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Key words: nanosilver, *Vibrio alginolyticus*, *Epinephelus coioides*, grouper aquaculture.

ABSTRACT

This study evaluated the efficacy of nanosilver in grouper aquaculture. The commercial nanosilver solution was trailed for antibacterial activity against *Vibrio alginolyticus*, one of the most serious threats for grouper survival. The *in vitro* studies showed that nanosilver possessed Minimum Inhibitory Concentration (MIC) value of 8 ppm, Minimum Bactericidal Concentration (MBC) value of 16 ppm and Zone of Inhibition (ZOI) of 14 mm (20 μg silver loading). In addition to assessment of antibacterial activity in culture medium, it was also found that the dosage of nanosilver above 8 ppb were effective for growth inhibition of *V. alginolyticus* in seawater (10^3 CFU mL^{-1}). The growth inhibition was more significant with the lower salinity in seawater. In the disinfectant application of living feed, exposure of 16 ppb nanosilver to *Brachionus plicatilis* for 15 minutes could accomplish a good sterile effect as well as maintain the vitality. In the safety issues, it was shown that the growth and survival of *Epinephelus coioides* were not affected after 8 weeks of dietary addition of nanosilver. Moreover, the accumulation of silver in the muscle tissues was not observed by the analysis of inductively coupled plasma mass spectrometry (ICP-MS). Therefore, nanosilver can be used as a suitable antibacterial agent in grouper aquaculture.

I. INTRODUCTION

Nanosilver has been identified as an effective antimicrobial agent (Siddiqi et al., 2018) and has been widely utilized as a bactericide in many fields such as medical use, agriculture, livestock, and aquaculture (Huang et al., 2015; Khan et al., 2018). The antimicrobial mechanisms of nanosilver include the process of impairment of cell membrane, dysfunction of intracellular structures, induction of reactive oxygen species and denaturation of biomolecules (Dakal et al., 2016). Many literatures have also been indicated that nanosilver is potential antimicrobial agent against wide range bacteria, fungi, parasites and viruses (Khezerlou et al., 2018). Although nanosilver with remarkable physical and chemical properties, is less reactive than silver ions and become low cost on the market (Magesky and Pelletier, 2018), there is a serious concern on its toxicity toward environment (Ivask et al., 2014; Yue et al., 2017). Release of biocidal nanosilver into the environment may threat to non-target organisms, such as natural microbes and aquatic biota. It was found that silver concentrations in tissues of wild yellow perch (*Perca flavescens*) were 3 orders of magnitude greater than the concentrations in natural lake ecosystem (Martin et al., 2017; Martin et al., 2018). The whole-lake experiment showed that releases of nanosilver at ppb concentrations in water can result in the accumulation of Ag to ppm levels in the liver tissues of a piscivorous fish species (3-8 years old). Literatures also reported that the induction of toxic effects in several aquatic species were accompanied with silver accumulation (Khan et al., 2015; Rajkumar et al., 2016). However, little information is available about the amount of nanosilver accumulated within marketed organisms (Khosravi-Katuli et al., 2017).

This study focused on the risk assessment of using antimicrobial nanosilver toward aquaculture of the orange spotted grouper (*Epinephelus coioides*), which was one of the most important economic fishes in Taiwan. The fish growth performances, accumulation of silver and disinfectant of living feed were evaluated here. Since the use of silver based nanoparticles as an alternative antimicrobial agent has received increasing attention in aquaculture due to the rapid development of antibiotic resistance (Swain et al., 2014). *Vibrio alginolyticus*, one of the most dangerous pathogens for the grouper rearing (Lee, 1995), was first investigated here for evaluating the antimicrobial effect of nanosilver.

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II. MATERIALS AND METHODS

1. Antimicrobial Experiments

Table 1. The antimicrobial effects of different concentrations of nanosilver against *Vibrio alginolyticus* (10^3 CFU/mL) in seawater during 24hr.

