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Bioavailability of modified forms of methionine in yellowtail and rainbow trout

Toshiro MASUMOTO[1], Jay Dev BISTA[2], Yoshiaki ITOH[3],
Hidetsuyo HOSOKAWA[1] and Sadao SHIMENO[1]

[1] *Laboratory of Fish Nutrition, Kochi University, Nankoku, Kochi, Japan (correspond author's e-mail address: tosh@cc.kochi-u.ac.jp)*
[2] *Natural Water Fisheries Development, Pokhara, Nepal*
[3] *Laboratory of Fisheries Utilization, Nankoku, Kochi, Japan*

Abstract: Bioavailability of modified methionine products, oligo-L-methionine (OM) and β-1,3-glucan (curdlan) coating methionine was examine by absorbability study in yellowtail (exp-1) and growth study in rainbow trout (exp-2). Effects of feeding frequency on crystaline methionine utilization was also examined in the rainbow trout (exp-2). Plasma free methionine levels increased in yellowtail fed curdlan coating methionine but the levels did not change in OM fed fish. In the feeding study, despite a good absorption of curdlan coating methionine, growth rate of curdlan coating methionine was inferior to crystalline methionine fed group. No growth difference was noted in fish fed a crystalline methionine supplement group either two or four times feeding a day. These results suggested that bioavailability of OM is inferior to curdlan coating methionine. However, the latter product did not give the same growth performance as crystalline methionine.

Key words: Yellowtail, rainbow trout, methionine

INTRODUCTION

Fish meal is a major dietary protein source in fish feed, yet short supply and high prices of fish meal has made it apparent that other sources of protein must be found. Plant protein source such as soybean meal is one of the candidates for the protein source though, methionine must be supplemented because it is deficient in soybean and almost all plant protein sources. Due to a deficient of methionine in almost all plant protein, methionine supplementation is needed. Yamada et al. (1981) comapred absorption rate of casein and similar profile of amino acid consists of crystalline amino acid and turned out that crystalline amino acids had a faster absorption than protein. Similarly, Murai et al. (1981) reported in carp and catfish that absorption rate of crystalline amino acids by two fish were extremely fast. It has been postulated that the observed lower supplementary effect of crystalline amino acids compared to intact protein is due to lack of synchronization of amino acid absorption between amino acids supplied in the crystalline form and those supplied from intact protein. Because simultaneous presentation of amino acids to the tissues is necessary for optimal protein synthesis (Murai et al. 1982). In this context, present study was designed to reduce this time lag during absorption between intact protein and supplemented methionine by modify it with either peptide or coating. The two modified methionine products used in this study are, oligo-L-methionine (OM) and curdlan coating methionine. The OM is a methionine peptide synthesized through plastin reaction. Curdlan is a bacterial product of β-1,3-glucan (Nakao, 1991) which dissolves in alkaline solution and it is not absorbed in an intestinal wall. The first purpose of this study is to assess absorbability of two modified methionine products. The second purpose is to examine bioavailability of the product by feeding study, also to test if crystalline methionine utilization is improved only by increasing a daily feeding frequency.

MATERIALS AND METHODS

Oligo-L-methionine(OM) and curdlan coating methinine preparation

L-methionine ethylester, which is a starting material of OM, and OM were synthesized according to the method described by Arai et. al. (1979). Fifteen gram of a reagent grade L-methionine was dissolved into 87ml ethanol and made cool less than -10°C with a dry ice-acetone bath. Then, 9.8ml of thionyl chloride was added dropwise to the solution. The solution was warmed up on a hot-stirrer until boiling point of the thionyl chloride then excess ethanol was evaporated with a rotary evaporator. Ten gram of the methionine ethylester was incubated with 50ml of 1mol $NaHCO_3$ (pH 9) containing 40mM L-cystein at 37°C for 24h with mechanically shaking incubator. After the incubation, the solution was centrifuged at 10000rpm for 30min. and resultant sediments were washed three times and freeze dried. Molecular weight of OM was estimated by using Sephadex G-25 chromatography with phosphate buffer containing 0.1M NaCl as an elluent. Each 1ml of ellute was collected and peptide content was determined by Lowry et al. (1951) at absorbance of 660nm, also ninhydrin reactive substances were monitored at 570nm. Result from above measurement, a single peak appeared between 2531d and 1350d (Fig.1). Number of methionine bind was estimated to be between 9-16. A curdlan coating L-methionine was prepared by the method according to Kanke et al. (1992).

