

## CORN AMINO ACID IMBALANCE AND THE ROLE OF LEUCINE EXCESS

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### SUMMARY

A study was conducted using two experimental models with weanling rats of the Wistar strain fed isocaloric diets containing 7% protein, *Model I*: two genetic varieties of maize were used; common corn (CC) or opaque-2-corn (M); and *Model II*: casein, 7% protein used without nicotinic acid in the diet in order to enhance the tryptophan limiting conditions like in the CC diet of Model I. PER, nitrogen balance, urinary urea and hepatic fat were determined. The results suggest that the imbalance of CC protein is related to lysine and tryptophan limitation, although the leucine excess content also plays an important role.

### INTRODUCTION

Protein malnutrition has been a subject of much concern in many parts of the world, due to its high priority. Nutritional

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disorders are dependent on socioeconomic and cultural conditions, as well as on human resources (1). In regions with easy access to animal protein sources where malnutrition exists, death and hunger are less frequent than among populations whose alimentary system is based mainly on vegetable foods, without any chance of obtaining foods of higher nutritional value.

Data from the Food and Agriculture Organization of the United Nations (2) show that maize consumption in Latin America is higher than any other food. In Guatemala, Mexico and Brazil, the average maize intake/person/day is 349, 272 and 200 g, respectively, quantities which provide 33.2, 25.2 and 19.3 g of protein. Thus, improvement of the protein quality of plants, especially cereals—one of the basic protein sources (3, 4)—would be extremely important in human nutrition mainly in developing countries (5).

For a long time it has been known that the quality of corn protein is not satisfactory (6), but it was until 1956 that the nature of the imbalance was identified (7-11) and others related to the fact that prolamines, which contribute with more than 50% of the corn proteins, are deficient in lysine and tryptophan. Mertz *et al.* (12-14) demonstrated that the gene Opaque-2 from Opaque-2 corn produced a genetic variety containing a balanced amino-gram. Several papers were subsequently published showing a better utilization of protein from these genetic variations (15-21).

The purpose of the present investigation was to study the nutritive value of the maize protein and the role played by the excess of leucine it contains.

## MATERIAL AND METHODS

### *Animals and Diets*

Weanling rats (*Rattus norvegicus albinus*, Wistar), of both sexes averaging 48 g in weight were used in this study.

Diet and water were provided *ad libitum* and the composition is shown in Table 1.

### *Experimental Design*

*Model I* — The animals of each group (10/group) were fed for eight weeks diets containing a) 70% protein from common

TABLE 1  
DIET COMPOSITION AND AMINO ACIDS ADDED (g/100 g)

	Protein <sup>1</sup> 70/o	Minerals <sup>2</sup>	Cod liver oil	Vegetable oil	Vitamins <sup>3</sup>	Vitamin-NA <sup>4</sup>	Lysine <sup>5</sup> (lys)	Tryptophan <sup>6</sup> (try)	Leucine <sup>6</sup> (leu)
Model I	CC	2	2	4	2	—	—	—	—
	CC	2	2	4	2	—	0.14	0.04	—
	CC	2	2	4	—	2	—	—	—
	M	2	2	4	2	—	—	—	—
	M	2	2	4	2	—	—	—	0.6
	M	2	2	4	—	2	—	—	—
	M	2	2	4	—	2	—	—	0.6
Model II	Cas	2	2	4	2	—	—	—	—
	Cas	2	2	4	2	—	—	—	1
	Cas	2	2	4	—	2	—	—	—
	Cas	2	2	4	—	2	—	—	1
	Cas	2	2	4	—	2	—	0.18	—

<sup>1</sup> CC = Common corn; M = Opaque-2 corn; Cas = Casein.  
<sup>2</sup> Phillips & Hart (1935) modified (37).  
<sup>3</sup> According to Ref. (37).

