The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from January 2010 The Israeli Journal of Aquaculture - Bamidgeh (IJA) has been published exclusively as an **online Open Access** scientific journal, accessible by all.
Please visit our IJA Website
http://www.aquaculturehub.org/group/israelijournalofaquaculturebamidgehija
for free publications and to enable you to submit your manuscripts.
This transformation from a subscription printed version to an online Open Access journal aims at supporting the concept that scientific peer-reviewed publications and thus the IJA publications should be made available to all for free.

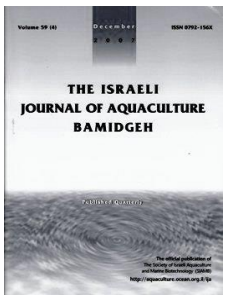
Published by the
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)
in partnership with the
University of Hawaii at Manoa Library
and the
AquacultureHub
A non-profit organization 501c3
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

Published as an open-access journal by the Society of Israeli Aquaculture & Marine Biotechnology (SIAMB).
To read papers free of charge, please register online at
http://www.aquaculturehub.org/group/israelijournalofaquaculturebamidgehija
The sale of *IJA* papers is strictly forbidden



Effects of Dietary Surfactin Supplementation on Growth Performance, Intestinal Digestive Enzymes Activities, and Hepatic Antioxidant Potential of American Eel (*Anguilla rostrata)* Elvers

Shao-Wei Zhai[1*#], Pan-Yue Zhao[1#], Ye Shi[1], Xue-Hao Chen[1], Ying Liang[1,2]

[1]Fisheries College of Jimei University, Engineering Research Center of the Modern Industry Technology for Eel, Ministry of Education, Xiamen, 361021, China

[2]Fujian Province Key Laboratory of Special Aquatic Formula Feed, Fuqing, 350308, China

Keywords: surfactin; American eel; growth; digestive enzymes; antioxidant potential

Abstract

The 70-day trial was conducted to investigate the effects of surfactin on growth performance, intestinal digestive enzymes activities, and hepatic antioxidant potential on American eel (*Anguilla rostrata)* at elver stage. Six hundred American eel elvers were randomly divided into five treatments with three replicates per group, 40 fish per replicate. Dietary surfactin levels of the five treatment groups were 0, 25, 50, 100, and 200 mg/kg, respectively. Final body weight, weight gain rate, and feed efficiency were significantly affected by surfactin supplementation ($P<0.05$). There were no significant differences in feeding rate and survival rate among all groups ($P>0.05$). Protease and lipase activity in the intestine were significantly increased by surfactin supplementation ($P<0.05$), and amylase activity was similar among all groups ($P>0.05$). Malondialdehyde level, total antioxidation capacity level, and activities of superoxide dismutase, and glutathione peroxidase in intestine of fish were significantly ($P<0.05$) affected. No significant differences in CAT activities were found among all groups ($P>0.05$). The results demonstrated that dietary 25 mg/kg surfactin supplementation improves growth performance, some digestive enzyme activities in the intestine, and hepatic antioxidant potential of American eel at elver stage.

[*] Corresponding author: Tel: +86 592 6181420, Fax: +86 592 6181476. e-mail: shaoweizhai@hotmail.com

[#]These two authors contributed equally to this work.

Introduction

Surfactin, the secondary metabolite of numerous strains of *Bacillus subtilis*, is a very powerful biosurfactant. The typical surfactin contains a structure in which a beta-hydroxy fatty acid moiety (C_{13}-C_{15}) is linked to seven amino acids arranged in a chiral sequence such as LLDLLDL (L-glutamic acid, L-leucine, D-leucine, L-valine, L-aspartic acid, D-leucine and L-leucine) (Seydlova and Svobodova, 2008; Zhao et al., 2017). Surfactin is distinguished by a broad spectrum of interesting biological activities such as anti-bacterial, antiviral, anti-fungal, biosurfactant, immunoadjuvant, anti-tumor, antimycoplasma, and hemolytic activities, which depend in the main on its membrane-active properties. As a membrane-active agent, it interacts with the cell membrane and disturbs its integrity (Seydlova and Svobodova, 2008; Chen et al., 2015; Zhao et al., 2017). This effect can explain those biological activities which are of high relevance in health care and biotechnology application.