Absorption study (exp.-1)

Absorbability of OM, curdlan and crystalline methionine was compared on yellowtail. Yellowtail weighing about 200g were obtained from a commercial fish farmer in Usa, Kochi and stocked in inside tank at Usa marine fisheries lab, Kochi University. Each 0.3g of methionine source was added to 10g of soy protein concentrate and mixed well with 32.4ml of water. The resultant slurry was force-fed to the fish by using a commercial disposable 5ml plastic syringe. On the day of the force-feeding, the fish were individually weighed and fin clipped to identified each fish. The fish were force-fed the slurry at a rate of about 4% wet weight (or 1.5% dry) per body weight and returned to the tank. Those fish were taken again at 1 and 4h after the force-feeding and blood sample was taken from caudal vein. The blood samples were immediately centrifuged and plasma was saved for the amino acid analysis. Amino acid analysis was according to previously described method

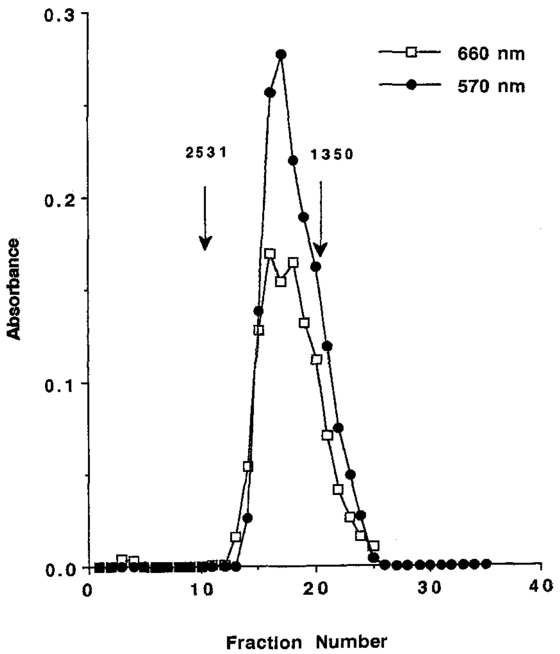

Fig. 1. Sephadex G-25 gel filtration of oligo-L-methionine with detection at 570nm and 660nm. The arrows indicate molecular weight marker.

(Masumoto et al., 1996).

Feeding Study (exp.-2)

Bioavailability of the modified form of methionine was examined by feeding experiment for rainbow trout. Due to the poor absorbability of OM which revealed from abosrbability study (exp-1), only curdlan coating methionine was tested. Rainbow trout 18-28g were obtained from a private fish farmer in Motoyama, Kochi and stocked in experimental laboratory tank of 25 liters capacity supplied with flow of fresh water with aeration. After acclimatize fish the experimental conditions, fish were separated into ten groups of 10 fish each, and duplicate tanks were used. The composition of the feed is shown in Table1. Fish were fed methionine-deficient (basal) diet, crystalline methionine supplement diet or curdlan coating methionine diet. Fish were fed twice a day 6 days a week for 30 days. For the crystalline methionine group only, fish were fed the twice or forth a day in order to compare effect of feeding frequency on crystalline methionine utilization. Every 10 days, all fish were individually weighed.

Table 1. Composition of basal diet.

Ingredient	g/100g dry diet
Brown fish meal	10.00
Soy protein concentrate	40.00
Wheat middings	10.00
Gelatin	5.00
α-Corn starch	8.50
Pollack liver oil	14.00
EAA mixture[1]	0.75
Glycine[2]	1.00
L-methionine source[3]	-
CMC-Na	2.50
Vitamin premix[4]	3.00
Mineral premix[5]	2.50
Guar gum	0.50
Feeding stimulant[6]	0.50
Cellulose	1.75
Total	100.00
Water	50.00

[1]As g/100 diet: L-leucine, 0.12; L-lysine, 0.38; L-threonine: 0.25

[2]Added to adjust methionine content

[3]Crystalline methionine and curdlan coating methionine were added 0.5 and 0.75(mg/100g dry diet), respectively.

