Note

Inclusion of Blue Mussels and Amino Acids in the Fish and Defatted Soybean Meals Based Diet for Tiger Puffer

*Takifugu rubripes*

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Abstract: Tiger puffer *Takifugu rubripes* of 6 g body weight were fed 6 experimental diets containing fish and defatted soybean meals as major ingredients and supplemented with blue mussel meat or mussel extract, free glycine or alanine, and both of the two amino acids for 8 weeks. Dietary inclusion of blue mussel meat or extract improved the growth of tiger puffer, and weight gain and specific growth rate of fish fed the extract diet were significantly higher than those for the control without supplementation. Inclusion of free alanine improved the growth of fish, however, little effects were observed for glycine group.

Keywords: Tiger puffer; Growth; Blue mussels; Amino acids

Commercial pellet diets for tiger puffer *Takifugu rubripes* are produced by several manufacturers in Japan. However, available information on their nutritional requirements is limited. Optimum protein level for the growth was reported to be 50% for 2 g initial body weight fish based on feeding casein and dextrin based diets (Kanazawa et al. 1980). Protein sparing effects of dietary carbohydrate and lipid were examined for dextrin and pollack liver oil and it was shown that these substances could be utilized as energy sources improving protein efficiency ratio up to a certain level, although effects on the growth were not clarified (Takii et al. 1995a, 1995b). Thus, commercial pellet diets for tiger puffer are commonly formulated as high protein and low calorie type and finding alternative protein sources for fish meal is urgently required to produce cost effective diets with a stable supply because of the worldwide shortage of fish meals.

Defatted soybean meal (SBM) is a widely available, economical protein source with an annual world production of about 150 million MT, and is considered to be one of the most suitable ingredients for replacing fish meal in commercial fish diets. Previous studies indicated that replacing dietary fish meal with SBM had adverse effects on the growth and feed utilization of tiger puffer without additional treatments (Kikuchi and Furuta 2009a, 2009b). However, dietary inclusion of blue mussel meat or mussel extract to the fish meal and SBM based diet resulted in comparable growth to the control with fish meal as the sole protein source mostly by increasing feeding. Increasing feed consumption by dietary mussel meat or extract was also reported for Japanese flounder, *Paralichthys olivaceus* (Kikuchi and Sakaguchi 1997; Kikuchi et al. 2002), and free alanine and
glycine were considered to be two of the causatives (Kikuchi 2003).

Six experimental diets were formulated to be isonitrogenous as shown in Table 1. The control diet (Diet 1) contained 50% fish meal and 27% SBM as protein sources. Five percent fish meal was replaced with freeze dried meat of blue mussels in diet 2. Major ingredients of diet 3 to 6 were similar to those of diet 1, and 10% mussel extract was added by outside remuneration to diets 3, 0.05 and 0.04% crystalline glycine and alanine for diet 4 and 5, respectively, and both for diet 6. Mussel extract was prepared according to Kikuchi and Furuta (2009a). All feed stuffs except pollack liver oil, were mixed, ground, minced, and formed into spheres about 2.2 and 3 mm diameter using laboratory scale pellet machine at the Laboratory of Nippon Formula Feed mfg. Co. Ltd (Ibaraki, Japan). Then the oil was added and the diets were dried and stored at ~30°C until use.

Juvenile tiger puffer of 3 g body weight was obtained from a commercial hatchery (Nissin Marine Tech, Aichi, Japan). Fish were reared in 2000 l closed recirculation culture systems with commercial diet for tiger puffer (Nissin-Marubeni, Tokyo, Japan) at 20°C until the start of feeding experiment. The feeding experiment was conducted for 8 weeks with closed recirculation systems of two 2000 l and one 1500 l total water volume at 20 ± 1°C. At the start of the feeding experiment, fish of 6 g body weight were stocked into three replicate cages per dietary treatment at a density of 20 fish per cage. Six cages with different dietary treatments were set in the same culture tank. Fish were fed each experimental diet to apparent satiation, twice daily at 9:00 and 16:00, 6 days per week. Body weight of fish was measured individually at the beginning and the end of the rearing after the fish were starved for 24 h.