Treatment (ppb)	Inoculum (×10^3 CFU/mL)								
	Time (hours)								
	0	1/6	1/2	1	2	4	8	16	24
0	1.11±0.03^{a}	1.24±0.03^{a}	1.57±0.02^{a}	2.03±0.05^{a}	2.95±0.04^{a}	3.89±0.53^{a}	4.65±0.07^{a}	5.95±0.03^{a}	7.19±0.13^{a}
1	1.21±0.08^{a}	0.98±0.08^{a}	0.56±0.04^{b}	0.47±0.01^{b}	0.95±0.03^{b}	1.46±0.08^{b}	3.71±0.10^{a}	3.79±0.05^{b}	5.61±0.16^{a}
2	1.24±0.04^{a}	1.01±0.10^{a}	0.57±0.10^{b}	0.57±0.12^{bc}	0.71±0.02^{b}	0.49±0.02^{bc}	0.29±0.06^{b}	2.39±0.10^{b}	5.92±0.04^{a}
4	1.22±0.03^{a}	0.88±0.03^{b}	0.48±0.09^{b}	0.25±0.14^{cd}	0.01±0.02^{c}	0.01±0.02^{c}	0.05±0.04^{b}	0.31±0.05^{c}	2.09±0.04^{b}
8	1.21±0.11^{a}	0.55±0.05^{c}	0.08±0.04^{c}	0.04±0.01^{d}	-	-	-	-	-
16	1.11±0.01^{a}	-	-	-	-	-	-	-	-

(1) Data are means ± SD.
(2) Means in the same column with the different letter (a,b,c,d) are significantly different ($p < 0.05$).
(3) " - " indicated no bacteria were detected.

The uncoated nanosilver solution (2000 ppm) with 60-100 nm particle size was obtained from Esther Material Technology Co., Ltd.. *V. alginolyticus* was provided by Dr. Ping-Chung Liu, Department of Aquaculture, National Taiwan Ocean University. The strain was collected by a previously described method (Samad et al., 2014) and cultured in tryptic soy agar (TSA) at 30°C. The bacteria were grown in tryptic soy broth (TSB) with 2.5% NaCl overnight. Then phosphate buffered saline (PBS) was used to adjust the bacterial concentration to 10^9 CFU (colony forming unit) mL^{-1} for the bacteriostatic studies. The Minimum Inhibitory Concentration (MIC) value of nanosilver was determined by two fold serial dilutions from 32 to 2 mg L^{-1}. The Minimum Bactericidal Concentration (MBC) was determined from the broth dilution of MIC tests by subculturing to TSA for 24 hr. In the well diffusion test, agar plates were inoculated with bacterial suspension followed by loading 10 μL of the nanosilver solution to the center of well with a diameter of 6 mm. The plates were incubated at 30°C for 24 hr and the Zone of Inhibition (ZOI) was measured. The bacteriostatic activities of nanosilver in sterile seawater were performed in various concentrations of treatment (0-16 μg L^{-1}), time of treatment (0-24 hr) and inoculum size (10^3, 10^5 and 10^7 CFU mL^{-1}). The bacteriostatic activities of nanosilver in seawater under various salinities (15, 25 and 35 ppt (part per thousand)) were also evaluated at 10^3 CFU mL^{-1} of inoculum size over a period of 7 days

2. Evaluation of Sterile effect on Rotifer

Rotifer, *Brachionus plicatilis* were obtained from Aquatic Animal Center, National Taiwan Ocean University. Living organisms with wet weight of 1 g were incubated in 1 L sterile seawater containing nanosilver concentration of 0, 8 and 16 ppb. 2 mL of experimental water was collected from each treatment every 15 min. Aliquots of experimental water was used for observation of *B. plicatilis* vitality under microscope and sterile effects after CFU counting. The vitality was calculated as following: (1- motionless/total) × 100%. After removing the silver containing water, *B. plicatilis* was re-incubated with sterile seawater. Then the non-silver containing water was spread on TSA. The sterile effect of nanosilver was measured by counting the CFU forming.

3. Fish rearing and dietary addition of nanosilver

Groupers (*E. coioides*) with average 7-8 cm length were obtained from Aquatic Animal Center, National Taiwan Ocean University and then acclimated in the hatchery of the Department of Aquaculture. Thirty fish of each group were distributed into tanks (45 × 45 × 40 cm) with 100 L of total water volume and fed commercial diet containing 0, 8 and 800 ppb nanosilver twice daily (3% body weight per meal). The growth performances were measured after 8 weeks as following. Weight gain (%) = [(final weight (g) – initial weight (g)) / initial weight (g)] × 100, Feed conversion ratio (FCR) = feed intake (g) / (final weight (g) – initial weight (g)).

