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The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

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AquacultureHub

http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH - Kibbutz Ein Hamifratz, Mobile Post 25210, ISRAEL

Phone: + 972 52 3965809

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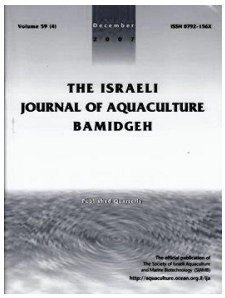

Effects of Dietary Surfactin Supplementation on Growth, Digestive Enzyme Activity, and Antioxidant Potential in the Intestine of Growth Retarded Marbled Eel (*Anguilla marmaorata*) at Elver Stage

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Keywords: *surfactin; marbled eel; growth; digestive enzyme; antioxidant potential*

Abstract

A 70 day trial was conducted to investigate the effects of surfactin on growth, digestive enzyme activity, and antioxidant potential in the intestine of growth retarded marbled eels (*Anguilla marmorata*) at the elver stage. Six hundred and forty marbled eels at elver stage were randomly divided into four treatment groups with four replicates per group, and 40 fish per replicate. The dietary surfactin level of the four experimental diets was 0, 25, 50, and 100 mg/kg, respectively. Final body weight, weight gain rate, survival rate, protease activity in the intestine, malondialdehyde level, total antioxidation capacity level, and activities of superoxide dismutase, and glutathione peroxidase in the intestine of fish, increased significantly with surfactin supplementation, and feed conversion rates improved significantly ($P<0.05$). No significant differences of growth performance, lipase activity (except in the 25 mg/kg surfactin supplementation group), amylase activity and CAT activities were found among surfactin supplemented groups ($P>0.05$). Results demonstrated that dietary surfactin supplementation could improve growth performance, some digestive enzyme activities, and antioxidant potential, in the intestine of growth retarded marbled eel at elver stage. Surfactin could be used as a dietary growth promoter for growth retarded eels.

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Introduction

Anguillid eels are very important for aquaculture. They are high in commercial value and have been cultured in both extensive and intensive systems mainly in Asia (Heinsbroek, 1991). Natural stocks of eels, especially European eel and Japanese eel, are decreased at a rapid rate due to over fishing, and environmental destruction (Arai et al., 2013). The price of eels has increased sharply. Some Chinese eel farmers have tried to culture other less expensive eel species. Marbled eel (*Anguilla marmorata*) is a typical catadromous fish. It is widely distributed throughout the tropical and subtropical regions of western-central Pacific and Indian Oceans as well as their freshwater systems (Ege, 1939; Luo et al., 2013). It is important to the aquaculture industry in Southeast Asia (Huang et al., 2016), and has been cultured in freshwater farms in China for many years (Luo et al., 2013).

In the cultivation process, about 10-30% of the eels are growth retarded causing significant economic losses. This may be due to mismanagement of starter feeding, contaminated environmental conditions, and disease (Huang, 2012). Results of research on feeds and drugs such as antibiotics and hormones have aided in promoting growth of retarded eels, however these may have health safety issues. (Siwicki et al., 2013; Ni et al., 1992; Wang et al., 1999). Results have indicated that there are differences in the digestive enzyme activities in the intestines of normal and retarded eels. This suggests that supplementation of intestinal health promoters in feeds might promote growth of these eels (Huang, 2012).

Surfactin is an antimicrobial lipopeptide produced by several strains of *Bacillus subtilis*. It has been characterized as anti-bacterial, anti-viral, anti-fungal, biosurfactant, immunoadjuvant, anti-tumor, antimycoplasma, and promoter of hemolytic activities (Seydlova and Svobodova,2008; Gao et al., 2013; Chen et al., 2015). In our previous studies, use of surfactin as antimicrobial peptides (AMPs) and feed emulsifier, improved growth, some digestive enzyme activities, and health status in the intestine of tilapia (*Oreochromis niloticus*) (Shi et al., 2014; Shi, 2015; Zhai et al., 2015a). Little information is available regarding surfactin supplementation in diets of retarded eels. The purpose of the present study was to investigate whether dietary surfactin supplementation may be beneficial to growth, digestive enzymes activity, and antioxidant parameters in the intestine of growth retarded marbled eel (*Anguilla marmorata*) at elver stage.

