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Garlic (*Allium sativum*) extract product enhancing digestive physiology and growth performance in marble goby (*Oxyeleotris marmorata*) juvenile

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Introduction

Marble goby (*Oxyeleotris marmorata*) is a carnivorous freshwater fish that important in financial side both local and export market. It is considered to be one of the most promising finfish for aquaculture in Thailand [1]. Culture of marble goby has been increased in several countries of Southeast Asia, especially in Thailand. This fish has been popular in Asian consumer because of its lean, boneless, firm, white flesh and delicious [2].

According to low-activity of marble goby behavior, its culture system and artificial feed has been attempted to develop. However, the yield was lower. Its production at Thailand was only average of 112 tons in 2008-2012 although its demand and price were high [3].

Various herb extracts were reported to improve the growth performance in animals by stimulating action on gut secretions. It was indicated that herbals induce secretion of digestive enzymes and high protein synthesis [4].

Garlic (*Allium sativum* L.) has been used for centuries as a flavoring agent, traditional medicine, and a functional food to enhance the physical and mental health [5]. Garlic composes of major bioactive components, such as specific sulfur compounds (allicin, ajoene, allyl polysulfides, and vinyldithiins) and selenium. Allicin has an effect on various enzymes that can affect metabolism of virulent bacteria [6]. Garlic was studied in different forms of extracts: aqueous, ethanol and dried powder [7]. However, there were few research that investigate its effect to digestive enzymes, in the aquatic animals.

Digestive enzyme is considered a good indicator of the nutritional status of fish [8-10], that are related to the efficacy of fish growth rate were investigated in many fishes to solve the nutritional problem [11]. Which the protease activity ratio of trypsin to chymotrypsin (T/C ratio) has been developed to indicate digestive efficacy and growth, independent of the specific activity levels of the two enzyme [12]. There was reported that intestine is the most important organs in digestion and absorption of nutrients from feed [13].

Therefore, monitoring digestive enzyme activity process is very important. The method of choice in assessing the effects of supplementation of garlic extract in commercial feed for rearing marble goby to increase the immune system. Therefore, we expect to use garlic extraction added-feed for that was applied in raw materials of the feed. This study aims to identify the effects of garlic extract supplement in diet on alterations of digestive enzyme activities in juvenile marble goby.

Materials and methods

Marble goby received from Department of Fisheries of Thailand. The fishes with size of 3.12±0.72 g were fed with natural food (blood worm, N), commercial diet (control feed, C), commercial diet mixed garlic aqueous extract (GAE) and ethanol extract (GEE) at concentration 0.5% (w/w) for 12 weeks. After 4, 8 and 12 weeks of feeding, major digestive organs (esophagus, stomach, midgut and hindgut) were collected for measurement of digestive enzyme activities (trypsin, chymotrypsin, amylase and lipase) and histology, prepared for H&E staining permanent slide and observed by compound microscope. The growth parameters including specific growth rate (SGR), weight gain (WG) and feed efficiency (FE)were calculated; and statistical analysis were performed with one-way ANOVA at confidence value of $P < 0.05$.

Results and discussion

For digestive enzyme activity, amylase showed the significant different ($p < 0.05$) at 4 and 8 weeks from GEE and GAE groups. Whereas lipase activity of GAE showed that not significant different ($P>0.05$) at 12 weeks. T/C ratio in the digestive tract of marble goby fed with GAE for rearing to 12 weeks were summarized in Table 1. Maliika et al. [16] found that groups of feed with diet containing garlic extracts induced activities of all four digestive enzymes in all sizes of sand goby every group of feed containing 3.0% (w/w) garlic extracts showed highest value of all digestive enzyme activities and significantly different ($P<0.05$).

This study results demonstrates strong activities for garlic aqueous extract (GAE) supplemented in fish feed for the marble goby. Due to the feed of carnivorous fish mainly composed of lipid and protein nutritional. This ratio was reflecting the secretion of pancreatic enzyme [17].

Table 1. Digestive enzyme specific activity (mean ± SD) of marble goby fed various experimental diets for 12 weeks

Digestive enzyme	Amylase (mg maltose/h/mg protein)	Lipase (OD increased/h/mg protein)	T/C
4 week			
N	5.92±0.20^{a}	58.33±16.50^{a}	1.14±0.47^{a}
C	7.71±1.53^{a}	70.93±13.73^{a}	2.50±1.32^{a}
GAE	8.98±0.61^{a}	82.45±20.15^{a}	5.75±2.54^{a}
GEE	20.06±1.69^{b}	90.13±18.32^{a}	6.94±4.90^{a}
8 week			
N	61.13±8.92^{a}	386.98±34.55^{a}	9.95±4.80^{a}
C	106.48±32.89^{ab}	324.77±137.98^{a}	7.33±1.40^{a}
GAE	141.70±29.22^{b}	382.24±303.36^{a}	5.21±4.77^{a}
GEE	98.06±20.75^{ab}	415.40±106.10^{a}	18.05±8.55^{a}
12 week			
N	29.81±8.94^{a}	146.11±21.19^{b}	2.63±0.34^{a}
C	40.98±3.97^{a}	5.19±0.12^{a}	1.34±0.20^{a}
GAE	32.21±11.02^{a}	173.65±69.40^{b}	3.13±1.43^{a}
GEE	29.11±5.52^{a}	11.15±1.54^{a}	2.94±1.40^{a}

N: natural food; C: commercial diet, GAE: commercial diet mixed garlic aqueous extract and GAE: commercial diet mixed garlic ethanol extract.

For histology analysis, GAE and GEE showed that villi heights were longer and tissue structure was more complete than N and C groups (Table 2). Midgut villi, the important role for nutrient absorption, were patently increased in GAE and GEE. Interestingly, all growth parameters of GAE and GEE were significantly different ($p<0.05$).

For growth performance, at the end of experiment; a trend of growth rates in fish fed with GEE showed the significantly increase in SGR (0.91±0.12), WG (2.14±0.30) and FE (5.86±0.68), respectively. This result is agreement with those in other fish species. Domg et al. [18] found that in dietary garlic extract (2.0%, w/w) could improve the growth of Juvenile sterlet sturgeon (*Acipenser ruthenus*), and Annita et al. [19] found that difference of levels dietary lipid feed in juvenile marble goby for 15 weeks. This study results showed that the highest growth performance and feed utilization in fish lipid 10% to be optimum for growth from 2.76 g to 13.76 g body weight.

Table 2. Length, width, and area (mean ± SD) of villi in midgut of marble goby fed various experimental diets for 12 weeks

Parameters	Length (mm)	Width (mm)	Area (mm^2)
Initial (4 wk)			
N	0.387±0.02	0.132±0.02	0.025±0.00^{a}
C	0.430±0.16	0.155±0.02	0.034±0.01^{ab}
GAE	0.510±0.19	0.262±0.07	0.063±0.02^{b}
GEE	0.457±0.07	0.158±0.01	0.036±0.01^{ab}
Week 12			
N	0.913±0.03	0.192±0.03	0.091±0.06^{a}
C	1.050±0.27	0.265±0.01	0.139±0.03^{a}
GAE	0.983±0.10	0.215±0.03	0.107±0.02^{a}
GEE	0.987±0.09	0.210±0.01	0.103±0.00^{a}

N: natural food; C: commercial diet, GAE: commercial diet mixed garlic aqueous extract and GAE: commercial diet mixed garlic ethanol extract.

Conclusions

The experimental study results suggested that garlic extract could increase the enzyme activities and morphology of digestive system. Therefore, this study performance and results could be available for development of artificial feed formulation that appropriate for marble goby.

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