Effects of Supplementation by the Limiting Amino Acids of a Low Gluten Diet on the Ratio of N\(^1\)-Methyl-2-pyridone-5-carboxamide plus N\(^1\)-Methyl-4-pyridone-3-carboxamide to N\(^1\)-Methylnicotinamide Excretion

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We have been investigating the probability that the increased ratio of N\(^1\)-methyl-2-pyridone-5-carboxamide (2-py) plus N\(^1\)-methyl-4-pyridone-3-carboxamide (4-py) to N\(^1\)-methylnicotinamide (MNA) excretion reflects improved protein nutrition.\(^1\)\(^4\) In this experiment, the effects of addition of the limiting amino acids to a 10% gluten diet on the ratio of 2-py plus 4-py to MNA excretion were investigated.

Male rats of the Wistar strain (four weeks old) were purchased from Clea Japan Inc. The rats were immediately kept individually in rat metabolic cages (CT-10, obtained from Clea Japan Inc.) and fed ad libitum a 10% gluten diet (gluten, 10%; \(\alpha\) -cornstarch, 52.7%; sucrose, 26.3%; corn oil, 5%; mineral mixture (Oriental's ratio), 5%; vitamin mixture (Oriental's ratio), 1%) for 28 days. Urine was collected in flasks containing 1 ml of 1 N HCl for the last day and stored at \(-25^\circ\)C.

The contents of nicotinamide (Nam), 2-py, and 4-py were simultaneously measured by the high-performance liquid chromatographic (HPLC) method of Shibata et al.\(^5\) The MNA content in urine was measured by the HPLC method of Shibata.\(^6\)

The significance of differences between means was carried out analysis of variance and was evaluated by Bartlett's test, and by Duncan's new multiple range test.\(^7\)

Table I shows the gain in body weight, food intake, and food efficiency ratio for 28 days. All of these values were significantly higher in the group with the 10% gluten diet supplemented with the limiting amino acids than in the group with the 10% gluten diet. In particular, the improvement of growth was noticed by supplementation of the limiting amino acids. This may be partly attributed to the increase in food intake.

Table II shows the urinary excretion of Nam and its metabolites, and the ratio of 2-py plus 4-py to MNA excretion. Values are means ± S.D. for 4 rats; values having different superscript letters in the same row are statistically significantly different at \(p<0.05\).
Table II shows the urinary excretion of Nam, MNA, 2-py, and 4-py when the 10% gluten diet and the 10% gluten diet supplemented with the limiting amino acids were fed to the rats. The urinary excretion of Nam and 2-py were higher in the group with the 10% gluten diet supplemented with the limiting amino acids than in the group with the 10% gluten diet, but these increases were slightly (about 100 nmol/daily urine in Nam; about 30 nmol/daily urine in 2-py). The urinary excretion of MNA was much lower in the group with the 10% gluten diet supplemented with the limiting amino acids than in the group with the 10% gluten diet (the difference was about 1600 nmol/daily urine). On the contrary, the urinary excretion of 4-py was much higher in the group with the 10% gluten diet supplemented with the limiting amino acids than in the group with the 10% gluten diet (the difference was about 600 nmol/daily urine). The total urinary excretion of Nam, MNA, 2-py, and 4-py was higher in the 10% gluten diet than in the 10% gluten diet supplemented with the limiting amino acids, although the intakes of tryptophan and nicotinic acid were higher in the group with the 10% gluten diet supplemented with the limiting amino acids than in the group with the 10% gluten diet (Tables I and II). In the group with the 10% gluten diet supplemented with the limiting amino acids, tryptophan could be used first to establish and maintain nitrogen equilibrium (namely body protein synthesis) and a little tryptophan used for the biosynthesis of niacin.

We have reported that the great reduction of MNA excretion and the great increase in the ratio of 2-py plus 4-py to MNA excretion were observed when a 10% casein diet was changed to a 20% casein diet and when a 20% SPI diet was changed to a 40% SPI diet. In this experiment, the ratio of 2-py plus 4-py to MNA excretion increased by 5.6 times and the MNA excretion decreased by 1/3.6 times upon addition of the limiting amino acids to the 10% gluten diet as shown in Table II. These findings indicate that the increased ratio of 2-py plus 4-py to MNA excretion reflects an improved protein nutrition.

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References
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