<sup>4</sup> Vitamins, but niacin free.  
<sup>5</sup> Matching lysine and tryptophan to M content.  
<sup>6</sup> Matching leucine to CC content corn starch was added until completing 1000/o.

corn (CC), b) opaque-2 corn (M), with or without addition of nicotinic acid (NA) and supplemented or not with the limiting amino acids in CC (lysine and tryptophan), and c) M additioned with the equivalent amount of leucine present in CC.

*Model II* — The animals of each group were fed for eight weeks diets containing 70/o protein as casein, supplemented or not with NA.

In both models the weight gain, PER (protein efficiency ratio), urinary urea, nitrogen balance, hepatic fat and plasma proteins were determined.

### *Determinations*

*PER.* This was evaluated by relating gain weight to grams of protein intake.

*Nitrogen Balance (NB).* Nitrogen retention was calculated by difference between ingested and excreted nitrogen. The collection period lasted four days, and nitrogen was determined using the Kjeldahl method (22).

*Urinary urea.* This was determined according to Levine *et al.* (23); *plasma proteins* by the biuret method (24), and *liver fat* as per the procedure of Kramer *et al.* (25).

The *statistical analyses* were performed using analysis of variance and the Tukey Test (26).

## RESULTS

### *Model I*

Table 2 presents the results of PER and urinary urea obtained at the 3rd and 8th week of experiment.

As the data show, the PER values for M diets were significantly higher than those obtained for CC and were not affected by the presence or absence of nicotinic acid in the diet. A decrease in PER was always observed when leucine was added to the M diet in an equivalent amount to that in CC. On the other hand, the addition of lys + try to CC improved the PER, but the values did not reach those obtained with M; these data agreed with other results

TABLE 2

DETERMINATION OF PER AND URINARY UREA IN TWO GROWTH PERIODS ON DIETS CONTAINING 7% PROTEIN OF DIFFERENT SOURCES (MODEL I)

Diet/group	PER	Urea (g/100 ml)	
		3rd week	8th week
CC <sup>1</sup>	0.94	2.40	2.50
CC + (lys) <sup>2</sup> + (try) <sup>3</sup>	1.45	1.20	1.80
CC - (NA)	0.94	2.20	2.90
M	2.15	1.63	1.83
M + (leu) <sup>4</sup>	1.83	3.92	4.19
M - (NA)	2.15	2.91	2.28
M - (NA) + (leu)	2.00	3.94	3.26

n = 5.

<sup>1</sup> Same as in Table 1.

<sup>2</sup> Lysine, 0.14%.

<sup>3</sup> Tryptophan, 0.04%.

<sup>4</sup> Leucine, 0.6%.

obtained in our laboratory (27, 28). In relation to the animals in the M group, an increase of urinary urea excretion in rats of the CC group without any supplementation was observed, as well as for M with leu addition.

Table 3 depicts the results of nitrogen balance performed at the 6th experimental week; the addition of lys + try to CC improved nitrogen retention, while that of leu to M decreased it. The best N retention was obtained with the M diet.

The results obtained for growth rate, total plasma protein (TPP), and hepatic fat (HF), for groups of Model I, are shown in Table 4. Growth rate was highest in the group fed the M diet, with or without additional NA, followed by M plus leucine, then by CC supplemented with lys + try, while the CC diet without supplementation produced the lowest weight gain. Hepatic fat was higher in animals fed the M + leu diet, reaching the results obtained by the CC group. The diet of CC prepared without NA gave the highest hepatic fat values of all groups.

TABLE 3

NITROGEN BALANCE (NB) OBTAINED IN RATS FED DIETS  
CONTAINING 7% PROTEIN OF DIFFERENT SOURCES  
(mg N/24 hr) AT THE 6th WEEK

Diet/group <sup>1</sup>	NI <sup>2</sup>	NB <sup>3, 6</sup>	D <sup>4</sup>	N retained <sup>5</sup>
CC	99.6	49.5(a)	71.3	49.6
CC + (lys) + (try)	119.6	72.5(b)	81.1	60.6
CC - (NA)	91.3	39.1(a)	65.4	42.8
M	133.0	81.1(c)	79.3	60.9
M + (leu)	129.0	48.0(a)	67.3	37.2
M - (NA)	138.0	70.4(b)	75.2	51.0
M - (NA) + (leu)	117.0	34.7(a)	65.7	29.6

<sup>1</sup> Same as in Table 1.