Surfactin, as an antimicrobial peptide or emulsifier in aquatic feed, has received much attention regarding its application in feed of some fish species (Shi, 2015; Sun, 2016), especially for the growth retarded marbled eel (*Anguilla marmorata*). Dietary surfactin supplementation has demonstrated beneficial effects on growth performance of growth retarded marbled eel, which might be due to enhanced nutrient availability for absorption via suppression of growth and metabolic activities of harmful gut microflora and ameliorations of the intestinal morphology and epithelium thickness (Zhai et al., 2016; 2017a). Little information is available about surfactin supplementation in diets of other eel species.

The American eel (*Anguilla rostrata*) is a widely distributed, facultatively catadromous fish that is reported to range from southern Greenland to the Gulf of Mexico and the Caribbean Sea (Benchetrit and McCleave, 2016). This species has been successfully cultured in freshwater farms in Fujian Province of China since 1996 (Xu, 1998), and is one of the main eel species cultured in China especially after a drastic decrease of natural stocks of European eel and Japanese eel. The quantity of glass eel of *Anguilla rostrata* cultured in China is about 10 tons each year (Fan et al., 2016). The purpose of the present study was to evaluate the effect of dietary surfactin supplementation on growth performance, digestive enzymes activities in intestine, and hepatic antioxidant potential of American eel at elver stage.

Materials and Methods

Experimental animals and husbandry

American eels (1200 in total) at elver stage, were purchased from the Development Center for Aquatic Animals of Putian (China). All fish were cultured in 15 circular PVC tanks, 80 fish per tank with recirculating water systems. After a four-week adaption period, 40 fish were left in each tank as trial subjects. The tanks (2m×2m×1.5m) were equipped with bio-filters, UV lights, heaters, and chillers (Huixin Marine Science and Technology Development Co., LTD, Dalian, China). Water temperature was kept at 24-28°C. Water quality measured variables were: salinity 0.2‰; pH 6.7-7.9; dissolved oxygen > 6.5 mg /L; total ammonia nitrogen 0.1-0.4 mg/L; nitrite nitrogen levels <0.7 mg/L. Water quality was monitored twice weekly with a multiparameter photome (HI9804N, HANNA, Baranzate, MI, Italy). Water exchange rate was 15% per day and photoperiod was maintained at 12 L:12D.

The fish were fed a commercial powder diet produced by Zhengyuan Feed Co., Ltd., Fujian, China. The commercial powder diet contained 47.6% protein, 4.8% lipids, 2.7% crude fiber, 16.8% ash, and 9.7% moisture. The powder diet was mixed with water 1.3 times the diet weight to form dough. The dough was placed on a feeding table and served to the eels. During the adaptation and trial periods, the fish were fed to apparent satiation twice daily (at 7:00 h and 19:00 h) and any uneaten feed was siphoned out 1 h after feeding, and dried. The total amount of feed consumed daily was calculated by subtracting the uneaten food from the eaten food.

Experimental design, animal and diets

After four weeks adaptation to the experimental conditions, 600 American eels (average body weight 6.79±0.12 g) were randomly selected and divided into five treatment groups with three replicates per group, and 40 fish per replicate. All treatment groups were fed the diets with the respective surfactin levels: 0 (control group), 25, 50, 100, and 200 mg/kg). The surfactin (content >95%) was provided by Anhui Kingorigin Biotechnology Co. Ltd., Hefei, China. The trial lasted for 70 days. The basal diet was a commercial powder feed and was the same as during the adaption period.

Sample collection and analysis

At the end of the trial, eight fish from each tank were sampled at random and anesthetized by dipping them into 50 μl/L of eugenol oil suspension for 30s. The fish from each tank were then weighed and killed in an ice bath for gathering of intestine and liver samples. Intestine and liver from the same tank 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 were collected and stored at -80˚C until assayed. The amylase, lipase, and protease activity in intestine were measured according to the methods of Zhai et al. (2016). Malondialdehyde (MDA) levels and total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, catalase (CAT) activity, and glutathione peroxidase (GSH-Px) activity, were measured according to the methods of Liu et al. (2018). The values of T-AOC, GSH-Px, SOD and CAT activities were expressed as units per mg protein. The level of MDA was expressed as nmol/mg protein, and the protein content of supernatant was assayed by method of the Bradford (1976).

