The Effect in Chicks of Feeding a Diet Containing Excess L-methionine on the Ability of the Intestine to Absorb Methionine

Hiro-omi YOKOTA and Hiroshi UEDA*

Laboratory of Animal Nutrition, School of Agriculture,
Nagoya University, Nagoya-shi 464

(Received July 30, 1980)

Abstract The ability of the intestine to absorb L-methionine was measured in chicks fed a diet containing excess L-methionine and then compared with the ability in chicks fed a control diet. Two days after the feeding of 1.5% L-methionine-excess diet to 15-day old chicks, the cumulative feed intake and body weight gain were significantly less than those of the control chicks. The L-methionine absorptive ability was measured between days 4 to day 6 by an *in situ* perfusion method at methionine concentrations of 2, 10 and 50 mM in the perfusate. At the two higher concentrations L-methionine absorptive ability of chicks fed a methionine excess diet was less than that fed a control diet, but not significantly. However, when the concentration of methionine in the perfusate was comparatively lower (2 mM), the ability of the chicks fed the excess diet was significantly smaller than that of the chicks fed the control diet during the first 15 minutes of perfusion. Intestinal (jejunum) weight/unit length was not as affected by the excess methionine in the diet as was body weight gain.

Jpn. J. Zootech. Sci., 52 (1): 53-57, 1981

Earlier studies showed that the ability of chickens to absorb L-methionine was a little inhibited by an increase of protein in the diet¹⁻³⁾. Chicks fed a methionine-deficient diet were fully able to digest and absorb dietary protein⁴⁾. In contrast amino acid absorptive ability in rats was increased with an increase in the protein content of the diet^{5,6)}. Similarly, histidine transport was increased with an increase in dietary excess histidine⁷⁾.

It has been reported that excess dietary methionine adversely affected chick performance and elevated plasma methionine level⁸⁻¹⁰⁾. There is, however, no information available concerning the effect of excess methionine on methionine absorptive ability in chicks.

In the present experiment we investigated the methionine absorptive ability of the small intestine *in situ* in chicks fed a methionine-excess diet.

Materials and Methods

Diet: The control diet contained (g/kg) isolated soybean protein 250, cornstarch 413.45, glucose 200, corn oil 40, cellulose 30, mineral mixture 61.74, vitamin mixture 0.75, choline chloride 2, L-methionine 5 and glycine 2.5. The mineral mixture contained (g/kg of diet) $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ 26.18, CaCO_3 14.8, KH_2PO_4 10.0, KCl 1.0, NaCl

* Present address: University Farm, Faculty of Agriculture, Kagawa-ken 769-28

6.0, MgSO_4 3.0, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.333, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.333, KI 0.0026, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.0167, ZnO 0.062, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.0017, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.0083 and Na_2SeO_3 0.0001. The vitamin mixture contained (per kg of diet) thiamin-HCl 100 mg, niacin 100 mg, riboflavin 16 mg, Ca-pantothenate 20 mg, cyanocobalamine 0.02 mg, pyridoxine-HCl 6 mg, biotin 0.6 mg, pteroylmonoglutamic acid 4 mg, myo-inositol 100 mg, para-amino-benzoic acid 2 mg, menaphthone 5 mg, ascorbic acid 250 mg, retinol acetate 10000 IU, cholecalciferol 600 IU and α -tocopherol acetate 20 mg and butylhydroxytoluene 125 mg as an anti-oxidant. The methionine-excess diet was formulated by adding 1.5% L-methionine to the control diet at the expense of cornstarch. The crude protein content was 21.5% in the control diet and 22.0% in the methionine-excess diet.

Chicks: Day-old SCWL male chicks were raised on a commercial chick starter diet (20% crude protein) for 12 days and then on the control diet for 3 days to adapt them to the semi-purified diet. On day 16, 30 chicks were selected on a weight basis and assigned into 6 groups of 5 chicks each so that the average body weight (129 g) was the same for each group. Three groups were fed either the control diet or the methionine-excess diet for 6 days. The chicks were housed individually and they were given free access to feed and water. Body weight and feed intake were measured daily.

Absorptive ability: YOKOTA²⁾ had previously reported that methionine absorptive ability in chicks became almost constant at 4 to 6 days after the diet was changed from non-protein to high-protein diet or vice versa. The methionine absorptive ability in the present experiment, therefore, was measured at 4 to 6 days of the experimental period after the chicks began to feed on the excess methionine diet. The chicks were placed on an operating table left side down so that an incision could be made on the right side of the abdomen. The jejunum was withdrawn from the abdominal cavity, and the upper and lower ends of the lower jejunum were cut but the mesenteric blood vessels were kept intact. Both ends of the segment were then connected to glass cannulas. The contents of the intestinal lumen were washed out with Krebs-Ringer phosphate solution. The glass cannulas were attached to a re-circulation apparatus¹¹⁾ which enabled fluid to be perfused through the jejunal segment by means of an air injector. Perfusion was carried on for 30 minutes with Krebs-Ringer phosphate solution containing L-methionine at concentrations of 2, 10 or 50 mM, and 5 ml samples of the perfusate were taken at 15 minutes intervals. Methionine concentration in the perfusate was measured colorimetrically¹²⁾ and the L-methionine absorptive ability was determined by the rate of disappearance of methionine from the perfusate. After the perfusion was finished, the mesentery and fat tissues were removed from the intestinal segment, and the weight and length of the intestinal segment were measured.