4. Quantification of silver concentration in fish tissue by ICP-MS

The concentrations of silver in fish tissues (muscle, liver,intestinal tract, brain and head kidney) were quantified by inductively coupled plasma mass spectrometry (ICP-MS). Nitric acid (67-70%, J. T. Baker® ULTREX™ II Ultrapure) and ultrapure water (18.2 MΩ · cm, ELGA system) were used for dilutions with elemental standard solution (AccuStandard®, ICP-MS Calibration Standard 2). Standard solutions over a range of silver concentrations (0.1-10 ppb) spiked with Rhodium (10 ppb) was used to generate a calibration curve (r^2 = 0.9994). The analytical procedure was validated by a matrix spike and silver recovery rate was found to be more than 95%. 1 g of tissue samples were digested with 6 mL of 67-70% nitric acid and heated at 95°C on a hot block until completely dissolved (2-4 hr). After cooling to room temperature, digests were diluted to 50 mL with ultrapure water and then filtered with a 0.22 μm Nylon disposable syringe filter for analysis. ICP-MS measurements were performed using an ICP

Table 2. The antimicrobial effects of different concentrations of nanosilver against *Vibrio alginolyticus* (10^5 CFU/mL) in seawater during 24hr.

Treatment (ppb)	Inoculum ($\times10^5$ CFU/mL)								
	Time (hours)								
	0	1/6	1/2	1	2	4	8	16	24
0	1.01±0.03^{a}	1.04±0.03^{a}	1.01±0.02^{a}	1.07±0.04^{a}	1.22±0.04^{a}	1.36±0.53^{a}	1.42±0.07^{a}	1.63±0.03^{a}	1.92±0.13^{a}
1	1.01±0.08^{a}	0.72±0.08^{b}	0.73±0.09^{ab}	0.82±0.07^{a}	0.48±0.03^{b}	1.36±0.08^{b}	0.73±0.10^{b}	1.02±0.05^{b}	1.32±0.16^{b}
2	1.03±0.04^{a}	0.64±0.05^{b}	0.45±0.11^{bc}	0.19±0.12^{b}	0.16±0.02^{c}	0.11±0.02^{c}	0.59±0.06^{b}	0.81±0.10^{b}	0.94±0.04^{b}
4	1.01±0.03^{a}	0.57±0.02^{b}	0.36±0.11^{c}	0.31±0.11^{b}	0.11±0.01^{cd}	0.01±0.02^{c}	0.08±0.04^{c}	0.18±0.05^{c}	0.26±0.04^{c}
8	1.05±0.11^{a}	0.32±0.05^{c}	0.13±0.10^{c}	0.09±0.04^{b}	0.02±0.01^{d}	-	-	-	-
16	1.02±0.01^{a}	0.01±0.01^{d}	-	-	-	-	-	-	-

(1) Data are means ± SD.

(2) Means in the same column with the different letter (a,b,c,d) are significantly different ($p < 0.05$).

(3) " - " indicated no bacteria were detected.

Table 3. The antimicrobial effects of different concentrations of nanosilver against *Vibrio alginolyticus* (10^7 CFU/mL) in seawater during 24hr.

Treatment (ppb)	Inoculum ($\times10^7$ CFU/mL)								
	Time (hours)								
	0	1/6	1/2	1	2	4	8	16	24
0	1.02±0.13^{a}	1.04±0.14^{a}	1.11±0.12^{a}	1.27±0.14^{a}	1.32±0.24^{a}	1.36±0.53^{a}	1.57±0.37^{a}	1.69±0.23^{a}	1.82±0.43^{a}
1	1.04±0.08^{a}	0.92±0.18^{ab}	1.12±0.19^{a}	1.17±0.17^{a}	1.21±0.21^{a}	1.32±0.28^{a}	1.47±0.28^{a}	1.42±0.28^{ab}	1.52±0.26^{ab}
2	1.03±0.14^{a}	0.82±0.15^{ab}	0.75±0.12^{b}	0.76±0.14^{b}	0.82±0.14^{b}	0.92±0.25^{a}	1.11±0.26^{a}	1.03±0.19^{b}	0.99±0.34^{b}
4	1.01±0.13^{a}	0.75±0.12^{b}	0.66±0.11^{b}	0.51±0.16^{bc}	0.50±0.21^{b}	0.55±0.12^{b}	0.62±0.14^{b}	0.88±0.25^{b}	1.01±0.28^{b}
8	1.04±0.09^{a}	0.32±0.07^{c}	0.33±0.03^{c}	0.29±0.14^{c}	0.21±0.07^{c}	0.20±0.04^{c}	0.24±0.08^{c}	0.22±0.06^{c}	0.25±0.11^{c}
16	1.02±0.07^{a}	0.21±0.11^{d}	0.20±0.04^{d}	0.14±0.05^{c}	0.11±0.05^{c}	0.09±0.02^{d}	0.12±0.09^{c}	0.11±0.05^{c}	0.18±0.03^{c}

(1) Data are means ± SD.

