2017 SCIENCELINE
Journal of Life Science and Biomedicine
J Life Sci Biomed, 7(5): 69-75, 2017

ISSN 2251-9939

Effect of Dietary *Scutellaria baicalensis* Root Water Extract against *Piscicola geometra* Infection of Cobia

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ABSTRACT

Piscicola geometra **is an ectoparasite and the causative agent of infection in fish. Heavy infestation of *P. geometra* significantly causing mortality and serious economic losses to cobia industry. However, no single drug available today has used for the treatment or prevention of *P. geometra* infestation in fish. Water extracts of *Scutellaria baicalensis* root were evaluated for their effect on cobia infected by *P. geometra*. Fish were divided into two groups (group A for healthy fish and group B for infected fish) before being fed for 30 days with 0% as control, 0.5%, 1%, and 2% of *S. baicalensis*. Cobia growth performance, mortality, and total number of parasite infestation on fish were investigated. Result showed that infestation of *P. geometra* significantly reducing the growth performance and survival rate of cobia. Cobia fed with 1% *S. baicalensis* showed highly significant differences (P<0.05) in growth performance (group A and B) and the number of parasite infestation (group B) compared to their respective controls. However, the growth performance of cobia fed with supplemented extract showed no significant differences in group A at the lowest concentration (0.5%) and the highest concentration (2%). Meanwhile group B showed significant different in growth performance among the treatment group (P<0.05). This study demonstrated that *S. baicalensis* root water extract administered as a dietary supplementation is one of the most practical methods to prevent *P. geometra* infestation in cobia culture.**

Original Article
PII: S225199391700011-7

Rec. 15 Aug. 2016
Acc. 10 Sep. 2017
Pub. 25 Sep. 2017

Keywords

Oral administration,
Piscicola geometra,
Scutellaria baicalensis,
Growth performance,
Cobia,
Parasite infestation

INTRODUCTION

Cobia (*Rachycentron canadum*), locally known as *badee* in Indonesia is one of the most promising candidate for marine culture industry in the world [1, 2]. The hardiness cobia is living in different environment and easy to adapt (distributed worldwide between in 50 and 1200 m depth, 16.8 to 32.2ºC of temperatures, and 5 to 44.5 of salinities [3, 4]), fast growth (up to 4 – 6 kg in a year with 90% of survival rate [5]), good conversion rate (1.6 to 1) [6], and high market value (excellent white meat flesh quality) [7]. Moreover, 100 g of cobia meat contains around 32 – 507 mg of docosahexanoic acid (DHA) and 280 – 485 mg of eicosapentanoic acid (EPA) which 24% higher than Atlantic salmon that contain 10 – 11.6 mg of DHA/100g and 76 – 83 mg of EPA/100 g [8, 9]. Cobia production is expanding rapidly in Taiwan, Vietnam, China, Philippines, Indonesia, Iran, and Reunion Island [4-6, 10].

However, intensification of aquaculture production has resulted in an increased incidence of disease outbreaks as the major constraints of the mariculture industry [4]. Although there has been no detailed review of the parasites disease of cobia, the wild cobia has been reported infect by parasite at gastrointestinal tract, gills, and skin [3, 11]. Parasites infestation causing mortality, growth retardation and serious economic losses to the cobia industry [12]. *Piscicola geometra* has found to infest the eye, gills, and skin of cobia culture in Taiwan and caused a lesion, erosion, and hemorrhage. Class of Hirudinea of the Rhynchobdellida is a blood sucker leech which utilizes a proboscis to penetrate the tissue of the host [13]. The wounding makes the growth of fish slower and changes their skin color become darker, also can cause the fall of the market value [4].