Growth performance of fish is shown in Table 2. All fish accepted the experimental diet and fed actively for the duration of the experiment. Survival rate was high in all dietary groups ranging from 97 to 100% and was not significantly different among dietary groups. Dietary inclusion of blue mussel meat or mussel extract improved the growth of tiger puffer, and weight gain and specific growth rate of fish fed diet 3 were significantly higher than those for the control ($P < 0.05$). Alanine supplementation improved the fish growth (diet 5), however, growth parameters were statistically similar to the control. Dietary glycine did not produce positive effect on the fish growth (diet 4). The growth of fish fed diet 6 was similar to that for the control, mussel and alanine groups.

<table>
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<th>Ingredient (%)</th>
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<th>4</th>
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<td>27</td>
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<tr>
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<tr>
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<td>Vitamin mixture</td>
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<td>α·Potato starch</td>
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<tr>
<td>Dextrin</td>
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<tr>
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<td>0.05</td>
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<td>0.05</td>
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<tr>
<td>Alanine</td>
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*Percent value except for blue mussel meat extracts. The mussel extract is percent rate of the addition done to total of other ingredients (wet basis).
Feed efficiency was not statistically different among dietary groups, and protein efficiency ratio for diet 4 was significantly lower than that for diets 2, 5, and 6. Supplements of blue mussel meat, extract or alanine increased feed consumption of tiger puffer and glycine had no positive effects, although statistical differences were not found among dietary groups.

As was shown for previous studies (Kikuchi and Furuta 2009a, 2009b), dietary inclusion of blue mussel meat or mussel extract to the fish meal and SBM based diet improved the growth of tiger puffer in this study. Because feed utilization in the dietary groups was similar to the control group, growth increment by the addition of the mussels is considered to mainly depend on increasing feed consumption. Similar growth enhancement effect was obtained for fish fed alanine supplemented diet, however, dietary glycine did not improve the growth with the lowest feed utilization. This is different from the case of Japanese flounder for which both alanine and glycine showed similar feeding and growth increment effects (Kikuchi 2003). Several amino acids such as alanine, betaine, glycine, and valine are known to attract feeding of aquatic organisms, however, their effects on feed consumption have not been clarified. Causative free amino acids for feeding stimulation is reported to change with the growth of fish (Sutterlin 1975), and may differ among fish species as was shown for feeding stimulants (Takii 1994). Effects of alanine supplementation on the growth of tiger puffer was slightly lower than that for the mussel extract (diet 3) with statistically similar results to the control, although alanine level did not differ between the two dietary groups (data were not shown). Takaoka et al. (1995) examining feeding stimulants for tiger puffer showed that mixture of serine, aspartic acid, glycine, and alanine improved the feeding in casein based diet, however, its activity was much lower than for synthetic clam extract which included several additional chemicals. They also indicated that addition of betaine to the amino acids mixture resulted in comparable or superior feeding activity to the clam extract depending on the betaine level. Amino acids mixture with betaine was used as feeding stimulants for tiger puffer in fish meal and SBM based diet (Ukawa et al. 1996). According to the pervious information, it is plausible that betaine supplementation to the alanine diet (diet 5) in this study produces further improvement in feed consumption and growth of tiger puffer. Because of limited available information, more research is required to clarify the effect of dietary blue mussels as well as free amino acids on the feeding and growth of tiger puffer.

### References


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魚粉と脱脂大豆を主体としたトラフガ飼料への
ムラサキガイガセミアミノ酸の添加効果

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魚粉と脱脂大豆を主成分とし、ムラサキガイセミアミノ酸、遊離のグリシン、アラニンならびに両アミノ酸を添加した6種類の飼料を作製し、初期体重6gのトラフガを8週間飼育した。ムラサキガイセミアミノ酸、エキスを含む飼料区の成長は対照区より優れていた。アラニンでも同様の成長促進効果が認められたが、グリシンでは効果がなかった。