[4]As mg/3.0 g: vitamin A palmitate, 4; cholecalciferol, 0.0045; menadione, 7.7; DL α-tocopherol, 176; thiamin HCL, 2.4; riboflavin, 4.4; pyridoxine HCl, 2.4; nicotinic acid, 7.2; Ca-panthotenate, 14, inositol, 169; biotin, 7; folic acid 2.4; choline chloride, 1168; cyanocobalamin 1.6; Ca-ascorbate, 178; cellulose 1255.8955 (Shimeno, et al., 1992)

[5]As mg/2.5g: KH_2PO_4, 412; Ca-lactate, 282; Ca (H_2PO_4). $2H_2O$, 618; iron proteinate, 166; $ZnSO_4$, 9.99; $MnSO_4$. $4H_2O$, 6.3; $CuSO_4$. $5H_2O$, 2; $CoSO_4$. $7H_2O$, 0.05; KIO_3, 0.15; dextrin, 450; cellulose, 553.51(Shimeno et al., 1992)

[6]As g/kg: L-proline, 354; L-alanine, 232; 5'-IMP, 414(Takeda, 1981)

Results

Absorption study (exp.-1)

Plasma methionine levels of fish force-fed a different diet are shwn in Fig. 2. the OM fed fish stayed at very low level and almost no changed between 1 and 4 h after feeding. The methionine levels of crystalline methionine group fed fish were higher than those of the OM fed fish but the former group of plasma concentrations were basically the same between 1 and 4h. The plasma methionine levels of curdlan coating methionine fed group were higher than other two groups and the levels of number 1 and 2 fish at 4h were higher than those at 1h. Total free amino acid minus methionine levels are shown in Fig. 3. It was relatively homogenous distribution between the groups at 1h after feeding though, there were wide range of variation at 4h. For example, crystalline methionine of 1 and 2 fish were lower than other fish.

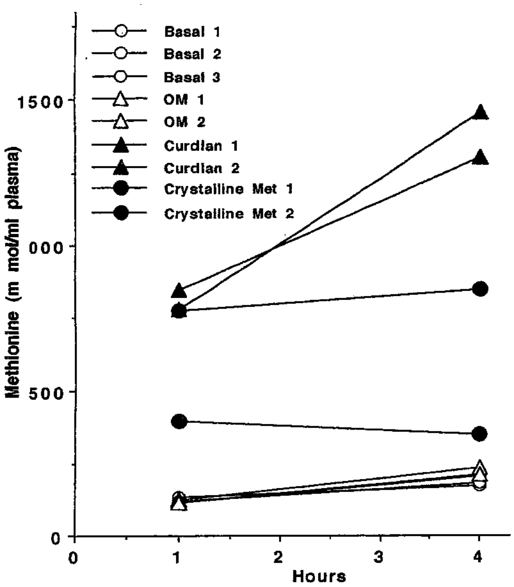

Fig. 2. Changes in the eplasma methionine levels of yellowtail force-fed a basal diet and diet containing various methionine sources.

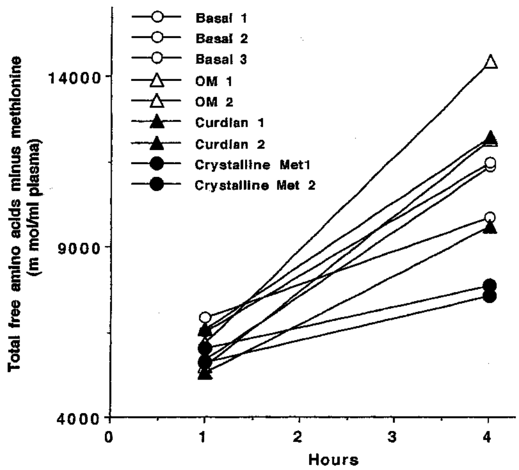

Fig. 3. Changes in the plasma free amino acid except methionine levels of yellowtail force-fed a basal diet and diet containing various methionine sources.