Antibiotics and the veterinary drug has been applied in aquaculture to treat fish disease [14, 15]. Although the fish disease is able to be healed, the incidence of water pollution, accumulation of chemical residues, drug resistant bacteria, and highly toxic substances has become a major problem in public health [16, 17]. Nowadays, humans have returned back to nature and started to use medicinal plants as a promising alternative method to control fish disease [18, 19]. The dried root of *S. baicalensis* has been listed in Traditional Chinese Medicine (TCM) [20]. A highly active compound such as alkaloids, flavonoids, phenolics, terpenoids, steroids, pigments, and essential oils contains in *S. baicalensis* root has provided a cheaper source to treat many diseases, reducing stress responses, enhancing immune responses and mitigate many side effects with greater accuracy [21-24]. The aim of this study is to evaluate the effects of dietary *S. baicalensis* root water extract on the growth performance and disease resistance of cobia against *Piscicola geometra* infestation.

MATERIAL AND METHODS

Ethical approval

The review board and ethics committee of Tungkang Marine Biotechnology Research Station Taiwan approved the study protocol and informed consent were taken from all the participants.

Preparation of plant extract

The dried root of *S. baicalensis* was powdered mechanically using an electrical stainless grinder. The powdered (10 gram) were extracted with distilled water (150 ml) and boiled at 95°C for 20 min. The extracts were divided into four 50 ml Falcon tube and centrifuged for 10 min at 4°C, 1000 xg. The clear supernatant was stored at -20°C of refrigerator for further uses. A standard commercial diet for cobia was used throughout and designed as the control diet. Different concentration of *S. baicalensis* root water extract was sprayed into the commercial feed slowly and mixing part by part (control, 0.5%, 1%, and 2%). The diets were dried under the sterile condition in a hot air oven at 60° for 24 h.

Parasites and hosts

A total of 240 healthy cobia, with 23.3 ± 2.3 gram of body weight and 10 ± 2 cm of body length were obtained from Tungkang Marine Biotechnology Research Station, Taiwan and maintained in 200-l of tanks with air stone and water circulation. The fish were acclimatized under laboratory conditions for 7 days and fed with commercial diet for cobia. After acclimation, 120 of fish cohabited with the ones infected with *P. geometra*.

Fish and experimental protocol

The experiments were carried out at Tungkang Marine Biotechnology Research Station, Taiwan. Experimental study was divided into two group treatment. After two weeks of post-infestation, healthy fish (without leech infestation) were randomly distributed in group treatment A and infected fish in group treatment B. Each group contains four treatment including control with three replicates. Each tank (200-l) was stocked with 10 fish. Fish were fed with experimental diet in twice a day at a rate of 5% body weight for 30 days. During the experimental period, the temperature was ranged from 26 to 28°C and salinity from 33 to 35 ppt. Total number and mean body weight of fish in each tank were measured in seven days interval. The effects of treatments on the number of parasites were analyzed by comparing with those in control group after 30 days of post-experimental diet feeding.

Fish growth performance calculation

Growth performance were assessed by net weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and survival growth rate (SGR) at seven days interval. Calculations were made using equation was describe

[25]. The amount of the fed was increasingly based on the fish body weight. Clinical history of lesion, behavior, and mortality of the fish was observed every day. The number of leech in each fish was randomly counted in the end of experiment.

$$\text{Survival growth rate (SGR)} \ \frac{\ln(Final\ weight\ (g)) - \ln(Initial\ weight\ (g))\ x\ 100}{total\ duration\ of\ the\ experiment\ (days)} \ldots.. \quad (1)$$

$$\text{Feed Conversion Ration (FCR)} = \frac{total\ amount\ of\ feed\ (g)}{weight\ gain\ (g)} \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$\text{Weight gain rate (\%)} = 100\ x\ \frac{Final\ body\ weight(g) - initial\ body\ weight(g)}{initial\ body\ weight\ (g)} .. \quad (3)$$

Statistical analysis

All data are presented as means ± standard deviation (SD). Statistical analysis was performed using SPSS 16.0 software by one-way ANOVA (analysis of variance) and accepted at the P<0.05 level. Significant differences between control and treatment groups were determined using post-hoc Duncan's test.