Data Calculation

At the beginning and at the end of the trial, body weight was measured for fish in each tank after 24 h of feed deprivation. Consumption of diet was recorded. Initial body weight (IBW) and final body weight (FBW) of fish, weight gain rate (WGR), feed efficiency (FE), feeding rate (FR), 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)×100%;

FE (%) = weight gain (g)/ feed intake (g) ×100%;

FR (%) = feed intake (g)/average body weight of fish (g) ×100%;

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

Statistical analysis

Data expressed as percentages or ratios were subjected to arcsine transformation prior to statistical analysis. Statistical analysis was performed with SPSS 11.5 statistical software (SPSS, Chicago, IL, USA). Results are presented as means ± SD of three replicates. Data from each 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.

Results

Parameters of growth performance and survival

Parameters of growth performance and survival of American eels are shown in table 1. There were significant differences ($P<0.05$) of FBW, WGR, and FE between control group and surfactin groups; 25 mg/kg surfactin group showed best growth performance among the five groups. FR and SR differences were not significant ($P>0.05$)among all the surfactin groups.

Table 1. Growth and survival parameters of American eel at elver stage fed diets with different surfactin levels.

	Surfactin level (mg/kg)				
	0	*25*	*50*	*100*	*200*
IBW(g/fish)	6.73±0.49	6.73±0.40	6.97±0.21	6.83±0.40	6.67±0.25
FBW(g/fish)	10.36±1.45^{b}	12.85±0.49^{a}	12.23±1.24^{a}	11.42±1.29^{ab}	10.43±1.02^{b}
WGR(%)	53.46±11.65^{b}	91.47±15.42^{a}	75.26±12.46^{ab}	67.00±12.53^{ab}	56.30±11.77^{b}
FE(%)	53.76±8.82^{c}	90.04±4.30^{a}	85.89±7.21^{a}	75.14±11.06^{ab}	59.55±11.31^{bc}
FR(%)	0.78±0.01	0.70±0.11	0.63±0.03	0.67±0.15	0.74±0.06
SR(%)	89.17±3.82	95.00±2.50	90.00±4.66	87.50±2.50	89.17±5.20

IBW=initial body weight; FBW= final body weight; WGR= weight gain rate; FE= feed efficiency; FR= feeding rate; SR= survival rate.

^{abc}Values within the same column without the same superscript were significantly different at $P < 0.05$ level. It's the same for the following table.

Activities of digestive enzymes in intestine

Digestive enzyme activities in intestine of American eels are shown in Table 2.

Compared with control group, lipase and protease activity in intestine of surfactin groups increased significantly ($P<0.05$); there were no significant differences ($P>0.05$) among the four surfactin groups. The value of lipase and protease activity of 25 mg/kg surfactin group was highest among all groups ($P<0.05$). Amylase activity was not significantly affected by surfactin supplementation ($P>0.05$).

Table 2. Digestive enzyme activities in intestine of American eel at elver stage fed diets with different surfactin levels

	Surfactin level (mg/kg)				
	0	*25*	*50*	*100*	*200*
Amylase (U/mg prot)	0.77±0.01	0.73±0.01	0.70±0.06	0.64±0.08	0.68±0.10
Lipase (U/mg prot)	25.57±3.80^{a}	31.82±4.99^{b}	32.93±0.65^{b}	33.06±2.10^{b}	34.70±2.74^{b}
Protease (U/mg prot)	23.74±3.75^{a}	55.22±5.62^{b}	51.71±4.87^{b}	46.48±2.11^{b}	41.97±6.18^{b}

^{ab}Values within the same column without the same superscript were significantly different at $P < 0.05$ level.

Parameters of hepatic antioxidant potential

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

Table 3. Parameters of hepatic antioxidant potential of American eel at elver stage fed diets with different surfactin levels

Item	*Surfactin level (mg/kg)*				
	0	*25*	*50*	*100*	*200*
MDA (nmol/ mg prot)	1.70±0.09^{b}	1.45±0.08^{a}	1.53±0.04^{a}	1.53±0.05^{a}	1.54±0.10^{a}
T-AOC (U/mg prot)	8.46±1.34^{a}	13.83±2.39^{b}	12.97±2.64^{b}	12.00±1.48^{b}	10.94±0.78^{ab}
SOD (U/mg prot)	245.48±1.04^{a}	278.21±27.02^{b}	277.05±10.68^{b}	256.29±8.76^{ab}	251.25±7.02^{ab}
CAT (U/mg prot)	27.81±1.84	35.11±4.25	33.50±5.77	34.19±5.51	31.17±1.09
GSH-Px (U/g prot)	13.51±0.75^{a}	23.95±3.53^{b}	22.09±1.41^{b}	22.03±3.49^{b}	22.21±1.28^{b}

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

^{ab}Values within the same column without the same superscript were significantly different at $P < 0.05$ level.