Results

Table 1 shows that body weight gain and cumulative feed intake were significantly smaller in the chicks fed the methionine-excess diet than those in the chicks fed the control diet starting from day 2 of the experiment. The findings were in agreement with the data of UEDA *et al.*¹³⁾

Methionine Absorption and Methionine-excess Diet

Table 1. Body weight gain and cumulative feed intake by chicks fed control diet or one containing excess methionine

	Diets	Days			
		2 ¹⁾	4 ²⁾	5 ²⁾	6 ²⁾
Body gain weight (g)	Control	18.8±1.0	41.4±2.0	51.4±4.9	61.8±4.1
	Methionine-excess	7.0±1.1*	23.6±2.2*	27.6±4.4*	36.0±3.3*
Feed intake (g)	Control	35.1±0.6	72.4±1.2	89.6±4.9	113.4±1.6
	Methionine-excess	24.5±0.7*	50.6±0.9*	65.6±4.0*	86.2±2.7*

1) Mean±standard error of mean of 20 chicks. 2) Mean±standard error of mean of 5 chicks. *Significantly different from corresponding control value: $P < 0.05$

The L-methionine absorptive ability calculated on the basis of length and wet weight of the intestinal segments from the chicks fed the control and the methionine-excess diets is shown in Table 2. When calculated in terms of length all the values for chicks given the methionine-excess diet were lower, but the difference was significant only after 15 minutes at the 2 mM concentration. When expressed on the basis of wet weight of intestine both the 15 and 30 minutes samples showed significantly reduced absorptive ability on the methionine-excess diet, but no other significant differences were observed.

Table 2 also shows that the methionine-excess diet caused a small but non-significant reduction in intestinal weight per unit length.

Discussion

Although the body weight of chicks fed the methionine-excess diet was between 86% to 90% of those fed the control diet, the weight of the jejunal segment per unit length was lowered to only 93% on a wet basis. Thus the effect of the methionine-excess diet was rather less on gut weight than on body weight. In the case of re-

Table 2. Effect of excess methionine in the diet of chicks on jejunal weight and ability to absorb methionine

				Methionine concentration in perfusate						
				2 mM		10 mM		50 mM		
	Intestinal weight	g wet weight /10 cm of intestine	Time after perfusion	Diets	Index of absorptive ability					
					A	B	A	B	A	B
Control	1.24±0.06 ¹⁾	(15) ²⁾	15 minutes	Control	11±2 ¹⁾	9±1	37±2	31±4	120±39	81±20
Methionine-excess	1.15±0.03 ^a	(15)		Methionine-excess	6±1*	5±1*	32±3	31±4	78±13	64±12
			30 minutes	Control	16±2	14±1	63±3	53±4	219±26	158±6
				Methionine-excess	12±1	10±1*	59±3	56±3	176±10	144±7

1) Mean±standard error of mean. 2) Number of chicks used. *Significantly different from corresponding control value: $P < 0.05$. Index of absorptive ability. A: $\mu\text{mole}/10\text{ cm}$ of intestine. B: $\mu\text{mole/g}$ of wet weight of intestine.

calculation for intestinal weight in terms of g/m of intestine/kg of body weight the value for chicks fed methionine-excess diet was a little heavier than that for control chicks, but not significantly. Because of no differences in carcass analysis for protein, fat and water content between chicks fed a control and a methionine-excess diet⁹⁾, their composition of the intestine may be the same between two groups of chicks, and might not influence the ability to absorb L-methionine of 2 groups of chicks.

YOKOTA¹⁴⁾ demonstrated that absorptive ability of methionine in the intestine significantly decreased in chickens having higher methionine concentration in the blood plasma than that of perfusate. As plasma methionine concentration in chicks fed a methionine-excess diet was 0.06 mM⁸⁾, it is not seemed that the inhibition of methionine absorption at 2 mM in the perfusate was due to the higher plasma methionine concentration in chicks fed the methionine-excess diet.

L-methionine absorption was generally lower in chicks fed the methionine-excess diet compared to that in chicks fed the control diet, although the difference was only significant at a concentration of 2 mM L-methionine. The effect was manifested 15 minutes after the commencement of the perfusion at the two indices of absorptive ability used here, but 30 minutes after the commencement it was seen only on the wet weight basis. In the case of recalculation in terms of $\mu\text{mole/g}$ of intestine/kg of body weight, absorption rate of methionine in chicks fed the methionine-excess diet (684 ± 39) was not significantly lower than that fed a control diet (801 ± 78) even after 30 minutes.