RESULTS

Body weight (BW), weight gain (WG), survival growth rate (SGR), feed conversion ratio (FCR) and survival rate of cobia fed the experimental diet in both of group are shown in Tables 1 and 2. *Scutellaria baicalensis* supplemented to the fish feed were significantly enhancing the growth performance of cobia compared with control feed (P<0.05) in both of the groups. Table 1 showed that, healthy cobia fed with 1% concentration of plant extract were significantly higher than other groups treatment at the growth performance with 35.6 ± 1.2 of final body weight, 74.34 ± 5.8 of weight gain, 50.60 ± 4.1 of survival growth rate, and the FCR around 1.14 (P<0.05). According to the statistical analysis, there was no significant differences of the growth performance between the lowest concentration (0.5%) and the higher concentration (2%) in group A (P<0.05). However, no fish die in group A during the experiment.

In contrast with group A, heavy infestation of *P. geometra* caused moderate hemorrhage on skin (Figure 1) and significantly reduce the body weight of cobia after 30 days of infestation (Figure 2). Administration of 1% of *S. baicalensis* to the infected cobia slightly enhance the growth performance of fish (Figures 1 and 2) compared with control. However, there were no significant differences in weight gain of fish in group B among all treatments. There were no significant differences in survival growth rate and feed conversion ratio among fish fed with control and 0.5% or among fish fed with 1% and 2% concentration of extract (P<0.05). In addition, the survival rate in fish fed with 1% concentration of extract was significantly higher than fish fed with control, 0.5%, and 2% (P<0.05; Table 2).

Infestation of *P. geometra* in the groups treated with 1% and 2% concentration of *S. baicalensis* root were significantly reduced compared with control. There were no significant differences in parasite infestation in fish fed with commercial diet and 0.5% concentration of *S. baicalensis* root (P<0.05; Table 3).

Table 1. Weight gain, specific growth ratio, feed conversion ratio, and survival of the healthy and infected cobia fed diets containing the various concentrations of *S. baicalensis* root for 30 days.

Treatment Group A	Final body weight (g)	Weight gain[1] (g)	SGR[2] (% BW day^{-1})	FCR[3] (g dry feed/g gain)	Survival (%)
Control	33.6 ± 1.1 ^{b}	55.05 ± 5.4^{b}	39.77 ± 3.8^{b}	1.46 ± 0.08^{b}	100 ^{a}
0.50%	33.77 ± 1.7^{ab}	62.31 ± 87.2 ^{ab}	44.49 ± 5.2 ^{ab}	1.33 ± 0.08^{ab}	100^{a}
1%	35.6 ± 1.2^{a}	74.34 ± 5.8 ^{a}	50.60 ± 4.1^{a}	1.14 ± 0.06 ^{a}	100^{a}
2%	34.7 ± 0.7^{ab}	54.43 ± 4.5 ^{ab}	40.77 ± 2.6^{ab}	1.53 ± 0.08^{ab}	100^{a}
SEM	0.54	2.56	1.78	0.03	0
[4]*P*-value	0.037	0.02	0.045	0.011	0

Values are means from triplicate groups of fish (mean ± standard deviations n=10), where the means in each row with different superscripts are significantly different (P<0.05). [1]Weight gain= 100 x (final body weight - initial body weight) / initial body weight. [2]Specific growth ratio = 100 x ln (final weight/initial weight)/ days of the experiment. [3]Feed conversion ratio= g dry feed consumed/g wet weight gain. [4]Values represent of five observations per treatment and their SEM.

Table 2. Weight gain, specific growth ratio, feed conversion ratio, and survival of infected cobia fed diets containing the various concentrations of *S. baicalensis* root for 30 days.

