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Changes in Enzymatic Activity of Small Intestine and Kidney of Rats by a Methionine Deficient Diet

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The effect of methionine dietary deficiency on food intake, weight gain, liver and kidney weight, feed conversion rate, protein efficiency ratio, maltase and leucineaminopeptidase (LAPase) activities of the intestinal mucosa as well as renal LAPase activity was studied. Three groups of female Wistar rats, weighing between 40-60 g, were fed for 25 days on either Diet A (casein supplemented with 0.6 % DL-methionine), Diet B (amino acid mixture simulating casein also supplemented with 0.6 % methionine) or Diet C (amino acid mixture with 0.67 % methionine deficiency with respect to Diet A). The results show no significant differences in either growth or enzymatic activity between the rats fed on Diet A and those on Diet B. The animals fed on Diet C show an increase in intestinal ($P<0.01$, vs Diet B) and renal ($P<0.005$, vs Diet A) LAPase activity, although intestinal maltase activity remained unchanged. Food intake, weight gain, organ weight and nutritional parameters obtained in rats fed on Diet C showed no statistically significant changes, with the exception of kidney weight which decreased ($P<0.005$) when compared to those fed on Diet B.

Key words: Methionine, Maltase, Leucineaminopeptidase

In previous studies (14) it has been reported that the intestinal maltase and leucineaminopeptidase (LAPase) as well as renal LAPase activities of rats fed with a fish meal diet derived from *Coryphaenoides rupestris*, decrease with respect to those fed on casein. The fish meal diet has a lower content of methionine (0.67 % less) than casein diet.

Since some authors observed changes produced in the activity of enzymes from animal tissues and organs as the result of deficiency or excess of essential amino acids in the diet (3,19), the aim of this work is to determine the effects of a methionine deficient diet (0.67 % less than the control diet) on maltase and LAPase activities of the intestinal mucosa, renal LAPase activity as well as on food intake, weight gain, liver and kidney weight, feed conversion rate (F.C.R. = food intake/weight gain) and protein efficiency ratio (P.E.R. = weight gain/protein intake).

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Furthermore, the effects produced by an artificially elaborated diet of amino acid simulating casein on the enzymatic activity, the weight organs and the above mentioned nutritional parameters, have been studied.

Materials and Methods

Recently weaned female Wistar rats, weighing between 40-60 g, were housed in individual cages at a room temperature of 22 ± 2°C, with a daily light period from 06.00 h to 20.00 h. The rats were divided into three groups and each group fed *ad libitum* for 25 days on either Diet A, Diet B or Diet C. The composition in percentage of dry matter of experimental diets was: protein, 12; fat, 4; raw fibre, 8; salt mixture and vitamin mixture (15), 5 each and vitamin A + D 30 and 10 IU/rat/day. The remainder was made up to 100 of equal parts of starch and saccharose. Microcrystaline cellulose was used as fibre and olive oil as fat.

The protein source of Diet A (control) was casein supplemented with 0.6 % DL-methionine (Merck); for the Diet B the protein source was an amino acid mixture simulating casein (15), also supplemented with 0.6 % of methionine, and for the Diet C the protein source was an amino acid mixture deficient in methionine (0.67 % less than the control diet), the deficiency being made up with non-essential amino acids. The composition of the amino acids used in Diets B and C is given in table I.

After the feeding period the rats were killed and the small intestine mucosa and kidney were removed, homogenized and centrifuged (15). Maltase and LAPase activities were determined using the method of DAHLQVIST (2) and GOLDBARG and RUTENBERG (5), respectively. Protein content was measured according to the technique of LOWRY *et al.* (10) using bovine serum albumin (Sigma) as a standard.

