Effects of dietary surfactin supplementation on growth performance, intestinal digestive enzymes activities and some serum biochemical parameters of tilapia (*Oreochromis niloticus*) fingerlings

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**ABSTRACT**

This trial was conducted to investigate the effects of supplementary surfactin on growth performance, intestinal digestive enzymes activities and some serum biochemical parameters of tilapia (*Oreochromis niloticus*) fingerlings. Three hundred and twenty fish were randomly divided into four treatment groups with four replicates in each group and 20 fish in each replicate. The dietary surfactin levels of four treatment groups were 0 (control group), 50, 100 and 200 mg/kg. The trial period was 7 weeks. Compared with the control group, final body weight, daily feed intake, weight gain rate, feed conversion ratio and protein efficiency ratio were improved significantly by surfactin supplementation (*p* < 0.05). No significant differences of survival rate were found between the control group and all surfactin supplementation groups (*p* > 0.05). The activities of protease and lipase in intestine of surfactin supplementation groups were significantly higher than those of the control group (*p* < 0.05), the amylase activities were similar among all treatment groups (*p* > 0.05). The activities of aspartate aminotransferase and alanine aminotransferase were decreased significantly by dietary surfactin supplementation (*p* < 0.05), the levels of blood urea nitrogen, triglyceride, total cholesterol and low-density lipoprotein cholesterol were also decreased significantly (*p* < 0.05), while lysozyme activity and the high-density lipoprotein cholesterol level were increased significantly by the surfactin supplementation (*p* < 0.05). In conclusion, we demonstrated a promotion of growth performance and improvement of intestinal digestive enzymes activities and some serum biochemical parameters of tilapia fingerlings by dietary surfactin supplementation.

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Digestive enzymes; growth performance; serum biochemical parameters; surfactin; tilapia

**Introduction**

The antibiotics have been routinely used in intensive aquaculture to promote health and enhance disease prevention for decades (Darwish & Hobbs 2005). However, the abuse of antibiotics has been reported to be associated with antibiotic residues and potential bacterial resistance, and this resistance was transferable to bacteria in humans (van den Bogaard & Stobberingh 2000). As a result, the widespread use of various antibiotic feed additives was banned by the European Union in 1997 (Acar et al. 2000). Thus, there is an urgent need for effective and environmentally friendly new additives to maintain the performance and health status of aquatic animal. In this context, antimicrobial peptides (AMPs) are believed to be one of the novel candidates, due to their natural antimicrobial properties, broad-spectrum activities, speed of action and a low propensity for the development of bacterial resistance. AMPs are produced by several species including bacteria, insects, amphibians and mammals as well as by chemical synthesis and genetically engineered micro-organisms (Wang et al. 2015). In recent years, studies on AMPs and their applications have become one of the hot spots in the areas of agricultural science, biology, medicine, and physiology as well as having potential applications in medicine and the food industry (Xiao et al. 2015). Supplementation with some AMPs has been reported to have positive effects on growth performance and immune function of fish (Peng et al. 2007; Zhou et al. 2008; He et al. 2013; Shi et al. 2014a).

Surfactin, an antimicrobial lipopeptide produced by several strains of *Bacillus subtilis*, has received much attention during the past two decades because it exhibits numerous pharmaceutical activities as AMPs. The chemical structure of surfactin is composed of seven amino acids that are bonded to the carboxyl and hydroxy groups on long chain fatty acids...
Materials and methods

Experimental design, diets and fish rearing conditions

After adaptation to experimental condition, 320 tilapia fingerlings with the initial average body weight of 12.01 ± 0.03 g were randomly divided into four treatment groups with four replicates in each group and 20 fish in each replicate. Fish were fed the diets with the surfactin levels being 0 (control group), 50, 100 and 200 mg/kg, respectively. The trial continued for 7 weeks.

Ingredients and proximate analyses of basal diet are presented in Table 1. Four experimental diets were formulated to contain various concentrations of surfactin (0, 50, 100 and 200 mg/kg of dry matter). The different levels of surfactin (content >80%, provided by Fujian Zhengyuan Feed Co., Ltd., Putian, China) were supplemented in the basal diet. All feed ingredients were thoroughly mixed and cold pelletized with a laboratory-pelletting machine using a 2.5-mm diameter module. After processing, the diets were packed into small bags and stored at −20 °C until used.