Treatment Group B	Final body weight (g)	Weight gain[1] (g)	SGR[2] (% BW day^{-1})	FCR[3] (g dry feed/g gain)	Survival (%)
Control	21 ± 0.2^{c}	21.83 ± 0.1^{a}	3.97 ± 0.1^{b}	3.25 ± 1.2^{b}	70^{b}
0.5%	21.42 ± 0^{c}	21.75 ± 1.1^{a}	1.54 ± 0.8^{b}	2.85 ± 1.1^{b}	80^{ab}
1%	21 ± 0.5^{a}	27.44 ± 2.3^{a}	30.69 ± 1.6^{ab}	1.02 ± 0.1^{a}	90^{a}
2%	22 ± 0.6^{b}	26.08 ± 5^{a}	18.56 ± 3.2^{ab}	1.78 ± 0.5^{a}	85^{ab}
SEM	0.15	1	0	1.46	3.3
[4]P - value	0.007	0.008	0.008	0.006	0.004

Values are means from triplicate groups of fish (mean ± standard deviations n=10), where the means in each row with different superscripts are significantly different (P<0.05). [1]Weight gain = 100 x (final body weight - initial body weight) / initial body weight. [2]Specific growth ratio= 100 x ln (final weight/initial weight)/ days of the experiment. [3]Feed conversion ratio= g dry feed consumed/g wet weight gain. [4]Values represent of five observations per treatment and their SEM.

Figure 1. Heavy infestation of *P. geometra* caused moderate lesion and hemorrhage on cobia skin

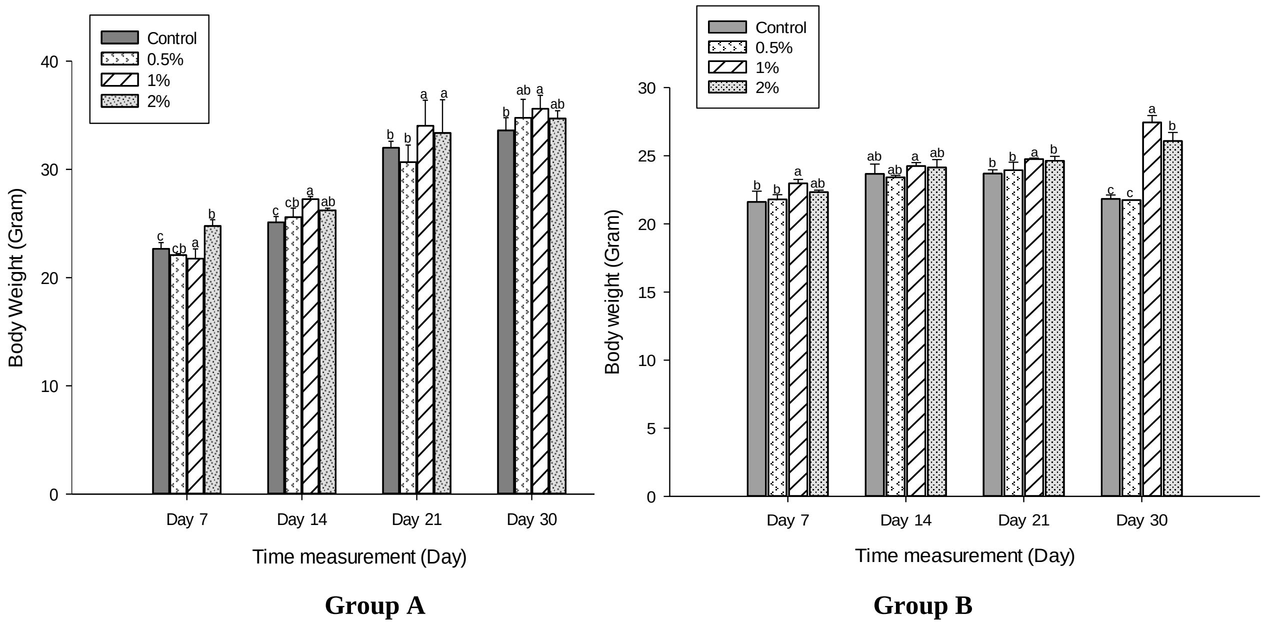

Figure 2. Body weight of healthy cobia (group A) and cobia infected by *P. geometra* (group B) fed with various concentration of *S. baicalensis* root water extract for 30 days (values are mean ± S. E). Mean value with different superscript with in a column for a parameter are significantly different (P < 0.05).

