Thesis for the Degree of Master of Science

Effects of dietary inclusion of various concentrations of *Scutellaria baicalensis* extract on growth, body composition, serum chemistry and challenge test of far eastern catfish (*Silurus asotus*)

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by

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요 약

본 연구에서는 국내 주요 담수양식어종 중 하나인 메기(*Silurus asotus*)를 대상으로 하여 천연 생약자원인 황금(*Scutellaria baicalensis*) 추출물의 사료 내 수준별 첨가에 따른 성장 효과, 체조성 및 혈액학적 변화 그리고 질병의 면역성에 미치는 영향을 조사하였다.

실험에 이용된 실험사료는 총 8종류의 사료[대조구(Con)-첨가제 무첨가구, 황금 추출물 0.25% 첨가 사료(SB-0.25), 황금 추출물 0.5% 첨가 사료(SB-0.5), 황금 추출물 1% 첨가 사료(SB-1), 황금 추출물 2% 첨가 사료(SB-2), 황금 추출물 3% 첨가 사료(SB-3)와 황금 추출물 5% 첨가 사료(SB-5)]를 준비하였으며, 황금 추출물 첨가제의 효능을 평가하기 위하여 상업용으로 널리 이용되고 있는 시판용 면역증강 첨가제를 권장양에 근거하여 0.1% 첨가한 사료(CP)를 준비하였다. 각 실험구는 3반복구를 두었다. 그리고

모든 실험어는 손으로 만복 시까지 1주일에 7일간 1일 2회 (07:00, 17:00) 매일 사료를 공급하여 주었으며, 사육실험 기간은 8주간이었다.

8주간의 사육실험 종료시 메기의 어체중 증가와 일일성장율(SGR)은 실험구간에 유의적인 차이는 없었다. 그리고 메기의 마리당 사료 섭취량, 사료전환효율(FER) 및 단백질축적율(PR)은 실험구간에 유의적인 차이가 없었지만 단백질전환효율(PER)은 SB-3사료 공급구에서 가장 높게 나타났다.

메기의 간을 제외한 전어체의 일반성분분석은 실험구간에 유의적인 차이를 보이지 않았다. 그러나 대조구와 SB-3사료를 공급한 실험구에서 간을 제외한 메기의 조지방 함량은 CP, SB-0.5, SB-2와 SB-5사료를 공급한 실험구에 비하여 유의적으로 높게 나타났다.

메기의 혈액성상학적 분석 결과 total protein, glucose와 triglyceride의 함량은 실험구간에 유의적인 차이를 보이지 않았다. 그러나 SB-2사료를 공급한 실험구에서 메기의 GOT 함량은 SB-1사료를 공급한 실험구를 제외한 모든 다른 실험구에 비하여 유의적으로 높은 값을 보였다. SB-2사료를 공급한 실험구에서 메기의 GPT 함량은 다른 모든 실험구에 비하여 유의적으로 높은 값을 보였다. 특히 SB-0.25사료를 공급한 실험구에서 메기의 GOT와 GPT 값은 동일하게 가장 낮은 값을 보였다.

사육실험 종료시 생존한 메기를 대상으로 하여 *Vibrio anguillarum*와 *Streptococcus iniae*의 인위적인 감염 이후 폐사율의 결과 *V. anguillarum*은 감염 이후 3일째부터 폐사가 관찰되기 시작하였으며, 세균감염 이후 10일 이후부터 대조구에 비하여 모든 실험구에서의 누적폐사율이 유의적으로 낮게 나타났다. 그리고 *S. iniae*도 감염 이후 2일째부터 폐사가 관찰되기 시작하였으며, 세균감염 이후 25일째부터 무 첨가구인 대조구에 비하여 모든 실험구에서의 누적폐사율이 유의적으로 낮게 나타났다.

본 연구 결과 천연 생약자원인 황금 추출물이 어류 항균제로 효과가 있다고 판단되며 수입의존적인 항생제 대체용 천연항균제 시장에서의 수입대체 효과를 기대하고 있다.