Healthy tilapia (O. niloticus), purchased in Development Center for Aquatic Animal of Zhangzhou (China), were acclimatized in two plastic tanks (200 cm × 90 cm × 100 cm), and, during the adaptation period, were fed a commercial diet (produced by Zhengyuan Feed Company, Fujian, China) three times daily for 4 weeks. The commercial diet contained 29% protein, 6% lipids, 4% crude fiber, 13% ash and 10% moisture. After adaptation to experimental condition, the fish were kept in 16 rectangular aquaria (120 cm × 60 cm × 70 cm). The volume of aquaria was about 500 L. Aerated water was supplied to the culture system with additional aeration provided by an air pump. The amount of the daily changing water was 50%. Fish were fed to satiation three times daily (at 8:00 h, 13:00 h and 19:00 h). Uneaten pellets were drawn out through a siphon. The water quality was monitored twice weekly with a multiparameter photometer (HI9804N, HANNA, Baranzate, MI, Italy). The values of dissolved oxygen, pH and ammonia-N ranged between 7 and 8 mg/L, 6.8 and 7.5 and 0 and 0.2 mg/L, respectively. The water temperature ranged from 22 to 28 °C.

Sample collection and analysis

At the end of the trial, five fish were sampled at random from each replicate and anaesthetized by dipping in 50 μL/L of eugenol oil suspension in water for 30 s, and then the blood sample was collected from the caudal vein of each anaesthetized fish. After kept at 4 °C for 30 min, the blood sample was centrifuged at 3500 rpm for 10 min at 4 °C, and the supernatant serum was collected and stored at −80 °C prior to analysis of serum biochemical parameters. After collecting blood samples, then the fish from each replicate were weighed and killed in an ice bath for intestines samples. The intestine from same replicate were pooled and homogenized in 10 volumes (v/w) of ice-cold normal saline (0.68%). The homogenates were centrifuged at 10,000 rpm for 15 min at 4 °C and the supernatants with the enzyme extracts collected and stored at −80 °C until assayed.

Table 1. Ingredients and proximate analyses of basal diet for tilapia fingerlings.

| Ingredients | g/kg | Nutrient level |
|-------------|------|----------------|
| Fish meal   | 50   | Crude protein, % 30.4 |
| Soybean meal| 150  | Crude fat, % 5.7 |
| Rapeseed extraction | 200 | Crude ash, % 12.3 |
| Cotton seed meal | 200 | Digestible energy 12.8 |
| High-gluten flour | (calculated value), MJ/kg |
| Rice bran   | 150  |               |
| Soybean oil | 20   |               |
| Monocalcium phosphate | 20 |               |
| Choline chloride | 2  |               |
| Vitamin premixa | 2 |               |
| Mineral premixb | 6 |               |

*Vitamin premix (mg/kg diet): thiamin, 0.25; lactoflavin, 0.25; Nicotinic acid, 1.0; pantothentic acid calcium, 1.25; folic acid, 0.075; biotin, 0.03; hydrochloric acid pyridoxine, 0.2; cobalt amine, 0.0005; vitamin C, 5; vitamin K, 0.2; inositol, 10; vitamin E, 2; vitamin A, 0.2.

*Mineral premix (mg/kg diet): NaCl, 1.0; MgSO4·7H2O, 15; NaH2PO4·2H2O, 25; KH2PO4, 32; Ca(H2PO4)2·H2O, 20; FeSO4, 2.5; calcium lactate, 3.5; ZnSO4·7H2O, 0.353; MnSO4·4H2O, 0.162; CuSO4·5H2O, 0.031; CoCl2·6H2O, 0.01; KIO3, 0.003.
The activities of amylase, lipase and protease in intestine were measured according to method of Zhai and Liu (2014). The measurement of serum parameters and proximate composition of experimental diets were performed according to the method as previously described (Zhai et al. 2014). The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lysozyme (LZM) and levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and blood urea nitrogen (BUN) in serum were determined with commercial clinical investigation kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All serum parameters were analyzed by an automatic biochemical analyzer (Hitachi 7020, Tokyo, Japan).

Data calculation

At the beginning and at the end of the trial, body weight was measured for the fish in each aquaria after 1 d of feed deprivation. The consumption of diet was recorded. The initial body weight (IBW) and final body weight (FBW) of fish, daily feed intake (DFI), weight gain rate (WGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR) were calculated as follows:

\[
\text{IBW(g/fish)} = \frac{\text{initial body weight of fish (g)}}{\text{initial number of fish}};
\]

\[
\text{FBW(g/fish)} = \frac{\text{final body weight of fish(g)}}{\text{final number of fish}};
\]

\[
\text{DFI(g/d)} = \frac{\text{feed consumption per fish(g)}}{\text{trial period (d)}};
\]

\[
\text{WGR(\%)} = 100 \times \frac{\text{final wet weight(g) – initial wet weight (g)}}{\text{initial wet weight (g)}};
\]

\[
\text{FCR} = \frac{\text{feed intake(g)}}{\text{weight gain(g)}};
\]

\[
\text{PER(\%)} = \frac{\text{final wet weight(g) – initial wet weight (g)}}{\text{feed protein intake(g)}};
\]

\[
\text{SR(\%)} = 100 \times \left(\frac{\text{final number of fish}}{\text{initial number of fish}}\right).
\]

Statistical analysis

Statistical analysis was performed with SPSS 11.5 statistical software (SPSS 2002). The results are presented as means ± SD of four replicates. 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 (Zhai et al. 2014).