Materials and Methods

Experimental design and animal. After a four week adaptation period to experimental conditions, 640 growth retarded marbled eels (*Anguilla marmorata*) at elver stage with an initial average body weight of 3.02±0.02 g were randomly divided into four treatment groups with four replicates in each group and 40 fish per replicate. Fish were fed diets with surfactin levels of 0 (control group), 25, 50, and 100 mg/kg, respectively. The trial continued for 70 days.

Diets and fish rearing conditions. The basal diet was a commercial feed produced by Zhengyuan Feed Company, Fujian, China. The feed contained 47% protein, 4% lipids, 3% crude fiber, 17% ash, and 10% moisture. Four experimental diets were formulated to contain various concentrations of surfactin. The different levels of surfactin (content >80%, provided by Fujian Zhengyuan Feed Co., Ltd., Putian, China) were well-mixed in the basal diet.

The marbled elvers were purchased at the Development Center for Aquatic Animals of Putian (China). Before the initiation of the experiment, the fish were acclimated in 16 circular PVC tanks (2m×2m×1.5m) with recirculating water systems equipped with bio-filters, UV lights, heaters, and chillers (Huixin Marine Science and Technology Development Co., LTD, Dalian, China). The water temperature was kept at 28-30°C. The water exchange rate was 10% per day; the photoperiod was maintained at 12L:12D; water quality variables were: salinity b 0.2‰; pH 6.6-8.0; dissolved oxygen > 6.3 mg /L; total ammonia nitrogen 0.2-0.5 mg/L; nitrite nitrogen levels <1.0 mg/L. Water quality was monitored twice weekly with a multiparameter photome (HI9804N, HANNA, Baranzate, MI, Italy).

The feed was prepared by adding water to the powder diet to form dough. During the adaptation and trial periods, the fish were fed to apparent satiation twice daily (at 8:00 h and 18:00 h). Any uneaten feed was siphoned out 1 h after feeding and dried. Total amount of daily consumed feed was calculated.

Sample collection and analysis. At the end of the trial, six fish were randomly collected from each replicate and anesthetized by dipping in 50 µl/L of eugenol oil suspension in water for 30s. The fish from each replicate were then weighed and killed in an ice bath for intestine samples. The intestines from each replicate were pooled and homogenized in 10 volumes (v/w) of ice-cold normal saline (0.68%). The homogenates were centrifuged at 10,000 g for 15 min at 4°C and the supernatants with the enzyme extracts were collected and stored at -80 °C until assayed.

The amylase, lipase, and protease activities in the intestine were measured according to the methods of Zhai and Liu (2014). The malondialdehyde level (MDA), total antioxidant capacity level(T-AOC), glutathione peroxidase (GSH-Px) activity, superoxide dismutase (SOD) activity and catalase (CAT) activity were measured according to the methods of Zhai et al. (2015b). Total protein content of supernatant was assayed by method of the Bradford (1976). The level of MDA was expressed as nmol/mg protein. The values of T-AOC, GSH-Px, SOD and CAT activities were expressed as units per mg protein.

Data Calculation. At the beginning and the end of the trial, body weight was measured for fish in each tank after 1 day of feed deprivation. Feed consumption was recorded. Initial body weight (IBW) and final body weight (FBW) of fish, weight gain rate (WGR), feed conversion ratio (FCR), and survival rate (SR) were calculated as follows:

IBW (g/fish) = initial body weight of fish (g)/ initial number of fish;

FBW (g/fish) = final body weight of fish (g)/ final number of fish;

WGR (%) = 100× [final wet weight (g) - initial wet weight (g)]/ initial wet weight (g);

FCR = feed intake (g)/weight gain (g);

SR (%) = 100× (final number of fish/initial number of fish).

Statistical analysis. Results are presented as means ± SD of four replicates. Statistical analysis was performed with SPSS 11.5 statistical software (SPSS, Chicago, IL, USA). Data from each treatment group were subjected to one-way analysis of variance (ANOVA).When overall differences were significant ($P<0.05$), Duncan's multiple range test was used to compare the mean values among the treatment groups. Data expressed as percentages or ratios were subjected to arcsine transformation prior to statistical analysis.

Results

Growth performance and Survival. Parameters of growth performance and survival of marbled eels are shown in table 1. The FBW, WGR, and SR in the surfactin treatment groups were significantly higher than in the control group ($P<0.05$), and FCR was significantly lower than the control group ($P<0.05$). No significant differences in FBW, WGR, FCR and SR were found among the surfactin supplemented groups ($P>0.05$).