(2) Means in the same column with the different letter (a,b,c,d) are significantly different ($p < 0.05$).

(3) " - " indicated no bacteria were detected

quadrupole MS instrument (ThermoFisher X Series II). ThermoPlasmaLab 2 software was used for data processing. All measurements were performed independently in triplicate (three fishes were selected randomly in each treatment). The reproducibility CV (coefficient of variation) was 5.3%.

5. Statistical analyses

Each experiment was repeated in triplicate and data was shown as mean ± SD (standard deviation). Statistical analysis was performed using SAS (Statistical Analysis System) Software Version 9.0. Statistical significance was set at $\alpha = 0.05$ for the One-way ANOVA with a subsequent Tukey's honest significant difference test.

III. RESULTS

1. Bacteriostatic or Bactericidal effects of nanosilver against *Vibrio alginolyticus*

The antimicrobial activities against Gram-negative fish pathogenic bacteria *Vibrio alginolyticus* were examined in culture medium. MIC and MBC of nanosilver were 8 and 16 ppm, respectively. 20 μg of nanosilver exhibited the inhibitory effect with ZOI of 14 mm. The antimicrobial effects of nanosilver were also observed in seawater at various inoculum sizes. Without the complex component in culture medium, Table 1 showed that the bactericidal effects of nanosilver within 24 hr can be achieved in ppb level at inoculum size of 10^3 CFU mL^{-1}. It was noted that the bactericidal effects were presented in treatment of 8 ppb for 2 hr or 16 ppb for 1/6 hr. The similar bactericidal effects were also shown in treatment of 8 ppb for 4 hr or 16 ppb for 1/2 hr at inoculum size of 10^5 CFU mL^{-1} (Table 2). At inoculum size of 10^7 CFU mL^{-1} (Table 3), the bacteriostatic effects or inhibitions of bacterial growth were observed in the dosage of nanosilver treatment above 8 ppb. In the experiment to figure out the antimicrobial effects of nanosilver on different salinities, it was shown that the bacteriostatic effects could last more days as decreasing the salinity

Table 4. The bacteriostatic effects of different concentrations of nanosilver against *Vibrio alginolyticus* in 35 ppt seawater during 7 days.

Treatment (ppb)	Inoculum ($\times 10^3$ CFU/mL)							
	Time (days)							
	0	1	2	3	4	5	6	7
0	1.12±0.00[a]	6.54±1.12	7.95±1.45[a]	9.31±2.61[a]	9.45±2.94[a]	14.88±3.14[a]	13.27±3.95[a]	17.11±6.11[a]
8	1.12±0.00[a]	-	0.12±0.08[b]	0.78±0.21[b]	1.54±0.39[b]	1.74±0.24[b]	3.45±1.06[b]	5.12±1.64[b]
16	1.12±0.00[a]	-	-	-	-	0.11±0.06[c]	0.58±0.13[c]	0.87±0.21[c]

(1) Data are means ± SD.
(2) Means in the same column with the different letter (a,b,c) are significantly different ($p < 0.05$).
(3) " - " indicated no bacteria were detected.

Table 5. The bacteriostatic effects of different concentrations of nanosilver against *Vibrio alginolyticus* in 25 ppt seawater during 7 days.

Treatment (ppb)	Inoculum ($\times 10^3$ CFU/mL)							
	Time (days)							
	0	1	2	3	4	5	6	7
0	1.03±0.00[a]	5.68±0.82	7.26±1.33[a]	8.71±3.51[a]	9.91±2.61[a]	12.62±5.54[a]	15.71±5.45[a]	16.21±6.31[a]
8	1.03±0.00[a]	-	-	0.58±0.16[b]	1.24±0.35[b]	1.96±0.24[b]	2.65±0.76[b]	4.22±1.14[b]
16	1.03±0.00[a]	-	-	-	-	0.54±0.13[c]	0.68±0.12[c]	1.27±0.31[c]

(1) Data are means ± SD.
(2) Means in the same column with the different letter (a,b,c) are significantly different ($p < 0.05$).
(3) " - " indicated no bacteria were detected.

Table 6. The bacteriostatic effects of different concentrations of nanosilver against *Vibrio alginolyticus* in 15 ppt seawater during 7 days.