I. Experiment

Effects of dietary inclusion of various concentrations of *Scutellaria baicalensis* extract on growth, body composition, serum chemistry and challenge test of far eastern catfish (*Silurus asotus*)

Abstract

Effects of various concentrations of *Scutellaria baicalensis* (SB) extract in the diets on growth, body composition, serum chemistry and challenge test of far eastern catfish (*Silurus asotus*) were determined and compared to commercially available immune enhancer. Eight experimental diets were prepared in triplicate: Con diet without supplementation of SB and SB-0.25, SB-0.5, SB-1, SB-2, SB-3 and SB-5 diets containing SB at the concentrations of 0.25, 0.5, 1, 2, 3 and 5%, respectively. In addition, 0.1% commercial product of immune enhancer was included into the diet (CP). At the end of the 8-week feeding trial, ten externally normal fish from each tank were infected by *Vibrio anguillarum* and *Streptococcus iniae*. No significant difference in weight gain of fish was found. Feed consumption, feed efficiency ratio and protein retention of fish was not affected by the experimental diets. Cumulative mortality of fish fed the Con diet was higher than that of fish fed the all other diets since 10 and 25 days after *V. anguillarum* and *S. iniae* infectionm 5. Results of this study indicated that dietary inclusion of

SB extract was effective to improve survival of fish after *V. anguillarum* and *S. iniae* infection, but the various concentrations of SB did not affect fish performance.

Keywords: far eastern catfish (*Silurus asotus*), *Scutellaria baicalensis*, dietary additive, challenge test, *Vibrio anguillarum*, *Streptococcus iniae*

1. Introduction

Far eastern catfish (*Silurus asotus*) is widely distributed throughout freshwater of Korea, China and Japan and commercially important freshwater finfish for aquaculture in Korea (KNSO, 2010) and Japan (Miwa, Yoshizaki, Naka, Nakatani, Sakai, Kobayashi & Takeuchi 2001) because of its fast growth and high resistance to disease. However, only a few feeding trials such as effect of fishmeal size on postprandial metabolic response (Fu, Cao & Peng 2006), dietary soybean meal substitution for fishmeal (Kim, Lim, Hwang, Kim & Kang 2009) and dietary and seasonal effects on dorsal meat lipid of far eastern catfish (Shirai, Suzuki, Tokairin, Ehara & Wada 2002) have been performed. In considering its importance as aquacultural fish species, more studies to improve its production and develop an effective culture method are highly needed.

Herbs have received more attention not only for their immunostimulatory functions, but also for their growth enhancing effects. In addition, application of herbs as dietary additive for fish farming is safer than that of antibiotics or other chemicals used as immunostimulants or growth promoters due to food safety of fish for human consumption. Dietary inclusion of Chinese herb mixture *Astragalus radix* and *Ganoderma lucidum* for carp (*Cyprinus carpio*) (Yin, Ardo, Thompson, Adams, Jeney & Jeney 2009), *Astragalus membranaceus* and *Lonicera japonica* for Nile tilapia (*Oreochromis niloticus*)

(Ardo, Yin, Xu, Varadi, Szigeti, Jeney & Jeney 2008) and *Radix astragalin seu Hedysari* and *R. angelicae sinensis* for yellow croaker (*Pseudosciaena crocea*) (Jian & Wu 2003), and Indian herb *Ocimum sanctum* Linn (Logambal, Venkatalakshmi & Michael 2000), *Azadirachta indica* (Logambal & Michael 2001) and *Solanum trilobatum* (Divyagnaneswari, Christybapita & Michael 2007) for tilapia (*Oreochromis mossambicus*) were reported to improve immune response and/or survival of fish after artificial pathogen infection.

An extract of *Scutellaria baicalensis* (SB) in which baicalin and baicalein are the major active components has been known to have antimicrobial, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant and anticancer activities (Chang & But 1986; Huang 1993; Kim, Yeom, Hahm, Lee & Kim 2000b; Tan & Vanitha 2004; Hwang, Lee & Yang 2006; Kintzios 2006; Kang, Fong & Tsang 2010) and recently developed as additive for aquafeed. Yin, Jeney, Racz, Xu, Jun & Jeney (2006) reported that administration of 0.5 and 1% Scutellaria Root (*S. radix* extracted from SB) showed an inhibition of phagocytosis and respiratory burst activity in tilapia.

In this study, therefore, effects of dietary inclusion of various concentrations of SB extract on growth, body composition, serum chemistry and challenge test of far eastern catfish were determined and compared to commercially available immune enhancer.