Table 1. Effects of dietary surfactin supplementation on growth and survival parameters of growth retarded marbled eel at elver stage

Item	*Surfactin level (mg/kg)*			
	0	*25*	*50*	*100*
IBW(g/fish)	3.00±0.02	3.06±0.01	3.02±0.06	3.01±0.04
FBW(g/fish)	$3.21±0.04^{a}$	$6.35±0.33^{c}$	$5.28±0.06^{b}$	$5.01±0.21^{b}$
WGR(%)	$7.40±0.63^{a}$	$107.59±9.91^{c}$	$76.27±0.83^{b}$	$69.01±3.73^{b}$
FCR	$8.10±0.42^{b}$	$0.82±0.03^{a}$	$1.04±0.01^{a}$	$1.16±0.09^{a}$
SR(%)	$81.25±1.77^{a}$	$91.25±1.77^{b}$	$90.00±3.53^{b}$	$91.67±1.44^{b}$

IBW= initial body weight; FBW= final body weight; WGR= weight gain rate; FCR= feed conversion ratio;SR = survival rate.

^{abc}Values within the same column with different superscripts were significantly different at $P<0.05$ level.

Digestive enzyme activity in intestine. Digestive enzyme activities in the intestine of marbled eels are shown in Table 2. Compared with control group, protease activity in the intestine of surfactin supplementation groups was significantly higher than the control group ($P<0.05$). There were no significant differences among three surfactin supplementation groups ($P>0.05$). Lipase activity of 25 mg/kg surfactin supplementation group was significantly higher than the control group and the other two surfactin supplementation groups ($P<0.05$). Amylase activity was not significantly affected by surfactin supplementation ($P>0.05$).

Table 2. Effects of dietary surfactin supplementation on digestive enzymes activities in intestine of growth retarded marbled eel at elver stage.

Item	*Surfactin level (mg/kg)*			
	0	*25*	*50*	*100*
Amylase (U/mg prot)	0.90±0.10	0.97±0.07	1.01±0.08	1.00±0.18
Lipase (U/g prot)	77.99±2.48^{a}	117.76±3.82^{b}	76.37±5.42^{a}	79.41±5.56^{a}
Protease (U/mg prot)	381.22±12.02^{a}	454.22±9.15^{c}	416.39±9.61^{b}	410.34±15.67^{b}

^{abc}Values within the same column with different superscripts were significantly different at $P < 0.05$ level.

MDA levels and antioxidant potential in intestine. The MDA levels and antioxidant potential in the intestine of marbled eels are shown in table 3. Compared with control group, the MDA level of surfactin supplementation groups decreased significantly ($P<0.05$), and the T-AOC level, SOD activity and GSH-Px activity were significantly increased ($P<0.05$). No significant differences of CAT activities were found between the control treatment and surfactin supplementation groups ($P>0.05$).

Table 3. Effects of Effects of dietary surfactin supplementation on MDA levels and antioxidant potential in intestine of growth retarded marbled eel at elver stage

Item	*Surfactin level (mg/kg)*			
	0	*25*	*50*	*100*
MDA (nmol/mg protein)	3.61±0.41^{b}	2.66±0.20^{a}	2.87±0.21^{a}	2.62±0.19^{a}
T-AOC (U/mg protein)	0.70±0.08^{a}	1.33±0.05^{c}	1.15±0.06^{b}	1.11±0.05^{b}
SOD (U/mg protein)	19.99±2.06^{a}	32.72±0.85^{c}	28.40±2.45^{b}	29.64±3.08^{bc}
CAT (U/mg protein)	6.06±0.55	5.80±0.66	5.94±0.58	5.83±0.87
GSH-Px (U/mg protein)	113.39±13.76^{a}	182.13±8.5^{b}	192.58±17.44^{b}	196.68±19.39^{b}

MDA= malondialdehyde;T-AOC= total antioxidation capacity;SOD= superoxide dismutase; CAT= catalase; GSH-Px =glutathione peroxidase.

^{abc}Values within the same column with different superscripts were significantly different at $P < 0.05$ level.