<sup>2</sup> NI = Ingested nitrogen.

<sup>3</sup> NB = Nitrogen balance = ingested nitrogen - (fecal + urinary nitrogen).

<sup>4</sup> D = Absorbed nitrogen: digestibility.

<sup>5</sup> N retained = (NB/ingested nitrogen) x 100.

<sup>6</sup> Numbers with different letters are significantly different at the 5% level. Numbers with the same letters = NS.

### Model II

In this Model it was decided to use casein instead of CC in the diet without added NA in order to decrease the available tryptophan pool with the results shown in Table 5. As observed, the growth rate decreased mainly due to leu addition, and not to nicotinic acid absence. Aside from this fact, the added leucine in the absence of nicotinic acid further decreased the growth rate, while the addition of tryptophan reverted the effect of absence of nicotinic acid.

### DISCUSSION

All diets used in this investigation were prepared to contain 7% protein, since usually diets based on maize as the staple food

TABLE 4

GROWTH RATE, TOTAL PLASMATIC PROTEIN (TPP) AND  
HEPATIC FAT (HF) OBSERVED IN ANIMALS AT THE 6th WEEK  
(MODEL I)

Diet/group <sup>1</sup>	Growth rate (g/day)	TPP (g/100 ml)	HF (g/100 g)
CC	0.51 <sup>(a)</sup> <sup>2</sup>	4.60 <sup>3</sup>	7.90 <sup>(a)</sup> <sup>2</sup>
CC + (lys) + (try)	1.14 <sup>(b)</sup>	5.10	5.50 <sup>(b)</sup>
CC - (NA)	0.54 <sup>(a)</sup>	4.60	9.70 <sup>(c)</sup>
M	1.78 <sup>(c)</sup>	6.40	4.10 <sup>(d)</sup>
M + (leu)	1.48 <sup>(d)</sup>	4.70	7.60 <sup>(a)</sup>
M - (NA)	1.85 <sup>(c)</sup>	5.70	5.40 <sup>(b)</sup>
M - (NA) + (leu)	1.46 <sup>(d)</sup>	5.10	7.60 <sup>(a)</sup>

n = 5.

<sup>1</sup> Same as in Table 1.

<sup>2</sup> Numbers with different letters are significantly different at the 50/o level. Numbers with the same letters = NS.

<sup>3</sup> No statistical analysis was performed, as this item represents the average obtained from a pool of samples in each group.

are low in it. CC is limited in lys and try, as the data on Table 2 reveal, and the supplementation of lys and try to CC improved the results by increasing the PER and decreasing the urinary urea. On the other hand, the absence of nicotinic acid in the diet did not influence the PER values, although leucine addition to the diet without NA, in the M diet led to an increase in urinary urea.

The nitrogen balance results in animals fed the CC diet improved significantly by supplementation (lys + try) (Table 3), even though it did not reach the nitrogen retention obtained by those fed the M diet. It was surprising to obtain equivalent nitrogen retention in the M and in CC groups supplemented with the limiting amino acids, as well as with the CC diet free of nicotinic acid, and M without it but with the addition of leu. These data suggest the important role leu excess plays in increasing the nicotinic acid demand. As a protein-free group was not included, it was impossible to determine the real NB, but the

relative NB of the different groups showed the differences in protein quality.