Feeding study (exp.-2)

The growth of rainbow trout fed different diet is shown in Fig. 4. The growth of crystalline methionine fed groups, both the twice and forth daily feeding, were higher growth rate than the other two groups. The growth rate of crystalline methionine was the same irrespective to the feeding frequency. The curdlan coating methionine fed fish were higher growth rate than the basal diet fed group though, it was inferior to the crystalline mthionine fed group.

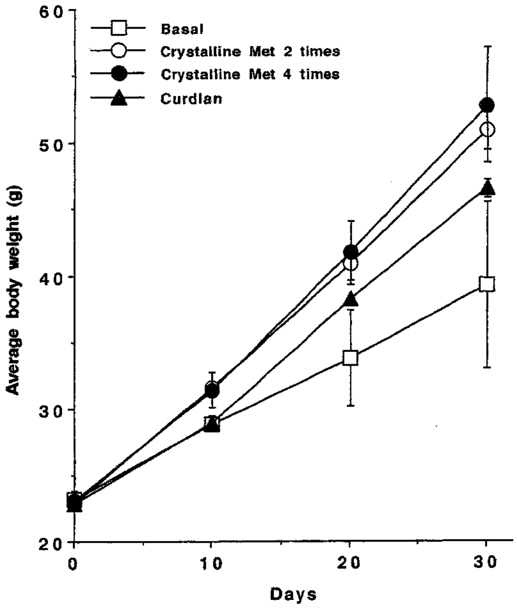

Fig. 4. Effect of curdlan coating methionine and feeding frequency on growth of rainbow trout.

Discussion

Plasma methionine levels of curdlan coating methionine fed fish at 1 h were higher than those of the basal diet fed fish, moreover the former levels increased at 4h except one fish. These results suggested that curdlan coating methionine was absorbed well. On the other hand, the OM had very low absorbability. This can not attribute to be force-feeding error (the quantity of force-feeding was different) becausece almost all amino acids derived from fish meal except methionine (Fig.3) at 4h of the OM was similar to the basal and the curdlan fed groups. Low absorbability of the OM was consistant with previously reported in Amago (*Oncorhynchus rhodurus*), (Takiwaki, 1993). In contrast to the present study, high availability of OM has been reported in rats (Hara and Kiriyama, 1991). The blood methionine levels of those rats increased after feeding of OM supplemented with casein or soybean, furthermore comparable growth was reported between the OM and crystalline methionine supplement diet fed rats. In rats, it has been also noted that OM availability is influenced by the protein source supplement with. The OM is highly digestible when it fed with fish meal compare to crystalline amino acid mix or soy protein concentrate (Chiji et al. 1990). In spite of the OM supplement with fish meal in the present study, methionine was not liberated from the OM. Therefore, absorbability and bioavailabvility of OM may be very low in fish. The reason why yellowtail and amago had low availability was uncertain. One explanation would be the low solubility of the OM. Methionine peptide reduces its water solubility when the peptide length exceeded 8. The length of the present OM was estimated 9-16 based on the gel chromatography (Fig.1). The low solubility may prevent OM from protease attack.

The plasma methionine level of crystalline methionine fed fish were unexpectedly low (Fig.2). This may be due to error in force-feeding because the total plasma free amino acid minus methionine levels were lower than other groups (Fig.3). Effects of bioavailability of the curdlan coating methionine and of feeding frequency were examined in the exp.-2. The best growth was obtained from the crystalline methionine groups irrespective to the feeding frequency. In carp, as the feeding frequency of crystalline amino acid mixture diet increased, their weight gain and feed efficiency improved proportionally (Yamada, et al. 1981). In rainbow trout, feeding twice a day may be optimum to maximize crystalline methionine utilization. Efficacy of the curdlan coating methionine was unexpectedly low, although absorbability of methionine was good in the exp.-1. Since curdlan is an undigestible materials, the curdlan itself might have an undesirable effects on growth of rainbow. Morphological characteristic of digestive tract in yellowtail and rainbow trout are similar though, feeding study for yellowtail is necessarily before conclude that abvailability of curdlan coating methionine is low.

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