher lime concentrations during cooking and longer steep times (Serna Saldivar et al 1990).

FM and RDMF diets contained lower amounts of lysine and tryptophan than the FEDMF diet; therefore, these diets had lower essential amino acid scores (EAAS). Values for these diets were 56.3 and 53.4, respectively. Fortification with 6% defatted soybean meal improved the EAAS to a value of 75.1 (Table III). This clearly shows the beneficial effect of soybean fortification in improving the essential amino acid pattern. In human nutrition, especially for the early stages of life, it is more important to provide high amounts of essential amino acids than high protein content. These observations agree with previous research studies (Serna Saldivar et al 1988a,b). The FEDMF diet provided the daily requirements of vitamins B_1, B_2, niacin, and folic acid and minerals iron and zinc for a 1–3 year old child (Table IV). None of these micronutrients were provided in enough quantities by the regular tortilla diets.

First Generation Growth Study

A highly significant difference ($P < 0.01$) in body weight after 28 days of growth, daily gains, and efficiency of food conversion was observed (Table V). Growth rates expressed in terms of protein consumed by rats fed FM and RDMF tortilla diets were 1.59 and 1.46, respectively ($P > 0.05$). Counterparts fed FEDMF and control diets had a significantly higher ($P < 0.05$) growth performance (2.05 and 2.34). This confirms the enhanced EAAS. Rats fed the control casein diet had the best growth performance ($P < 0.05$) confirming results of other researchers (Serna Saldivar et al 1987, 1988a,b; Sproule et al 1988). Rats fed the regular tortilla diets had opaque, kinky hair coat appearance compared with the other treatments that were smooth and shiny. During the second month of growth, rats fed regular tortilla diets lost their hair and had severe dermatitis. This effect probably occurred due to poor quality protein and deficiencies of vitamins and minerals. It is well known that the lack of niacin produces dermatitis (Neldner 1988).

Reproductive Performance

Rats fed FEDMF and control diets had 100% pregnancy rate while rats fed RDMF and FM tortilla diets had 40 and 33% pregnancy rates, respectively (Table VI). The lower pregnancy rate was associated with the lower body weight produced by diets deficient in protein, vitamins, and minerals. The lack of good quality protein, iron, and folic acid have been associated with reproduction failure. In addition, females fed FEDMF and control diets had 9 and 10 newborns, respectively, in contrast with fertile counterparts that had 4 and 7 newborns. Due to the lower number of pups/litter, the newborns from the RDMF and FM fed dams had slightly higher birth weight and survival rate than counterparts fed the other diets. At the end of lactation, only 4 and 7 pups were weaned by females fed FM and RDMF, respectively. In contrast, 32 and 45 rats were weaned by mothers fed FEDMF and casein-control diets, respectively. In addition, the average weight of the litters was higher for rats fed the FEDMF and control diets (43.6 and 50.2 g) than for pups of the RDMF and FM diets (21.1 and 31.0 g). Maternal protein and energy malnutrition negatively affect reproduction performance and litter weight (Alexander et al 1988; Friggers et al 1993; Sakanashi et al 1987; Rasmussen 1988). These studies

TABLE VII
Effect of Tortilla Protein Fortification and Enrichment on Growth of Second Generation Postweaned Rats[a]

Diets[b]	Initial Wt[c] (g)	Final Wt[d] (g)	ΔWt[e] (g)	FI[f] (g)	PI[g] (g)	ΔWt/PI
RDMF	14.20	27.20b	13.00b	316.00a	25.44a	0.51c
FM	18.25	28.75b	10.50b	359.80a	29.58a	0.36c
FEDMF	14.33	39.60b	25.27b	198.43b	18.22b	1.73b
Control	35.40	96.72a	61.32a	322.82a	31.67a	2.03a

[a] Means with different letters within column were statistically different ($P < 0.05$).
[b] RDMF = tortilla-based diet from regular dry masa flour; FM = tortilla-based diet from fresh masa; FEDMF = tortilla-based diet from protein fortified and enriched dry masa flour; control = casein-based diet.
[c] Initial Wt = initial rat weight.
[d] Final Wt = final rat weight after 28 days of growth.
[e] ΔWt = weight gain.
[f] FI = food intake (28 days).
[g] PI = protein intake (28 days).

