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Review

# Effects of different modified diets on growth, digestive enzyme activities and muscle compositions in juvenile Siamese fighting fish (*Betta splendens* Regan, 1910)

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ABSTRACT

The effects of four modified diets (gamma-irradiated, microwave-irradiated, probiotic-supplemented and carbohydrase-supplemented diets) were studied on digestive enzyme specific activities and growth performance quality of juvenile Siamese fighting fish (*Betta splendens* Regan, 1910) during 2 weeks of critical and intensive rearing period. The modified procedures did not change biochemical compositions and gross energy of diets, but generally resulted in relatively higher in vitro digestibilities of protein and carbohydrate and fish survival rate, albeit insignificant. Only gamma irradiation significantly increased in vitro protein digestibility of the diet, and microwave irradiation increased starch gelatinization and water solubility ($P<0.05$). Fish fed microwave-treated diet showed highest values in all studied growth indicators and digestive enzyme specific activities (except lipase), with significantly higher amylase specific activity and activity ratio of amylase to trypsin (A/T ratio). Correlation analysis indicated significant relationships ($P<0.05$) among the levels of total protease, amylase and trypsin, and between SGR and A/T ratio. Muscle and body compositions of juveniles fed on microwave- or gamma-irradiated diets were similar to the control, while the juveniles fed on probiotic- or carbohydrase-supplemented diets showed lower protein depositions ($P<0.05$). Similar levels of RNA, RNA/Protein ratio, and Protein/Lipid ratio in body and muscle in all dietary groups fed ad libitum suggested that the improved growth performance in juvenile Siamese fighting fish fed on microwave-irradiated diet may not be only due to improved physicochemical properties of the diet but also improved fish consumption rate.

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## 1. Introduction

Production of Siamese fighting fish (*Betta splendens* Regan, 1910) has been providing the highest income among exported ornamental fish in Thailand. During the fish life span, live diets such as rotifers, infusorians, water fleas (*Moina* sp.) and mosquito larvae are mainly used. Propagation of the live diets mostly uses the wastes from avian and porcine farms that cause the incidence of diseases and environmental impacts. These have contributed to the decrease in survival rate of juveniles and slow growth rate in maturing fish. In order to increase successive growth and survival of juvenile fish, artificial diets with improved nutrient utilization are important. Many methodologies were used to increase nutrient utilization, such as microwave cooking (Alajaji and El-Adawy, 2006; Hu and Wen, 2008; Khatoon and Prakash, 2006; Ma et al., 2009; Negi et al., 2001; Sadeghi and Shawrang, 2006), gamma irradiation (Al-Masri and Guenther, 1999; Chung et al., 2010; Ebrahimi et al., 2009; El-Niely, 2007; Fombang et al., 2005; Yoon et al., 2010), probiotics (Son et al., 2009; Yanbo and Zirong, 2006) and digestive enzymes supplementation (Kumar et al., 2006; Lin et al., 2007; Mohapatra et al., 2002). Carnivorous fishes, including Siamese fighting fish, have limited ability for carbohydrate digestion, especially at the juvenile stage, due to their short intestine and the low activity of carbohydrate digestive enzymes. However, dietary carbohydrate appears to be necessary for improving growth and protein utilization in many fish species (Wilson, 1994). Mohapatra et al. (2002) reported a significant increase in carbohydrate utilization in *Labeo rohita* fry with increasing the level of gelatinized carbohydrate or by supplementing carbohydrases.

The objective of this study was to select a suitable modified method for improving nutrient utilization, based on equal nutritional values, in juvenile Siamese fighting fish. The main feed ingredients were selected based on in vitro digestibility studies of protein and carbohydrate using trypsin activity and amylase activity for standardization, respectively (Thongprajukaew, 2011). The formulated feed was then treated by different procedures for improving nutrient utilization. Digestive enzyme specific activities and muscle compositions were used for determining physiological alterations during the experiment. Digestive enzyme indicators were measured; activity ratio of amylase to trypsin (A/T ratio) for feeding habit and metabolic flexibility of carbohydrate–protein utilizations (Hofer and Schiemer, 1981) and activity ratio of trypsin to chymotrypsin (T/C ratio) for evaluating growth efficiency (Rungruangsak-Torrissen, 2007; Sunde et al., 2001; Sunde et al., 2004). The diet with high carbohydrate content was chosen to test the hypothesis. Two week experiments were conducted at intensive rearing period after juvenile digestive tract was completely developed. These studies could provide knowledge for improving diet quality for rearing juvenile Siamese fighting fish.

## 2. Materials and methods

### 2.1. Experimental diets

#### 2.1.1. Preliminary study

In vitro digestibility was performed for screening appropriate dose for gamma irradiation and appropriate time for microwave irradiation for modifying diets. Appropriate feedstuffs for culturing juvenile Siamese fighting fish were selected based on the in vitro protein and carbohydrate digestibilities, as described by Thongprajukaew (2011). The main feed mixture used for gamma or microwave irradiation contained fish meal (30%), soybean meal (20%), wheat gluten (12%), squid meal (5%) and wheat flour (20%), as shown in Table 1. For gamma irradiation, the main feed mixture was irradiated at the dose of 20, 40, 60 or 80 kGy using $^{60}$Co as gamma irradiation source

**Table 1**
Ingredients of the experimental diets for rearing juvenile Siamese fighting fish.

| Ingredients | Inclusion (%) |
|---|---|
| Fish meal[a] | 30 |
| Soybean meal[a] | 20 |
| Wheat gluten[a] | 12 |
| Squid meal | 5 |
| Wheat flour[a] | 20 |
| Lecithin | 2 |
| Fish oil | 1 |
| Soybean oil | 2.6 |
| Mineral mixture[b] | 0.05 |
| Vitamin mixture[c] | 0.25 |
| Vitamin C | 0.1 |
| Fermented red rice | 2 |
| Cellulose | 5 |

[a] Main ingredients selected from in vitro screening of suitable feedstuffs using the enzyme extracts from juvenile Siamese fighting fish, as described by Thongprajukaew (2011).
[b] Mineral mixtures, 1 kg of feed contained 30 mg iron, 20 mg zinc, 25 mg manganese, 5 mg copper, 5 mg iodine and 0.2 mg selenium.
[c] Vitamin mixtures, 1 kg of feed contained 4000 IU vitamin A, 2000 IU vitamin $D_3$, 50 mg vitamin E, 10 mg vitamin K, 20 mg thiamine, 20 mg riboflavin, 20 mg pyridoxine, 200 mg calcium panthothenate, 150 mg niacin, 2 mg biotin, 5 mg folic acid, 0.2 mg vitamin $B_{12}$, 400 mg inositol and 200 mg ethoxyquin.

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