Results

Growth performance and survival rate

The values of growth performance parameters are presented in Table 2. The FBW and WGR of surfactin supplementation groups were significantly higher than those of the control group (p < 0.05), and there were significant differences among the surfactin supplementation groups (p < 0.05). The DFI of surfactin supplementation groups were significantly higher than those of the control group (p < 0.05), and the PER of surfactin supplementation groups (except 200 mg/kg supplementation group) were significantly higher than those of the control group (p < 0.05). Significant differences of DFI were found between 50 mg/kg surfactin supplementation group and the other two surfactin supplementation groups (p < 0.05), the differences of PER were significant between 50 mg/kg surfactin supplementation group and 200 mg/kg surfactin supplementation groups (p < 0.05). The FBW, DFI, WGR and PER of the 50 mg/kg surfactin supplementation group were highest among all groups. FCR of surfactin supplementation groups were significantly lower than that of the control group (p < 0.05), there were significant differences between the 50 mg/kg surfactin supplementation group and the other two surfactin supplementation groups.

Table 2. Effects of dietary surfactin supplementation level on growth performance of tilapia fingerlings.

| Items                        | Dietary surfactin supplementation level (mg/kg) |
|------------------------------|-------------------------------------------------|
|                             | 0      | 50    | 100   | 200   |
| IBW, g/fish                 | 12.05 ± 0.03a | 12.02 ± 0.02a | 11.99 ± 0.02a | 11.98 ± 0.02a |
| FBW, g/fish                 | 73.18 ± 0.73a | 95.93 ± 0.70a | 87.50 ± 1.12a | 81.41 ± 1.17a |
| DFI, g/fish                 | 1.72 ± 0.03a | 2.04 ± 0.04c | 1.91 ± 0.03ab | 1.30 ± 0.03bc |
| WGR, %                      | 507.30 ± 5.94a | 698.09 ± 6.72a | 629.77 ± 15.44c | 579.55 ± 20.13b |
| PER, %                      | 238.37 ± 7.84a | 276.43 ± 9.10c | 265.28 ± 10.04bc | 253.04 ± 9.53ab |
| SR, %                       | 100a   | 100a   | 100a   | 100a   |

Values in the same line with different superscripts significantly differ (p < 0.05).

IBW: initial body weight; FBW: final body weight; DFI: daily feed intake; WGR: weight gain rate; FCR: feed conversion ratio; PER: protein efficiency ratio; SR: survival rate.
groups ($p < 0.05$), FCR of the 50 mg/kg surfactin supplementation group were lowest among all treatment groups. SR of all treatment groups were not affected by surfactin supplementation ($p > 0.05$).

### Activities of digestive enzymes

Digestive enzymes activities in intestine of tilapia fed with different level of surfactin are showed in Table 3. Compared with the control group, the activities of protease and lipase in intestine of surfactin supplementation groups were significantly higher than those of the control group ($p < 0.05$), there were no significant differences among three surfactin supplementation groups ($p > 0.05$). The amylase activities were not significantly affected by surfactin supplementation ($p > 0.05$).

### Serum biochemical parameters

Serum biochemical parameters of tilapia fed with different level of surfactin are showed in Table 4. Compared with the control group, the activities of ALT and AST of surfactin supplementation groups were significantly decreased ($p < 0.05$). There were significant differences of ALT activities among three surfactin supplementation groups ($p < 0.05$), the differences of AST activities between the 50 mg/kg surfactin supplementation group and other two surfactin supplementation groups were also significant ($p < 0.05$). The levels of TC, TG and LDL-C of surfactin supplementation group were significantly lower than those of the control group ($p < 0.05$), the HDL-C levels and LZM activities were only found between the 50 mg/kg surfactin supplementation group and other treatment groups ($p < 0.05$).