TABLE VIII
Effect of Tortilla Protein Fortification and Enrichment on Hemtocrit Values

Diet[a]	Adult Males	Lactating Females	Weanling Rats[b]	Growing Rats[c]
RDMF	42	37	31	42
FM	43	37	—[d]	41
FEDMF	47	41	33	43
Control	45	40	35	43
Standard error	3.38	4.25	2.88	3.25

[a] RDMF = tortilla-based diet from regular dry masa flour; FM = tortilla-based diet from fresh masa; FEDMF = tortilla-based diet from protein fortified and enriched dry masa flour; control = casein-based diet.
[b] Second generation rats were weaned at 28 days of age.
[c] Second generation rats were subjected to growth for 28 additional days postweaning.
[d] Lost observation.

showed that malnourished animals produce less milk, thus affecting survival rate and litter weight gains. As observed with the first generation rats, the hair coat development was retarded in pups from mothers fed FM or RDMF diets.

Second Generation Growth Study

Growth of second generation weanling rats fed RDMF or FM diets was 0.51 and 0.36 g of gain/g of protein consumed, respectively (Table VII). The growth was not statistically different ($P > 0.05$). In contrast, counterparts fed FEDMF or control diets had higher ($P < 0.05$) growth rates (1.73 and 2.03 g of gain/g of protein consumed, respectively). Therefore, weanling rats fed these diets gained from three to four times more weight than counterparts fed regular tortilla diets. This difference is higher than that documented in the first generation growth study (Table V), demonstrating that prolonged malnutrition affects each generation more severely. Other researchers (Marichich et al 1979; Browning et al 1988; Kubena et al 1988; Desai et al 1996; Hals et al 1996; Gressens et al 1997) concluded that protein and energy malnutrition and deficiency of vitamins (B_{12}) and minerals (Zn, Mg) of mothers significantly retarded growth in second generation individuals.

Hematocrit and Bone Development

Hematocrit values of rats fed FM and RDMF diets were lower than counterparts fed FEDMF and control diets (Table VIII). Hematocrit values are positively related to iron and negatively related to anemia; the rats fed FEDMF diets had values similar to the control fed animals. Lactating females and second generation weanling rats had lower hematocrit values than other physiological stages, probably due to loss of iron reserves during pregnancy and lactation. Thus, newborns consumed milk deficient in iron.

Femur weight and length and force required to break bones was higher ($P < 0.05$) in first generation adult rats fed the control and FEDMF diets than counterparts fed RDMF and FM (Table IX). This indicates a positive interaction between protein quality and bone development. Serna-Saldivar et al (1991, 1992) found that calcium was more available in quality protein maize than in regular maize tortillas. According to Ranhotra et al (1999), there is a direct relationship between femur ash content and force required to break it. Femurs of adult males fed the control and FEDMF diets contained more ash and required more force to break. For lactating females, bone moisture and ash and break force was similar ($P > 0.05$) for all tortilla diets. These values were lower than the values observed in adult males, probably due to bone decalcification because of excessive milk production to sustain greater numbers of pups/litter. For second generation weanling rats, femur weight, length, ash, and break force was higher for control-fed animals. Breaking femurs of control rats required approximately three times as much force when compared with the average of the tortilla diets. A similar trend was observed when femur values of second generation growing rats were compared.

Although the difference was nonsignificant ($P > 0.05$), femurs of second generation rats fed FM tortillas were stronger probably due to the higher calcium content of the tortillas (Table IV). From the nutritional viewpoint, more lime should be added to dry masa flours to increase calcium content at least to the same level of fresh masa.