Treatment (ppb)	Inoculum ($\times 10^3$ CFU/mL)							
	Time (days)							
	0	1	2	3	4	5	6	7
0	0.96±0.00[a]	2.14±1.12	4.25±1.45	6.31±2.61[a]	7.45±2.94[a]	9.18±3.14[a]	12.67±3.95[a]	14.11±2.81[a]
8	0.96±0.00[a]	-	-	0.18±0.05[b]	0.44±0.19[b]	1.14±0.19[b]	1.45±0.76[b]	2.13±0.47[b]
16	0.96±0.00[a]	-	-	-	-	-	0.72±0.21[b]	0.97±0.18[c]

(1) Data are means ± SD.
(2) Means in the same column with the different letter (a,b,c) are significantly different ($p < 0.05$).
(3) " - " indicated no bacteria were detected.

in seawater. Table 4 showed the bacteriostatic effects under normal salinity (35 ppt) could last 1 and 4 days for nanosilver treatment of 8 ppb and 16 ppb, respectively. As shown in Table 5 and Table 6, the bacteriostatic effects under lower salinities sustained for 2 days at the nanosilver treatment of 8 ppb. Moreover, the effect under 15 ppt of salinity was improved to last 5 days at the treatment of 16 ppb, whereas the effects under 25 ppt and 35 ppt of salinities last 4 days.

2. The effect of nanosilver on *Brachionus plicatilis*

Nanosilver was used for the disinfectant application of living feed, *Brachionus plicatilis*. The optimal concentration of treatment was evaluated between the vitality and the sterile effect. As shown in Table 7, the rotifer's activities decreased with increasing the time or dosage of nanosilver treatment. Moreover, the excellent sterile effects can be found in treatment of 16 ppb over 45 minutes (Table 8). Our data suggested that exposure of 16 ppb nanosilver to *B. plicatilis* for 15 minutes could be an optimal condition for disinfectant. Under the condition, the significant inhibition of *Vibrio* growth as well as the maintenance of vitality was achieved.

Table 7. The effects of nanosilver on the vitalities of *Brachionus plicatilis*.

Treatment (ppb)	Activities (%)			
	Time (mins)			
	15	30	45	60
0	99.05 ± 0.94[a]	97.14 ± 1.33[a]	94.29 ± 2.57[a]	84.76 ± 4.12[a]
8	99.05 ± 0.94[a]	97.14 ± 0.83[a]	91.43 ± 2.88[a]	78.67± 2.09[b]
16	98.01 ± 0.87[a]	81.90 ± 2.53[b]	72.38 ±5.34[b]	69.52 ± 3.26[c]

(1) Data are means ± SD.
(2) Means in the same column with the different letter (a,b,c) are significantly different ($p < 0.05$).

Table 8. The sterile effects of nanosilver on *Brachionus plicatilis*.

Treatment (ppb)	Bacteria counts ($\times 10^4$ CFU/mL)			
	Time (mins)			
	15	30	45	60
0	3.52±1.45[a]	4.12±1.33[a]	4.04±1.73[a]	6.31±3.13[a]
8	2.41±0.53[a]	1.62±0.43[b]	0.46±0.23[b]	0.12±0.13[b]
16	0.02±0.01[b]	0.01±0.01[c]	-	-

(1) Data are means ± SD.
(2) Means in the same column with the different letter (a,b,c) are significantly different ($p < 0.05$).
(3) " - " indicated no bacteria were detected.

Table 9. The effects of dietary supplement of nanosilver on the growth performances of *Epinephelus coioides*.

Treatment (ppb)	Initial weight (g)	Final weight (g)	Weight gain (%)	FCR	Survival (%)
0	42.27±5.32[a]	127.64±8.83[a]	201.96±5.23 [a]	1.92±0.13 [a]	100
8	39.63±4.71[a]	118.36±12.51[a]	198.66±9.64 [a]	1.88±0.24 [a]	100
800	45.69±5.83[a]	129.82±11.57[a]	184.13±15.82 [a]	1.89±0.21 [a]	100

(1) Date are means ± SD.
(2) Means in the same column with the different letter (a,b,c) are significantly different ($p < 0.05$).
(3) Weight gain: [(Final weight – initial weight) / initial weight] × 100 %.
(4) FCR (Food conversion rate): feed intake (g) / weight gain (g).
(5) Survival rate = (Final no. of fish / initial no. of fish) x 100 %

Table 10. Silver accumulation in various tissues of *Epinephelus coioides* after 8 weeks of dietary supplement with nanosilver.