Discussion

In this study, the growth performance and survival rate of American eels at elver stage were significantly affected by dietary surfactin supplementation. The growth performance of 25 mg/kg surfactin group was the best among the five groups. This was consistent with the results of growth retarded marbled eel (Zhai et al., 2016). The phenomenon of growth promotion of dietary surfactin was also found in other fish species. It was found that the surfactin supplementation level for the best growth performance of tilapia (*Oreochromis niloticus*) and orange-spotted grouper (*Epinephelus coioides*) were 50 mg/kg and 100 mg/kg, respectively (Shi, 2015; Sun, 2016). It was obvious that there were some differences in surfactin supplementation levels to promote the best growth performance of different fish species. The biological function of surfactin is dependent on the surface activity in the cell membrane (Seydlova and Svobodova, 2008; Chen et al., 2015). It was concluded that the surface activity of surfactin might be different on cell membranes of different animal species (Shi, 2015; Wang et al., 2015).

The emulsification activity of surfactin no longer increases in relation to the increase of surfactin concentration when the concentration exceeds the critical micelle concentration (Li et al., 2009). Future studies are needed to reveal the complex mechanisms of surfactin on cell membranes of different fish species.

Digestive enzymes activities, apart from amylase in intestine of American eel, were significantly affected by surfactin supplementation. This was consistent with previous studies of surfactin supplementation in diets of tilapia, orange-spotted grouper, and growth retarded marbled eel (Shi, 2015; Sun, 2016; Zhai et al., 2016). Those research studies also reported that the lipase and protease activities in the intestine of those fish species increased significantly by surfactin supplementation, and amylase activity was not significantly affected. The increase in activities of some digestive enzymes induced by surfactin could have promoted digestion and absorption of nutriments from diets and improved the growth of fish (Zhai et al., 2016). The increase of those enzyme activities might be related to the improvement in intestinal health by surfactin supplementation. Surfactin, for its excellent antimicrobial activity, could play an important role in maintaining normal gut homeostasis, including maintenance of intestinal epithelial barrier integrity by stimulation of mucus synthesis, promoting the production of tight junction proteins and repair of the intestinal barrier, regulating mucosal immunity function, and protecting the intestinal physical surface (Shi, 2015; Wang et al., 2015).

SOD, CAT, and GSH-Px are the main enzymatic antioxidants that eliminate free radicals in cells. The antioxidant enzymes play a crucial role in the inhibition of radical generation and prevention of oxidative damage in fish (Ozluer-Hunt et al., 2016; Xie et al., 2017; Liu et al., 2018). Lower MDA levels and higher T-AOC levels implied that fewer free radicals were generated, and antioxidant potential of fish was improved (Lee et al., 2013; Xie et al., 2017; Liu et al., 2018). In the present study, the hepatic antioxidant potential of the American eel was ameliorated with the increasing activities or level of SOD, GSH-Px, and T-AOC and the decreasing level of MDA by surfactin supplementation. Those might be attributed to the antimicrobial activity and the lowering effect of lipid accumulation in liver by surfactin supplementation. The regulatory effects on microflora were further proven in previous studies showing that dietary surfactin supplementation could reduce harmful microflora such as *E.coli* and increase beneficial microflora such as *Lactobacillus* in the intestine of tilapia and growth retarded marbled eel (Shi, 2015; Zhai et al., 2017a). The decreased number of harmful bacteria could generate fewer free radicals to avoid hepatic oxidative stress damage (Mandal et al., 2013; Sun, 2016). Some research has shown that surfactin could enter the cell lipid bilayers, solubilize the fluid phospholipid phase, chelate monovalent, and divalent cations, and modify membrane permeability by channel formation or membrane solubilization by a detergent-like mechanism (Deleu et al. 2013; Ines and Dhouha, 2015). Further study should help to investigate the detailed mechanisms of surfactin in improving liver antioxidant status of American eel. In addition, the appropriate supplementation level of surfactin in diets might exert beneficial effects on lipid metabolism to improve hepatic health status of orange-spotted grouper by lowering fatty acid synthetase levels and increasing of hepatic lipase and protein lipase activities (Zhai et al., 2017b).