Cerebral DNA

Total content and concentration of DNA in first generation adult males and lactating females were similar for all diets (Fig. 1). However, a significant change in these variables was observed in brains of second generation subjects. For animals fed FEDMF and control diets, total DNA concentration and number of neurons were significantly ($P < 0.05$) higher than for counterparts fed FM or RDMF. These results agree with previous observations of Shils et al (1994) and Gressens et al (1997), who concluded that the amount of cerebral DNA strongly depends on the availability of nutrients (amino acids, vitamins) during intrauterine development and lactation. A severe protein malnutrition causes adverse effects on brain development and a reduction in cerebral DNA because essential amino acids are required for DNA synthesis. Important alterations occur in the hippocampal, cerebellum, and basal ganglia (Andrade et al 1996; Cintra et al 1997; Viana et al 1997). A recent neuropharmacological investigation revealed permanent negative changes in the neuron receptors due to malnutrition in the early stages of the life cycle (Levitsky and Strupp 1995). The number of neurons affects nerve impulses and, therefore, attitude and intellectual quotient (Williams and Rakic 1988; Williams and Herrup 1988). The clear differences observed between first and second generation subjects are attributed to the development of nerve tissues during the first stages of life (< 2 years for humans) (Shils et al 1994).

TABLE IX
Effect of Protein Fortification and Enrichment on Moisture, Ash, Thickness, Length, Weight, and Break Force of Femurs[a]

Diet[b]	Moisture (%)	Weight (mg)	Thickness (cm)	Length (cm)	Organic Matter (%)	Ash (mg)	Break Force (N)
Adult males							
RDMF	37.5a	688.5bc	0.85a	3.73b	47.4a	218.9b	103.15c
FM	36.5a	598.3c	0.78a	3.60c	46.3ab	198.2b	97.02c
FEDMF	37.2a	760.6ab	0.85a	3.80b	48.4a	225.3b	142.28b
Control	35.3a	896.0a	0.85a	3.98a	41.5b	330.0a	192.87a
Lactating females							
RDMF	36.3a	595.1a	0.80b	3.60ab	51.2a	178.7ab	67.38a
FM	35.0a	459.6b	0.80b	3.50b	49.7a	141.2b	56.16a
FEDMF	38.1a	536.5ab	0.80b	3.64ab	49.6a	159.9b	64.46a
Control	37.8a	628.6a	0.87a	3.78a	45.3a	214.8a	82.30a
Weanling rats[c]							
RDMF	54.3a	55.7b	0.60b	1.65b	74.3a	5.4b	3.55b
FM	48.9a	46.7b	0.60b	1.68b	65.9b	7.3b	7.48b
FEDMF	57.3a	60.0b	0.61b	1.70b	74.1a	6.2b	4.67b
Control	56.1a	140.5a	0.67a	2.11a	59.7c	23.4a	17.68a
Growing rats[d]							
RDMF	54.2a	105.9b	0.65b	2.00c	66.7a	14.8b	10.40b
FM	54.8a	137.0b	0.68ab	2.25bc	58.6b	24.9b	18.67b
FEDMF	56.6a	163.3b	0.68ab	2.42b	64.8a	23.4b	13.13b
Control	43.7b	348.3a	0.76a	3.13a	49.7c	97.1a	45.52a

[a] Means with different letters within column and physiological stage were statistically different ($P < 0.05$).

[b] RDMF = tortilla-based diet from regular dry masa flour; FM = tortilla-based diet from fresh masa; FEDMF = tortilla-based diet from protein fortified and enriched dry masa flour; control = casein-based diet.

[c] Second generation rats were weaned at 28 days of age.

[d] Second generation rats were subjected to growth for 28 additional days postweaning.