Treatment (ppb)	Silver concentration (ppm)				
	Muscle	Liver	Intestinal tract	Brain	Head kidney
0	0.007±0.002[a]	0.070±0.021[b]	2.135±0.539[b]	0.042±0.008[b]	0.019±0.005[b]
8	0.004±0.002[a]	0.119±0.017[a]	2.826±0.712[ab]	0.065±0.015[b]	0.022±0.009[b]
800	0.004±0.002[a]	0.125±0.011[a]	3.749±0.690[a]	0.113±0.021[a]	0.054±0.012[a]

(1) Date are means ± SD.
(2) Means in the same column with the different letter (a,b) are significantly different ($p < 0.05$).

3. The effect of nanosilver on Groupers

The growth performance of the Orange-spotted grouper *Epinephelus coioides* under dietary supplement of nanosilver were evaluated for the safety issue (Table 9). The concentrations by orders of magnitude above environmental concentrations were employed for the dietary supplement (8 and 800 ppb). The parameters such as weight gain, FCR and survival rate was shown no negative effect in nanosilver feeding. Among these, 100% survival rates were found during the 8 weeks of feeding. In addition, the weight gain of individuals fed with diets containing nanosilver was not significantly lower than that of individuals fed with normal diets. No significant difference was also found between FCR of individuals fed with nanosilver containing diets and that of individuals fed with normal diet. To assess the hazard of nanosilver, internal transfer to target tissues are quantified. The concentrations of silver in various tissues of grouper throughout the experiment are summarized in Table 10. Although the accumulation of silver was found in target organs such as liver, intestinal tract, brain and head kidney after 8 weeks of dietary nanosilver exposure. It was noted that the accumulation of silver was not observed in muscle tissue, while the silver concentration in groups of dietary exposure of nanosilver were similar to that in group of no exposure of nanosilver. Our data demonstrated that the use of nanosilver could be safe for grouper aquaculture and edible concern.

IV. DISCUSSION

The grouper aquaculture was ravaged by various pathogenic diseases. Thus, the production of groupers continued to encounter increasing difficulties (Harikrishnan et al., 2010). The excellent antimicrobial activities of nanosilver promoted us to apply it in the grouper aquaculture. Since nanosilver or silver-based nanocomposites have been studied in antibacterial activity against various aquaculture pathogens, including *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Aeromonas bestiarum*, *Pseudomonas fluorescence*, *Flavobacterium branchiophilum*, *Edwardsiella tarda*, *Edwardsiella ictaluri*, *Yersinia rukeri*, *Vibrio parahaemolyticus*, *Vibrio salmonicida*, *Vibrio tapetis* and *Vibrio cholera* (Dananjaya et al., 2017; Krishnaraj et al., 2010; Pallela et al., 2018; Pugazhendhi et al., 2018; Shaalan et al., 2017; Velmurugan et al., 2014). The *in vitro* antimicrobial activities of nanosilver against *V. alginolyticus* were first reported in the present study. Our data have shown that the concentrations of 16 ppb were effective in growth inhibition of *V. alginolyticus* under seawater environment as well as in sterile effect on *B. plicatilis*. The effective dosage below ppm level was commonly found in other reports evaluating the antibacterial activity of nanosilver against Gram-negative pathogens (Franci et al., 2015). Our results also agree with previous studies that bactericidal activity of nanosilver (60-100 nm) was optimal against *Vibrio* species (Salem et al., 2015).

The toxic effects of nanosilver on fish should be concerned about the sublethal dosage, uptake and accumulation (Joo et al., 2013; Kleiven et al., 2018; Martin et al., 2018). The usage of nanosilver (60-80 nm) here could be advantageous because small dimensions (10-20 nm) of silver nanoparticles was reported to be highly cytotoxic toward rainbow trout hepatocytes (Farkas et al., 2010; Ostaszewska et al., 2018). In addition, the accumulation of silver in our studied tissues was concentration-dependent based on the following order: liver > brain > kidney > muscle. The extremely low concentration of silver accumulated in muscle tissue was also shown in rainbow trout (Joo et al., 2013; Shaalan et al., 2018) and Atlantic salmon (Kleiven et al., 2018) after dietary or waterborne exposure to nanosilver. To our knowledge, the understanding of nanosilver toxicity and accumulation in *E. coioides* is unknown until now. Only a few studies reported the toxicity of nanocopper on *E. coioides* (Wang et al., 2016; Wang et al., 2015a; Wang et al., 2015b). Therefore, the present study could provide a risk assessment for the practical use of silver nanomaterials in grouper aquaculture.

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