In the present study, growth performance and antioxidant parameters were not improved by higher surfactin supplementation levels. Similar results were also reported in the studies of tilapia (Shi, 2015), orange-spotted grouper (Sun, 2016, Zhai et al., 2017b), and growth retarded marbled eel (Zhai et al., 2016). The safety of surfactin oral administration was reported in some acute and sub-acute toxicity studies of experimental animals. (Hwang et al., 2009; Sahnoun et al., 2014; Ben Ayed et al., 2015). It is well known that the toxic effects of surface active agents on biological membranes are clearly dependent on its concentration in the different media. At low concentrations, surfactin inserts exclusively in the outer leaflet of the membrane inducing only a limited perturbation (Francius et al. 2008). However, further addition of surfactin leads to a transient permeabilization of the membrane, or even the complete disruption and solubilization of the lipid bilayer with formation of mixed micelles. Toxic activity of biosurfactant produced by *Bacillus subtilis* A1 was found on all young instars of *A. stephensi*, as well as on adult longevity and fecundity (Parthipan et al. 2018). More

research should be conducted to explain the detailed mechanism of higher surfactin levels on cell membranes of American eels.

In conclusion, the present study showed that dietary 25 mg/kg surfactin supplementation could promote growth performance and improve some digestive enzymes activities, and hepatic antioxidant potential of American eel at elver stage.

Acknowledgement

This study was supported by China Agriculture Research System (CARS-46), Key Science and Technology Project of Fujian Province (2018N0020), open fund of Fujian Province Key Laboratory of Special Aquatic Formula Feed (Fujian Tianma Science and Technology Group Co., Ltd.) (TMKJZ1703) and Innovation and Entrepreneurship Training Program for College Students of Jimei University (201710390291).

References

Ben Ayed H., Nasri R., Jemil N., Ben Amor I., Gargouri J., Hmidet N., Nasri M., 2015. Acute and sub-chronic oral toxicity profiles of lipopeptides from *Bacillus mojavensis* A21 and evaluation of their in vitro anticoagulant activity. *Chem-Biol. Int.*, 236: 1-6.

Benchetrit J., McCleave J.D., 2016. Current and historical distribution of the American Eel *Anguilla rostrata* in the countries and territores of the wider Caribbean. *ICES J. Mar. Sci.,* 73: 122-134.

Bradford M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72:248–254.

Chen W.C., Juang R.S., Wei Y.H., 2015. Applications of a lipopeptide biosurfactant, surfactin, produced by microorganisms. *Biochem. Eng. J.*, 103:158-169.

Deleu M., Lorent J., Lins L., Brasseur R., Braun N., El Kirat K., Nylander T., Dufrêne Y.F., Mingeot-Leclercq M.P., 2013. Effects of surfactin on membrane models displaying lipid phase separation. *BBA –Biomembranes*, 1828(2):801-815.

Fan H.P., Wang M.F., Qin Z.Q., 2016.The development of the world's eel industry from the perspective of eel seedling cultured. *Sci. Fish Farming*, (12):4-5.

Francius G., Dufour S., Deleu M., Paquot M., Mingeot-Leclercq M.P., Dufréne Y.F., 2008. Nanoscale membrane activity of surfactins: influence of geometry, charge and hydrophobicity. *Biochim. Biophys. Acta Biomembr.*, 1778:2058–2068.

Hwang Y.H., Kim M.S., Song I.B., Park B.K., Lim J.H., Park S.C., Yun H.I., 2009. Subacute (28 day) toxicity of surfactin C, a lipopeptide produced by *Bacillus Subtilis*, in rats. *J. Health Sci.,* 55(3): 351-355.

Ines M., Dhouha G., 2015. Lipopeptide surfactants: production, recovery and pore forming capacity. *Peptides*, 71:100-112.

Lee J.S., Cheng H., Damte D., Lee S.J., Kim J.C., Rhee M.H., Suh J.W., Park S.C., 2013. Effects of dietary supplementation of *Lactobacillus pentosus* PL11 on the growth performance, immune and antioxidant systems of Japanese eel *Anguilla japonica* challenged with *Edwardsiella tarda*. *Fish Shellfish Immun.*, 34(3):756-761.

Li Y., 2009. Micellization activity of surfactin in aqueous solution. *Degree Diss.,* East China University of Science and Technology, China.