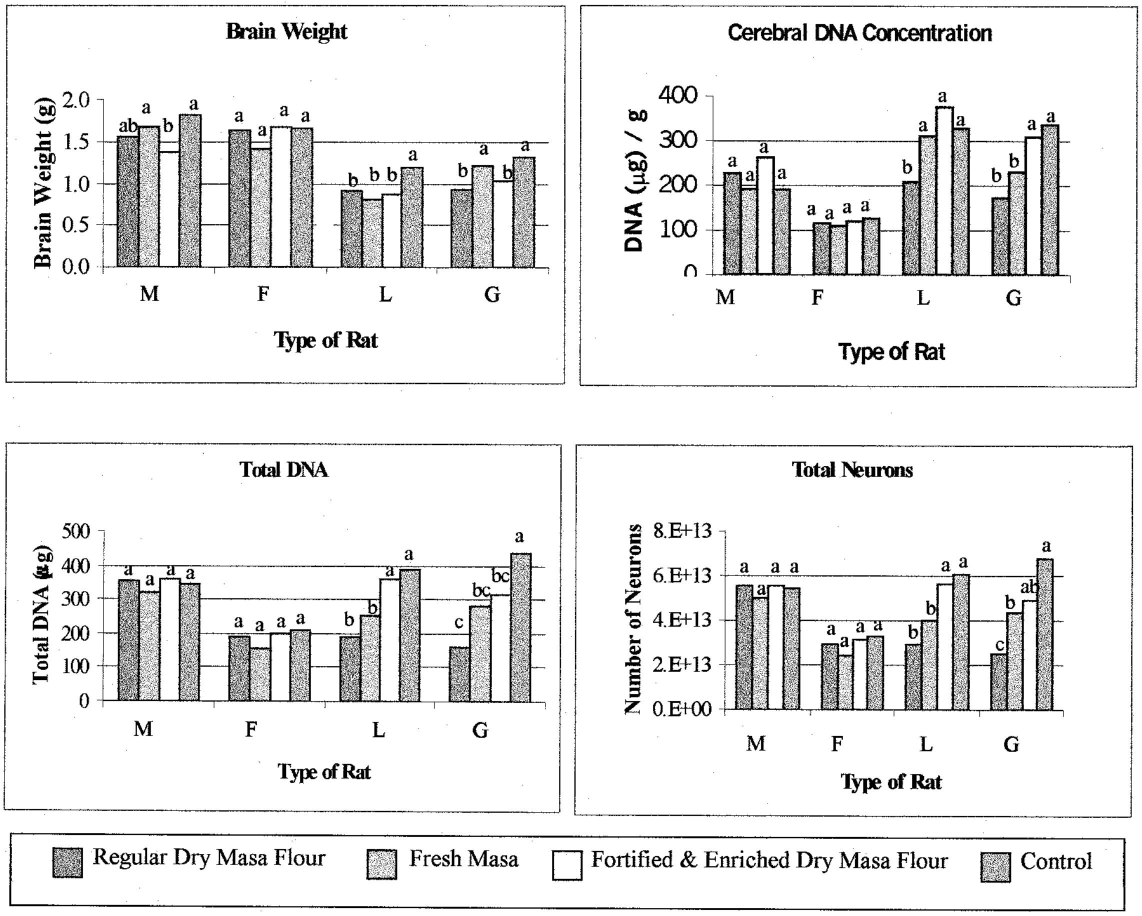

Fig. 1. Effect of tortilla fortification and enrichment on brain weight, cerebral DNA, and number of neurons. M = first generation adult males, F = first generation adult females, L = second generation lactating pups (28 days old), G = second generation rats after 28 days postweaned growth. DNA (µg) = DNA in the brain. Within physiological stage, means with different letters are statistically different ($P < 0.05$).

CONCLUSIONS

This study clearly demonstrates that the growth of rats fed FEDMF was higher than counterparts fed RDMF or FM and only slightly lower than control animals. The better amino acid profile of the fortified tortilla improved weight gains and food conversion. In addition fortification and enrichment improved hematocrit and bone development in first generation adult animals. Adult females had a high pregnancy rate, higher number of pups/litter with higher survival rate. The difference in growth of second generation weanling rats was more evident. The total DNA concentration was similar for first generation subjects belonging to the same physiological stage. However, a higher total DNA concentration and number of neuron cells was observed in weanling rats fed the fortified and enriched tortilla diets than counterparts fed regular tortillas. The data obtained clearly shows the beneficial effects of soybean protein fortification and enrichment with essential micronutrients to malnourished organisms. The cost of fortification and enrichment is easily paid by the massive nutritional and health benefits that occur, especially for children and pregnant or lactating women.

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