Table I. *Composition of amino acid mixture in diets B and C*

Amino acid	Diet B %	Diet C %
L-Aspartic acid	0.71	0.79
L-Threonine	0.46	0.46
L-Serine	0.66	0.73
L-Glutamic acid	2.12	2.36
L-Proline	1.04	1.16
Glycine	0.22	0.24
L-Alanine	0.41	0.45
L-Valine	0.65	0.65
L-Cystine	0.08	0.09
L-Methionine	0.33	0.26
L-Isoleucine	0.55	0.55
L-Leucine	1.02	1.02
L-Tyrosine	0.52	0.58
L-Phenylalanine	0.48	0.48
L-Lysine HCl	1.10	1.10
L-Histidine HCl.H_2O	0.38	0.38
L-Arginine HCl	0.47	0.52
L-Tryptophan	0.20	0.20
DL-Methionine	0.60	—
	12.00	12.02

Statistical analysis was carried out by paired Student's t-test.

Results

The results show no significant differences for food intake, liver and kidney weight, F.C.R. and P.E.R. of the rats fed on the experimental diets, except for kidney weight of animals fed on Diet C, which show a lower increase, with respect to those on Diet B (table II).

Table III shows the results obtained on maltase and LAPase activities of the intestinal mucosa as well as renal LAPase increases in the animals fed on methionine deficient diet (C) compared to the other diets. This increase is significant ($P < 0.005$) in renal LAPase of rats fed on Diet C with respect to those fed on Diet A and in intestinal LAPase of rats fed on Diet C compared to those fed on Diet B. No sig-

Table II. *Food intake, weight gain, liver weight, kidney weight, feed conversion rate (F.C.R. = food intake/ weight gain), protein efficiency ratio (P.E.R. = weight gain/protein intake) in rats fed with casein (Diet A), amino acid mixture simulating casein (Diet B) and amino acid mixture deficient in methionine (Diet C).* Values are means ± SEM (n = 10).

	Diet A	Diet B	Diet C
Food intake (g/25 days)	261 ± 13	267 ± 9	262 ± 11
Weight gain (g/25 days)	60.24 ± 4	58.46 ± 3	50.73 ± 5
Liver weight (g)	4.51 ± 0.18	4.37 ± 0.15	4.08 ± 0.24
Kidney weight (g)	0.53 ± 0.04	0.52 ± 0.02	0.45 ± 0.03**
F.C.R.	4.64 ± 0.55	4.63 ± 0.23	5.70 ± 0.63
P.E.R.	1.98 ± 0.19	1.83 ± 0.09	1.58 ± 0.12

** P<0.005 (vs Diet B).

nificant difference in LAPase activity was found between animals fed on casein and those receiving amino acid mixture simulating casein.

The intestinal maltase activity was not affected.

Discussion

Animal experiments showed that the amino acid mixture simulating casein was utilized as efficiently as intact casein by weanling rats (17), adult rats (11) as well as by litter-mate female rats (13).

In the present work, the food intake, weight gain, liver and kidney weight, F.C.R. and P.E.R. show no significant difference in animals fed on casein with respect to those fed on amino acid mixture simulating casein. Similar results were reported by ITOH *et al.* (7) at 0.8 and 1.6 % dietary nitrogen levels.

However, FORSUM and HAMBRAEUS (4) using a diet with protein level of 10 % reported an increase in food intake and weight gain in rats fed on amino acid mixture simulating casein compared to those fed on protein. This discrepancy between results may be attributed to the absence of asparagine and glutamine in the amino acid mixture simulating casein in the present work; but although the addition of asparagine and glutamine in the diet led to a slight improvement in growth with an increase of food intake, the difference is not significant (7). On the other hand, the differences in dietary energy levels may be another explanation of the disagreement in the results.

ROSE *et al.* (16) reported similar results in humans fed diets containing 45 Kcal/Kg of body weight/day between casein and its corresponding amino acid mixture. Later, AHRENS *et al.* (1) obtained the same effect in rats for an energy level of 3.4 Kcal/g.