Leu excess causes adverse effect under different experimental conditions. For example, Benton *et al.* (7) showed a sensible reduction of the growth rate by a 30% or higher addition of leu, to a diet containing 90% casein; nevertheless, Smith (29) noticed a small effect on diets containing 120% casein. Maramatsu *et al.* (30) suggest that the leu excess effect may be due to contamination by isoleucine. On the other hand, Bressani *et al.* (15), point out that the amino acid imbalance difficults removal of fat from the liver. Whenever nicotinic acid ingestion is adequate, its synthesis from tryptophan becomes inhibited through a feed-back reaction. However, under a low nicotinic acid diet, more tryptophan must be metabolized to nicotinamide, thus decreasing availability of the tryptophan pool.

The purpose of the experimental Model II was to enhance the effect of lack of tryptophan and leucine excess by reducing the NA of the diet. According to results presented in Table 4, it can be observed that the absence of nicotinic acid by itself does not impair casein results, either for growth rate, HF or TPP. Nevertheless, nicotinic acid absence and leucine excess in the diet decrease growth rate of the animals, and promote an increase in the hepatic fat.

Children with protein-energy malnutrition often present increased fat deposits in the liver, the amount varying according to the type of carbohydrate in the diet (31-34). In our study, the carbohydrate did not vary (Table 5) and the amount of hepatic fat increased according to the imbalanced aminogram of the protein in the diet (33).

The addition of tryptophan to the "cas + leu" diet improved the growth rate and TPP, suggesting competition between nicotinic acid and tryptophan. The addition of tryptophan, however, did not decrease liver fat infiltration. This fact suggests that the excess of leucine is involved in the fat deposit in the liver, while nicotinic acid does not play an important role in this process. Sakakibara suggests that the decrease in the hepatic NAD content in rats fed a leu-supplemented diet is mainly due to competitive inhibition of the intestinal absorption of tryptophan (35).

Our results suggest that the excess of leucine in CC diets, impairs growth rate, and increases the nitrogen excretion, decreasing nitrogen retention and plasma protein and increasing the fat liver deposit. A leucine excess also increases nicotinic acid demand.

TABLE 5

GROWTH RATE, TOTAL PLASMATIC PROTEIN (TPP),  
AND HEPATIC FAT (HF OF ANIMALS) FED DIETS CONTAINING  
7% PROTEIN (CASEIN) AT THE 8th WEEK (MODEL II)

Diet/group <sup>1</sup>	Growth rate (g/day)	TPP (g/100 ml)	HF (g/100 g)
Cas	1.36(a) <sup>2</sup>	4.98 <sup>3</sup>	3.86(c) <sup>2</sup>
Cas + (leu)	0.94(b)	4.90	5.50(b)
Cas - (NA)	0.97(b)	4.97	3.71(c)
Cas - (NA) + (leu)	0.79(c)	4.43	5.57(b)
Cas - (NA) + (leu) + (try)	0.94(b)	4.95	6.40(b)

n = 5.

<sup>1</sup> Cas = Casein.

Leu = Leucine.

NA = Niacin free.

<sup>2</sup> Numbers with different letters are significantly different at the 5% level.

Numbers with the same letters = NS.

<sup>3</sup> No statistical analysis was performed, as this item represents the average obtained from a pool of samples in each group.

## RESUMEN

### IMBALANCE DE AMINOACIDOS EN EL MAIZ Y EL PAPEL QUE DESEMPEÑA EL EXCESO DE LEUCINA

Se llevó a cabo un estudio en dos modelos experimentales usando ratas recién destetadas de la cepa Wistar, las que se alimentaron con dos dietas isocalóricas que contenían 7% de proteína. En la elaboración de la dieta *Modelo I* se utilizaron dos variedades genéticas de maíz, maíz común (MC) o maíz Opaco-2 (M). La dieta *Modelo II* contenía: caseína al 7% de proteína libre de ácido nicotínico, a fin de realzar las condiciones limitantes del triptofano, al igual que en la dieta MC del *Modelo I*. Luego se determinó el balance de nitrógeno, urea urinaria y grasa hepática. Los resultados sugirieron que el desbalance de la proteína del MC está relacionado con las limitaciones en cuanto al contenido de lisina y triptofano, aun cuando el exceso de leucina también desempeña un papel de importancia.

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