Table 3. The effectiveness of administration of *S. baicalensis* root water extract of cobia to treat *P. geometra*

Experimental group B	Number of parasites per fish for 30 days of feeding
Control	56.70 ± 7.8 [b]
0.5%	53.57 ± 9.6 [b]
1%	35.45 ± 13.5 [a]
2%	28.98 ± 18.7 [a]

Different superscripts are shows the significantly different (P < 0.05), intensity ± SD

DISCUSSION

Piscicola geometra is a significant cause of disease in fish, livestock and serious economic losses to the aquaculture industry. Since the used of veterinary drugs has attracted criticism, there was an urgent need to find new drugs to treat and prevent fish disease [17]. The root of *Scutellaria baicalensis* has been listed in a traditional Chinese medicine to treat human disease [24]. Although *S. baicalensis* root has been common use for human therapy for millennia, there has been tested for controlling and against fish disease [26]. These results demonstrated essentially of dietary *S. baicalensis* root for normal physiological function and had activity against parasite infestation of cobia.

In this study, the effectiveness of *S. baicalensis* root on *Piscicola geometra* was evaluated using oral administration. This study showed that heavy infestation of *P. geometra* has inhibited the growth performance of cobia. However, cobia fed with *S. baicalensis* root water extract has increased the growth performance of healthy fish (group A) and slightly improve the specific growth rate of cobia in group B (parasite infected) compared to untreated control. Administration of *S. baicalensis* also reduced the number of living parasites of 1% concentration and significantly improved the survival rate of cobia infected by *P. geometra* (P<0.05). According to the Ramudu KR and Dash [27], active compounds of herbal plants supplemented on feed could induce the secretion of high protein synthesis of the digestive enzyme and stimulating the appetite and increasing food consumption and efficiencies.

The root of *S. baicalensis* produces more than 30 types of bioactive including 4'-deoxyflavones which promotes various activities such as anti-stress, growth promotion, immunostimulation, and antimicrobial activity [20]. Some study, administration of *S. baicalensis* root has significantly improved specific growth rate of olive flounder with 2% of concentration [28]. Moreover, oral administration of *S. baicalensis* root also can modulate the innate immune system of tilapia with an optimal feeding period of 3 weeks [29]. Some of the herbal extracts also have been reported to treat some parasitic disease in farm fish [26].

Water extracts of tropical seaweed *Asparagopsis taxiformis* showed the most potential for development as a natural treatment to manage the monogenean ectoparasite [30]. Dietary of garlic extract significantly reduced the infection of *Neobenedenia* sp in barramundi farmed [31]. Additionally, administrated of praziquantel resulted in over 80% reduction in worm intensity of chub mackerel culture [32]. However, freshwater bathing of praziquantel drastically increased the parasite intensity due to stress and loss of mucus during the bathing [32]. These results indicated that dietary *S. baicalensis* root could improve the growth performance and had anti-parasitic against *P. geometra* infestation with 1% of concentration.

CONCLUSION

The present study provided evidence that dietary *S. baicalensis* root could significantly enhance the growth performance and protection against parasite infection for cobia. Further work is needed to establish the stimulatory dose and optimal time of feeding of *S. baicalensis* root.

Authors' Contributions

Putri Nurhanida Rizky participated in the performed experiments, drafting the manuscript, analyze and interpretation the data. Cheng Ta-Chih participated in the design of study and financial support. Happy nursyam participated in the critically revised the manuscript for important intellectual contents. All authors of this research paper have directly participated in the planning, execution, or analysis of this study and have read and approved the final version submitted.

Competing interests

All authors declare that they have no competing interests that might have influenced the performance or presentation of the work described in this manuscript.

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