Table III. *Intestinal mucosa and kidney leucineaminopeptidase (LAPase) activity and intestinal maltase activity (U/mg protein) in rats fed with casein (Diet A), amino acid mixture simulating casein (Diet B) and amino acid mixture deficient in methionine (Diet C).*
Values are means ± SEM. U = μmoles of substrate split/min. n = number of determinations.

	n	Diet A	Diet B	Diet C
Intestinal LAPase	8	0.28 ± 0.02	0.24 ± 0.02	0.33 ± 0.03*
Kidney LAPase	8	0.19 ± 0.01	0.22 ± 0.03	0.26 ± 0.03**
Intestinal maltase	9	0.94 ± 0.12	0.95 ± 0.13	0.94 ± 0.11

* P<0.01 (vs Diet B); **P<0.005 (vs Diet A)

The energy level of the diets reported in this paper is 3.5 Kcal/g, while that of FORSUM and HAMBRAEUS (4) was 3.8 Kcal/g.

Some authors (6) reported that intestinal enzymatic activities may serve as an index to evaluate the nutritive value of dietary protein. In this work, no significant difference in intestinal maltase and LAPase activities was found between animals fed on casein (Diet A) and those receiving amino acid mixture simulating casein (Diet B); this suggests that casein is nutritionally similar to its corresponding amino acid mixture, so that the results obtained in rats fed on Diet C (methionine deficient diet) could be compared with those obtained in animals fed on Diet A or Diet B.

The food intake, weight gain, organ weight and nutritional parameters measured in rats fed on Diet C showed no significant variation in comparison with the control group (casein), although the weight gain and kidney weight is 15 % less. Similar results were obtained in rats fed on the methionine deficient diet as compared to those fed on amino acid mixture simulating casein; however, in this case, the lower increase of kidney weight is significant ($P<0.005$). These facts maybe due to the difference in methionine content (0.67 % greater in Diets A and B than in Diet C), because addition of methionine at low levels improves weight gain, increases the weight of internal organs and does not alter food intake (9, 12, 20).

On the other hand, intestinal and renal LAPase activity increase in animals fed on Diet C compared to the other diets. The addition of methionine 0.2 % to a 10 % protein diet produces an increase in intestinal LAPase activity (8), but, the results of the present work indicate the opposite. This may be explained by the higher amount of methionine used, which produces an inhibition of LAPase activity in small intestinal mucosa and kidney by competition for the substrate of the enzyme (18).

As the results show, the intestinal maltase activity was not affected by a dietary deficiency of 0.67 % of methionine.

These results seem to confirm, that the low content of methionine in the fish meal diet, derived from *Coryphaenoides rupestris*, is not responsible for the inhibitions produced by these diets on intestinal maltase and LAPase activities as well as renal LAPase activity (14).

Resumen

Se estudia el efecto de una dieta deficiente en metionina sobre el alimento ingerido, ganancia de peso, peso de hígado y de riñón, conversión del alimento, coeficiente de eficacia en crecimiento, actividad de la leucinaminopeptidasa intestinal y renal, y sobre la maltasa intestinal. Se utilizaron tres grupos de ratas hembras Wistar (40-60 g), alimentadas durante 25 días con una Dieta A (caseína suplementada con 0,6 % de DL-metionina), Dieta B (mezcla de aminoácidos simulando caseína, suplementada con 0,6 % de metionina) o Dieta C (mezcla de aminoácidos simulando caseína, con un 0,67 % menos en metionina que la Dieta A). No se observan diferencias significativas en el crecimiento, ni en la actividad enzimática, entre los animales alimentados con la Dieta A y Dieta B. Los de la Dieta C, muestran aumento en la actividad de la leucinaminopeptidasa intestinal ($P<0.01$, vs Dieta B) y renal ($P<0.005$, vs Dieta A), aunque la actividad de la maltasa intestinal no se ve afectada. El alimento ingerido, ganancia de peso, peso de los órganos y parámetros nutricionales obtenidos en las ratas alimentadas con la Dieta C no varían significativamente, con excepción del peso del riñón el cual disminuye ($P<0,005$) con respecto a las de la Dieta B.

Palabras clave: Metionina, Maltasa, Leucin-aminopeptidasa.

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