Liu Y., Hu J., Zhou S., Yang R., Qin J.G., Ma Z., Yang Q., 2018. Effect of Acute ammonia stress on antioxidant enzymes and digestive enzymes in Barramundi *Lates calcarifer* larvae. *Isr. J. Aquacult-Bamidgeh, AquacultureHub,* IJA_70.2018.1508, 12pages.

Mandal A., Paul T., Roy S., Mandal S., Pradhan S., Mondal K., Nandi D.K., 2013. Effect of newly isolated *Lactobacillus ingluviei* ADK10, from chicken intestinal tract on acetaminophen induced oxidative stress in Wistar rats. *Indian J. Exp. Biol.,* 51(2): 174-180.

Ozluer-Hunt A., Ozkan-Yilmaz F., Berkoz M., Engin K., Gunduz S.G., Yalin S., 2016. Effects of dietary nucleotide yeast on immune responses and antioxidant enzyme activities of rainbow trout juveniles (*Oncorhynchus mykiss*). *Isr. J. Aquacult-Bamidgeh, AquacultureHub,* IJA_68.2016.1264, 13pages.

Parthipan P., Sarankumar R.K., Jaganathan A., Amuthavalli P., Babujanarthanam R., Rahman P.K.S.M., Murugan K., Higuchi A., Benelli G., Rajasekar A., 2018. Biosurfactants produced by *Bacillus subtilis* A1 and Pseudomonas stutzeri NA3 reduce longevity and fecundity of *Anopheles stephensi* and show high toxicity against young instars. *Environ. Sci. Pollut. Res.*, 25(11SI): 10471-10481.

Sahnoun R., Mnif I., Fetoui H., Gdoura R., Chaabouni K., Makni-Ayadi F., Kallel C., Ellouze-Chaabouni S., Ghribi D., 2014. Evaluation of *Bacillus subtilis* SPB1 lipopeptide biosurfactant toxicity towards mice. *Int. J. Pept. Res. Ther.,* 20(3): 333-340.

Seydlova G. and Svobodova J., 2008. Review of surfactin chemical properties and the potential biomedical applications. *Cent. Eur. J. Med.*, 3(2):123-133.

Shi Q.C., 2015. The effects of dietary surfactin supplementation on the growth and intestinal health of GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Degree Diss.*, Jimei University, Xiamen, China.

Sun X.W., 2016. Effects of dietary surfactin supplementation on growth performance, lipid metabolism and liver health status of juvenile grouper (*Epinephelus Coioides*). *Degree Diss.*, Jimei University, Xiamen, China.

Wang S., Thacker P.A., Watford M., Qiao S., 2015. Functions of antimicrobial peptides in gut homeostasis. *Curr. Protein Pept. Sci.*, 16:582-591.

Xie J.J., Chen X., Liu Y.J., Tian L.Xia., Xie S.W., Niu J., 2017. Effects of dietary astaxanthin on growth performance, hepatic antioxidative activity, hsp70, and hif-1α gene expression of juvenile golden pompano (*Trachinotus ovatus*). *Isr. J. Aquacult.–Bamid.,* IJA_69.2017.1430, 12 pages

Xu J.R., 1998. A study on aquaculture technology of American eel. *Sci. Fish Farming*, (10):21-32.

Zhai S.W, Chen X. H, Wang, M.H., 2017a. Effects of Different levels of dietary surfactin supplementation on intestinal morphology, and intestinal microflora of growth retarded marbled eel juveniles (*Anguilla marmaorata*). *Isr. J. Aquacult-Bamid.*, IJA_69.2017.1433, 7 pages.

Zhai S.W., Shi Q.C., Chen X.H., 2016.Effects of dietary surfactin supplementation on growth, digestive enzymes activities and antioxidant potential in intestine of growth retarded marbled eel (*Anguilla marmorata*) at elver stage. *Isr. J. Aquacult-Bamid.*, IJA_68.2016.1282, 7 pages.

Zhai S.W., Sun X.W., Chen X.H., 2017b. Effects of antimicrobial peptides surfactin administration on growth performance, intestinal digestive enzymes activities and some serum biochemical parameters of orange-spotted grouper (*Epinephelus coioides*) juveniles. *Isr. J. Aquacult-Bamid.*, IJA_69.2017.1380, 7 pages.

Zhao H., Shao D., Jiang C., Shi J., Li Q., Huang Q., Rajoka M.S.R., Yang H., Jin M., 2017. Biological activity of lipopeptides from *Bacillus*. *Appl. Microbiol. Biotechnol.,* 101:5951-5960.