Because methionine concentration in the intestinal lumen may be rather high due to the feeding of the methionine-excess diet, the intestine may not adapt to absorb methionine at the lower concentrations. YOKOTA¹⁵⁾ reported that a carrier for methionine absorption from the intestine begins to function just after the introduction of the amino acid into the intestinal lumen. It is possible that when the concentration in the lumen of chicks fed the methionine-excess diet may inhibit the mobilization of the carrier during the first 15 minutes, but that the inhibitory effect gradually wears off.

As discussed above, methionine absorptive ability was not severely affected by feeding a methionine-excess diet in chicks, although it was a little inhibited particularly at a low concentration of methionine in the perfusate. These results are similar to those of PISANO *et al.*⁴⁾ who reported that chicks fed a methionine-deficient diet fully absorbed dietary nitrogen *in vivo*. In rats fed a histidine-excess diet, the transport activity measured using everted sacs was increased several times more than that in rats fed a control diet⁷⁾. It seems likely that there are some differences in the response of the amino acid absorptive ability to the feeding of amino acid excess or deficient diets between rats and chicks.

The authors wish to thank Dr. Marie E. Coates, National Institute for Research in Dairying for her critical reading the manuscripts. The present work was supported by a grant-in-aid (No. 236029) for scientific research from the Ministry of Education, Science and Culture in Japan.

References

- 1) YOKOTA, H. Jpn. J. Zootech. Sci., **47**: 233-235. 1976.
- 2) YOKOTA, H. Proc. Japan. Soc. Animal Nutr. Met., **21**: 13-22. 1977.
- 3) YOKOTA, H. XVI World's Poultry Congress Brazil, 1661-1667. 1978.
- 4) PISANO, J. J., C. M. PAINE and M. W. TAYLOR, J. Nutr., **67**: 213-222. 1959.
- 5) GOLDSMITH, R. M., K. A. MUNDAY and M. R. TURNER, Proc. Nutr. Soc., **30**: 80A. 1971.
- 6) LIS, M. T., R. F. CROMPTON and D. H. MATTHEWS, Br. J. Nutr., **27**: 159-167. 1971.
- 7) NAKAMURA, Y., K. YASUMOTO and H. MITSUDA, J. Nutr., **102**: 359-364. 1972.
- 8) UEDA, H. and I. TASAKI, Japan. Poultry Sci., **14**: 138-143. 1977.
- 9) UEDA, H. and H. YOKOTA, Japan. Poultry Sci., **14**: 236-239. 1977.
- 10) UEDA, H., H. YOKOTA and I. TASAKI, XVI World's Poultry Congress, Brazil, 1649-1655. 1978.
- 11) TASAKI, I. and H. YOKOTA, Jpn. J. Zootech. Sci., **41**: 104-109. 1970.
- 12) CSOKA, R. B. and C. A. DENTON, J. Biol. Chem., **163**, 329-338. 1946.
- 13) UEDA, H., H. YOKOTA and I. TASAKI, Japan. Poultry Sci., **16**: 113-120. 1979.
- 14) YOKOTA, H. Jpn. J. Zootech. Sci., In preparation.
- 15) YOKOTA, H. Jpn. J. Zootech. Sci., **47**: 81-86. 1976.

メチオニン過剰飼料摂取ヒナの腸管における メチオニン吸収能力

横田浩臣・上田博史

名古屋大学農学部, 名古屋市 464

L型メチオニンの腸管における吸収能力をメチオニン過剰飼料を摂取したヒナと対照飼料を摂取したヒナとで測定し比較した。対照飼料は抽出大豆蛋白質を蛋白質源とし、メチオニンおよびグリシンを添加した蛋白質含量21.5%の飼料であり、メチオニン過剰飼料は対照飼料に1.5%のL型メチオニンを添加した。過剰飼料を給与すると、2日後に増体重および飼料摂取量は対照と比較して減少したので、メチオニン吸収実験は過剰飼料給与開始後4日目から6日の間に行った。吸収能力は *in situ* において再循環型装置を用い、30分間に亘り測定し、環流液からのメチオニンの消失量をもって吸収量とした。環流液には Krebs-Ringer phosphate 溶液を用い、L型メチオニンを2, 10 および 50 mM の濃度に溶解した。過剰飼料摂取群のヒナのメチオニン吸収能力は対照飼料

摂取群のものより高くなることはなかった。2 mM の場合には、環流開始 15 分後において 過剰飼料摂取群のヒナの吸収量は、対照飼料摂取群のそれに比較して有意に減少した。この結果より、メチオニン過剰飼料摂取群のヒナの腸管内には、メチオニンが高濃度に存在し、高濃度のメチオニンを吸収するようにメチオニン吸収機構が働いていて、低濃度のメチオニンが与えられたときには、それに応答するような吸収機構がすみやかに働かず、吸収能力を十分発揮できず単位時間の吸収量が減少したものと推察される。また、メチオニン過剰飼料を摂取すると、単位長さ当りの腸管重量は減少する傾向にはあるが、有意差は認められなかった。

日畜会報, 52 (1): 53-57, 1981