### Discussion

In the present study, the growth performance of tilapia fingerlings was significantly improved by dietary surfactin supplementation. Similar results were found in the study of Shi et al. (2014a), who observed that the growth performance of tilapia could be significantly improved by 12.5 mg/kg surfactin supplementation in tilapia diet. Shi et al. (2014b) showed that 100 mg/kg NT-6 antimicrobial lipopeptide (the mixture of surfactin, iturins and fengycins) had significant improvement on growth performance of Litopenaeus vannamei. The positive effects of other AMPs on growth performance of aquatic animal were also demonstrated. Peng et al. (2007) found that 100 mg/kg antibacterial peptides (derived from intestine of chicken, pig and rabbit) could promote tilapia growth. Zhou et al. (2008) observed that dietary 15 mg/kg apidaecin (AMPs from bee) supplementation could improve growth performance of common carp (Cyprinus carpio). The study of He et al. (2013) suggested that feed supplementation with 0.2–0.4% antibacterial peptides extracted from Bacillus subtilis fmnJ had the potential to promote growth in M. amblycephala. Besides, supplementations with antimicrobial lipopeptide containing surfactins, iturins and fengycins were reported to have positive

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**Table 3. Effects of dietary surfactin supplementation level on activities of digestive enzymes in intestine of tilapia fingerlings.**

| Items        | Dietary surfactin supplementation level (mg/kg) |
|--------------|-----------------------------------------------|
|              | 0     | 50    | 100   | 200   |
| Amylase, U/mg prot | 1.09 ± 0.04<sup>a</sup> | 1.08 ± 0.06<sup>a</sup> | 1.06 ± 0.02<sup>a</sup> | 1.03 ± 0.04<sup>a</sup> |
| Lipase, U/g prot    | 4.51 ± 0.12<sup>b</sup>    | 5.21 ± 0.24<sup>b</sup> | 5.07 ± 0.28<sup>b</sup> | 5.22 ± 0.19<sup>b</sup> |
| Protease, U/mg prot | 24.94 ± 0.47<sup>c</sup> | 27.02 ± 0.19<sup>c</sup> | 26.38 ± 0.56<sup>c</sup> | 26.00 ± 0.23<sup>c</sup> |

<sup>a,b</sup>Values in the same line with different superscripts significantly differ ($p < 0.05$).

**Table 4. Effects of dietary surfactin supplementation level on serum biochemical parameters of tilapia fingerlings.**

| Items                  | Dietary surfactin supplementation level (mg/kg) |
|------------------------|-----------------------------------------------|
|                        | 0     | 50    | 100   | 200   |
| BUN, mmol/L            | 1.89 ± 0.07<sup>b</sup> | 1.63 ± 0.03<sup>b</sup> | 1.80 ± 0.01<sup>b</sup> | 1.87 ± 0.08<sup>b</sup> |
| ALT, U/mg prot         | 19.36 ± 0.82<sup>c</sup> | 10.71 ± 0.21<sup>c</sup> | 14.08 ± 0.61<sup>c</sup> | 16.72 ± 0.12<sup>c</sup> |
| AST, U/mg prot         | 49.96 ± 3.25<sup>c</sup> | 32.01 ± 1.48<sup>c</sup> | 37.31 ± 2.35<sup>c</sup> | 37.75 ± 1.43<sup>c</sup> |
| LZM, U/mL              | 11.84 ± 0.08<sup>a</sup> | 15.47 ± 0.52<sup>b</sup> | 12.29 ± 0.29<sup>b</sup> | 12.33 ± 0.08<sup>a</sup> |
| TC, mmol/L             | 7.69 ± 0.32<sup>a</sup> | 5.43 ± 0.16<sup>a</sup> | 5.85 ± 0.08<sup>a</sup> | 6.20 ± 0.10<sup>a</sup> |
| TG, mmol/L             | 4.42 ± 0.04<sup>c</sup> | 3.40 ± 0.26<sup>c</sup> | 3.44 ± 0.16<sup>c</sup> | 4.41 ± 0.04<sup>c</sup> |
| LDL-C, mmol/L          | 3.44 ± 0.29<sup>c</sup> | 1.45 ± 0.23<sup>c</sup> | 1.99 ± 0.03<sup>c</sup> | 1.95 ± 0.08<sup>c</sup> |
| HDL-C, mmol/L          | 2.30 ± 0.12<sup>c</sup> | 2.66 ± 0.12<sup>c</sup> | 2.74 ± 0.03<sup>c</sup> | 2.56 ± 0.09<sup>c</sup> |
| BUN: blood urea nitrogen; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LZM: lysozyme; TG: triglyceride; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