Discussion

In the present study, the growth performance and survival rate of growth retarded marbled eel at elver stage were significantly improved by dietary surfactin supplementation. The growth promotion effects of dietary surfactin have also been found in other aquatic animals. Growth performance of tilapia was found to be significantly improved by 12.5mg/kg surfactin supplementation in tilapia diet (Shi et al. 2014). Growth performance of 50 mg/kg surfactin group was best among all treatment groups (Shi, 2015; Zhai et al., 2015a). Growth performance of *Litopenaeus vannamei* was significantly improved with 100mg/kg NT-6 antimicrobial lipopeptide (the mixture of surfactin, iturins and fengycins) (Shi et al. 2014). While there were some differences between different surfactin supplementation levels in promoting growth performance of aquatic animals, these could be attributed to the surface activity of surfactin on different cell membranes of those animals. The biological function of surfactin is dependent on surface activity of the cell membrane (Seydlova and Svobodova, 2008; Chen et al., 2015). Previous reports have shown that surfactin concentration is critical for its effect on cell membranes. The presence of the rigid domains plays an essential role in initial insertion, and surfactin can interact both with the membrane polar heads and the acyl chain region (Seydlova and Svobodova, 2008; Deleu et al., 2013). The strong self-assembly ability of surfactin forms sphere-like micelles and some larger aggregates by adoption of a beta-sheet conformation even at low concentrations (Zou et al., 2010). Surface activity of surfactin may be different on the cell membranes of animal species

(Shi, 2015; Wang et al., 2015). Future studies may demonstrate the complex mechanisms underlying the growth promotion effect of surfactin in growth retarded eel.

In our study, intestinal protease activity was significantly enhanced in growth retarded marbled eel fed diets containing surfactin for 10 weeks. Previous studies with tilapia however have demonstrated that intestinal lipase and protease activities in tilapia increased significantly with 12.5, 25, and 50mg/kg surfactin supplementation, and amylase activity was not affected (Shi et al., 2014; Shi, 2015). Lipase activity in the intestine of tilapia was improved by 50, 100, and 200mg/kg surfactin supplementation (Zhai et al. 2015a). Digestive enzyme activity in growth retarded Japanese eels was lower than in normal eels (Huang, 2012). The increase in some digestive enzyme activities induced by surfactin could have promoted digestion and absorption of nutriments from diets and improved the growth of fish (Zhai and Liu, 2014). The improvement of growth retarded marbled eel elvers might be related to the increase of some digestive enzyme activities, which could be result of improved intestinal health arising from AMP supplementation. Surfactin, as one of the AMPs, could be important in maintaining normal gut homeostasis (Shi, 2015). There is emerging evidence that AMPs possess properties which maintain and repair the intestinal epithelial barrier integrity by stimulation of mucus synthesis, promoting the production of tight junction proteins. AMPs can also function as potent immune regulators and thereby protect the intestinal surface (Wang et al., 2015).

SOD, CAT, GSH-Px are the main enzymatic antioxidants produced endogenously in cells to eliminate excess free radicals. Lower levels of MDA and T-AOC have implied that less free radical were generated and antioxidation capacity of fish was improved (Lee et al., 2013; Zhai and Liu, 2014;Zhai et al., 2015b). In the present study, the antioxidant status in the intestines of eel was improved by surfactin supplementation. This might be attributed to the antimicrobial activity of surfactin in the intestine. AMPs have a broad spectrum of antimicrobial activity and may inhibit formation of microbial cells by interaction with their membranes or other mechanisms, such as inhibition of cell-wall synthesis or suppression of nucleic acid or protein synthesis (Wang et al., 2015). The number of *Escherichia coli* in tilapia intestine has been found to decrease and the number of *Lactobacillus* to increase with surfactin supplementation (Shi, 2015). The increase in number of *Lactobacillus* might produce antioxidant enzymes and scavenge the excessive free radicals released by intestinal cells to prevent intestinal oxidative stress damage (Forsyth et al., 2009; Lee et al., 2013; Mandal et al., 2013). Further study is needed to investigate the change of intestinal tract microflora and determine the detailed mechanisms of surfactin in improving intestinal antioxidation of growth retarded eel.

In conclusion, this study has demonstrated that supplementary surfactin in diets could promote growth performance, and improve some digestive enzyme activities and antioxidant potential in the intestines of growth retarded eel (*Anguilla marmorata*) at elver stage.

Acknowledgements

We gratefully acknowledge the financial support provided by Special Project of International Science and Technology Cooperation and Exchange of Ministry of Science and Technology (Grant No.2014DFT30150), Open found of Engineering Research Center of the Modern Industry Technology for Eel, Ministry of Education (Grant No.RE201508) and General Project of Fujian Province Educational Department (Grant No.JA15268).

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