Effect of feeding zinc-deficient Bengal gram *(Cicer arietenum)* diet to rats on the in vitro absorption of L-histidine monohydrochloride

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Summary. The in vitro absorption of L-histidine monohydrochloride from the duodenum was less in a group of rats fed a zinc-deficient bengal gram diet than in a group fed a zinc-supplemented bengal gram diet.

Zinc is an essential micronutrient for the human body. Zinc deficiency is reported to result in retarded growth, poor sexual development, poor wound healing[1] and disturbances in nucleic acid metabolism[2]. There are reports which indicate that zinc affects the utilization of nutrients, especially proteins[3,4]. The feeding of zinc-deficient and zinc-supplemented bengal gram *(Cicer arietenum)* diets to rats has indicated[5] that the amount of faecal nitrogen was significantly higher ($p < 0.05$) in the group fed the zinc-deficient bengal gram diet than that in the zinc-supplemented ad libitum and pair fed bengal gram diet fed groups. The bengal gram has a high phytate content which hinders the availability of zinc. The effect of dietary zinc deficiency on the absorption of amino acids is not known to any considerable extent. Therefore, the present study was planned to determine the effect of feeding a zinc-deficient bengal gram diet on the absorption of L-histidine mono-hydrochloride by in vitro method in the rat intestine.

Experimental. Animals and diets: Zinc-deficient (4 ppm) and zinc-supplemented (80 ppm) casein diets and zinc-deficient (10 ppm) and zinc-supplemented (80 ppm) Bengal gram diets were used for the present investigation. All the 4 diets contained protein at 10% level. The zinc-deficient casein diet had the following percentage composition: Casein, 12.9, corn starch, 62.1, groundnut oil, 10.0, cellulose powder, 5.0; sucrose, 5.0; vitamin mixture[6], 1.0; and salt mixture[7] (without zinc), 4.0.

The percentage composition of the zinc-deficient bengal-gram diet was as follows: Bengal gram, 59.0; corn starch, 16.0; groundnut oil, 10.0; sucrose, 5.0; cellulose powder, 5.0; vitamin mixture, 1.0; and salt mixture (without zinc), 4.0. The supplemented diets (80 ppm zinc) were prepared by addition of zinc sulphate.

Adult male albino rats were divided into 4 groups, each having 6 rats of nearly the same body weight. The animals were kept in aluminium cages and were fed ad libitum for a period of 4 weeks. Deionised water was supplied during the experimental period.

At the end of the experimental period, the rats were fasted for 30 h and anesthesized with solvent ether. The duodenum, about 4 cm in length, was removed, washed in Krebs' Ringer phosphate solution and then everted. The absorption of L-histidine monohydrochloride was studied according to the method of Akedo et al.[8]. On the mucosal side, Krebs-Ringer phosphate buffer (pH 7.4) containing 20 mg L-histidine monohydrochloride and 0.2 g glucose per 100 ml was used. To the serosal side of the sac, 0.5 ml of Krebs-Ringer phosphate buffer containing 0.2% glucose was added prior to incubation at 37 °C. During the incubation, oxygen was bubbled through the contents. At the end of incubation time (30 min), 0.05 ml of samples were taken from the serosal side and histidine was determined[9]. At the end of the incubation period, the intestinal segment was taken out of the buffer, blotted on filter paper and weighed. The results of the relative absorption of L-histidine from the duodenum from different groups were expressed[10,11] as mg of L-histidine/100 ml of serosal fluid/100 mg of fresh tissue/30 min of incubation at 37 °C.

Results and discussion. The rate of in vitro amino acid absorption was maximum in the group fed on the casein zinc-supplemented diet (table). The difference in the absorption of the amino acid in the Bengal gram zinc-deficient and zinc-supplemented groups was statistically ($p < 0.05$) significant. The relative amino acid absorption in the group fed on the zinc-deficient Bengal gram diet was only 70% of the absorption by the zinc-supplemented Bengal gram group. Koo and Turk[12] investigated the effect of zinc-deficiency on the fine structure of pancreatic acinar cells and intestinal epithelium and reported that the structural integrity of these organs was affected. Groth[13] reported that parakeratosis and inflammatory changes in subepithelial tissues and in the tongue, which occurred in the deficient state, regressed after the rats were again given zinc. When the protein source is predominantly of plant origin, the zinc may be the limiting nutrient for its utilization. Enrichment of a low protein diet with zinc improved the utilization of nitrogen[14] whereas the enrichment of the same diet with amino acids failed to do so. Thus the observed decrease in absorption of histidine in the present investigation may be attributed to some changes in the absorptive surface of the duodenum that might have been produced as a result of zinc deficiency.

The effect of feeding zinc-deficient and supplemented diets to rats on in vitro absorption of L-histidine monohydrochloride

Diets	L-Histidine (mg)*	Relative percentage absorption of the amino acid
D_1 Casein zinc-deficient diet	2.85±0.33	28.4
D_2 Casein zinc-supplemented diet	10.03±0.56	100.0
D_3 Bengal gram zinc-deficient diet	4.52±0.43	41.1 (70.0)**
D_4 Bengal gram zinc-supplemented diet	6.64±0.33	64.5

* These are the relative values expressed as mg L-histidine/100 ml of serosal fluid/100 mg fresh tissue/30 min incubation at 37 °C.

** The value given in parenthesis expressed as percentage of amino acid absorption in comparison to values of diet D_4 fed group.

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