<sup>a,b,c</sup>Values in the same line with different superscripts significantly differ ($p < 0.05$).
suggest a potential enhancement of synthesis of surfactin. The concentration of surfactin supplementation groups might be associated with the antimicrobial activity and function as potent immune regulators of AMPs (Hume 2011; Xiao et al. 2015; Wang et al. 2015). Further study is needed to reveal the detail mechanisms of surfactin-promoting growth. Besides, some intestinal digestive enzymes activities and serum biochemical parameters were improved in present study, which might also be related to the growth promotion effect of surfactin in tilapia diet (Li 2015; Zhai et al. 2015). Previous reports concerning the effects of surfactin on digestive enzymes in fish are scarce at present. In this study, except for amylase, the activities of digestive enzymes in intestine were significantly enhanced after tilapia was fed surfactin-supplemented diets for 49 d. The mechanisms of surfactin promoting the activities of digestive enzymes are probably involved in the improvement of intestinal morphology. The villus height of intestine is indicative of the gut health in fish. Increasing the villus height suggest an increased surface area capable of greater absorption of available nutrients (Caspary 1992). It was reported that tilapia fed diet supplemented with AMPs surfactin had increased villus height of intestinal tract, which was related to the functions of surfactin in gut homeostasis (Shi 2015). There is emerging evidence that AMPs possess properties in maintenance of intestinal epithelial barrier integrity by stimulation of mucus synthesis, promoting the production of tight junction proteins and repair of the intestinal barrier, and the AMPs can also function as potent immune regulators and protect the intestinal surface (Wang et al. 2015).

The measurements of blood biochemistry parameters are commonly used as diagnostic tools in biomonitoring, allowing the pathophysiological changes attributable to nutrition to be detected. Blood enzyme values (AST, ALT and LZ) and the levels of energetic and protein metabolites (TC, TG and BUN) of fish are considered important diagnostic characters. Often their values are used in estimating the health and condition of fishes (Coz-Rakovac et al. 2005; Zhai et al. 2014). Reports about effect of AMPs on blood biochemical parameters in aquatic animal were very limited. The change in serum BUN concentrations reflects the whole body status of amino acid metabolism and utilization in animals. Thus, decrease of serum BUN concentration of surfactin supplementation groups might suggest a potential enhancement of synthesis of protein in animals (Wu et al. 2013). The increased activity of AST and ALT in serum, in the absence of acute necrosis or ischaemia of other organs such as myocardium, suggests liver cell damage and leaching of these enzymes in blood (Kumar et al. 2013). The lower levels of AST and ALT of surfactin supplementation groups in this study indicated that tilapia hepatopancreas might have better health status. This point was proved by Li (2015), who reported that the improvement of antioxidant ability and decrease of lipid level in hepatopancreas of genetically improved farmed tilapia (Oreochromis spp) were caused by dietary surfactin supplementation. The serum LZM is used as an indicator of innate immune response in fish, the increased LZM activities indicate increased disease resistance (Tort et al. 2003; Zhai et al. 2014). The higher levels of LZM in surfactin supplementation groups are considered as the indicator of strong innate immune function (Li 2015; Shi 2015).

The results of present study showed that surfactin could decrease levels of serum TG, TC and LDL-C and increase HDL-C level in tilapia, which might be beneficial to avoid fatty liver pathological changes. Those results were consistent with the results reported by Li (2015), where tilapias were fed with dietary 50 mg/kg surfactin. The lowering serum lipids effect of surfactin might be related to its surface activity and the role of emulsifier. Because our observation was consistent with the emulsifier studies on rats conducted by Jimenez et al. (1990) and lwata et al. (1993), who observed that soy-derived emulsifier (lecithin) has beneficial effects on lowering serum TC and TG, while increasing high-density HDL-C level in the blood. The mechanism of how emulsifiers influence serum lipids levels is still unclear. Further study is needed to reveal the detail mechanisms of emulsifiers decreasing serum lipids.

From the results of present trial, we found that dietary surfactin under certain supplementation level could improve the growth performance and some intestinal digestive enzymes activities and serum biochemical parameters of tilapia, and the excess supplementation of surfactin in diet had no further improvement effect. These results were similar to the reports of previous studies (Sahnoun et al. 2014; Shi et al. 2014a; Li 2015; Zhai et al. 2015). They contributed this behaviour of surfactin to its surface activity, because all the biological activity of surfactin is based on the surface activity, and surfactin interaction with the cell membrane is highly dependent on the surfactin concentration, a threshold concentration of surfactin in the bilayer is needed for its solubilization (Seydlova & Svobodova 2008; Chen et al. 2015).
Conclusions
This study demonstrated that supplementary surfactin in tilapia diet had beneficial effect to promote the growth performance and intestinal digestive enzymes activities and improve some serum biochemical parameters of tilapia (O. niloticus) fingerlings.

Disclosure statement
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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