2. Materials and methods

2.1. Experimental conditions

Far eastern catfish were purchased from a private hatchery (Nonsan, Chungcheongnam-do, Korea), transferred into the laboratory at Inland Aquaculture Research Center (Changwon, Gyeongsangnam-do, Korea) and acclimated for 2 weeks before initiation of the feeding trial. During the acclimation period, fish were fed an extruded pellet containing 440 g/kg crude protein and 70 g/kg crude lipid (Suhyup Feed Co. Ltd., Korea) for catfish twice a day. The feeding trial was conducted in a water recirculating system in an outdoor greenhouse during the summer season. A unit of a recirculating system consisted of 24 fiber reinforced plastic rectangular glass tanks (80 cm × 80 cm × 70 cm, water volume: 200 L) and a circulation pump was used throughout the feeding trial with water exchange rate of 2.2 L/tank/min. Eight hundred forty fish averaging 0.96 g were randomly distributed into 24 tanks (thirty five fish per tank). Each tank was aerated and water temperature ranged from 20.8°C to 25.5°C (mean ± SD: 24.0 ± 1.16°C).

2.2. Preparation of SB and the experimental diets

A commercially available extract of SB (Beautiful Science & Technology Co. Ltd., Suwon, Gyeonggi-do, Korea) containing 100 ppm baicalin was

used as a dietary additive. Eight experimental diets were prepared in triplicate: Con diet without supplementation of SB and SB-0.25, SB-0.5, SB-1, SB-2, SB-3 and SB-5 diets containing SB at the concentrations of 0.25, 0.5, 1, 2, 3 and 5%, respectively (Table 1). The designated concentrations of SB were included into the experimental diets instead of the same amount of water. In addition, 0.1% commercial product of immune enhancer according to the manufacturer's recommended level was included into the diet, referred to as CP diet, instead of the same amount of water. Fishmeal and dehulled soybean meal were used as protein source for the experimental diets. Dextrin and wheat flour, and squid liver and soybean oils were used as carbohydrate and lipid sources, respectively. The ingredients of the experimental diets were mixed well with water at a ratio of 3:1 and pelletized with a pellet-extruder. The experimental diets were dried at room temperature overnight and stored at -20°C until use. All fish were hand-fed to apparent satiation twice a day (07:00 and 17:00), seven days a week, for 8 weeks.

Table 1. Ingredient and chemical composition (%, DM basis) of the experimental diets

	Experimental diets							
	Con[1]	SB-0.25[2]	SB-0.5[3]	SB-1[4]	SB-2[5]	SB-3[6]	SB-5[7]	CP[8]
Ingredient (%)								
Fishmeal	45	45	45	45	45	45	45	45
Dehulled soybean meal	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Dextrin	5	5	5	5	5	5	5	5
Wheat flour	30	30	30	30	30	30	30	30
Squid liver oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Soybean oil	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Choline	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[9]	2	2	2	2	2	2	2	2
Mineral premix[10]	2	2	2	2	2	2	2	2
Scutellaria baicalensis[11]	0	0.25	0.5	1	2	3	5	
Commercial product								0.1
Nutrients (%)								
Dry matter	90.4	90.3	89.2	89.3	89.3	90.4	89.6	89.3
Crude protein	45.1	45.6	46.8	46.1	45.4	45.0	45.1	45.0
Crude lipid	11.4	11.5	11.8	11.4	12.1	11.6	12.1	11.6
Ash	8.2	9.0	8.8	8.6	8.2	8.4	8.3	8.5

[1]Diet without supplementation of *Scutellaria baicalensis* extract.

[2]Diet containing *Scutellaria baicalensis* extract at the concentrations of 0.25%.

Table 1. Continued

[3]Diet containing *Scutellaria baicalensis* extract at the concentrations of 0.5%.

[4]Diet containing *Scutellaria baicalensis* extract at the concentrations of 1%.

[5]Diet containing *Scutellaria baicalensis* extract at the concentrations of 2%.

[6]Diet containing *Scutellaria baicalensis* extract at the concentrations of 3%.

[7]Diet containing *Scutellaria baicalensis* extract at the concentrations of 5%.

[8]0.1% commercial product of immune enhancer according to the manufacturer's recommended level was included into the diet.

[9]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

[10]Mineral premix contained the following ingredients (g/kg premix): NaCl, 43.3; MgSO4·7H2O, 136.5; NaH2PO4·2H2O, 86.9; KH2PO4, 239.0; CaH4(PO4)·2H2O, 135.3; ferric citrate, 29.6;ZnSO4·7H2O, 21.9; Ca-lactate, 304.0; CuCl, 0.2; AlCl3·6H2O, 0.15; KI, 0.15; Na2Se2O3, 0.01; MnSO4·H2O, 2.0; CoCl2·6H2O, 1.0.

[11]*Scutellaria baicalensis* (SB) extract supplied by Beautiful Science & Technology Co. Ltd. (Suwon, Gyeonggi-do, Korea), which was an aqueous type was included into the experimental diets in stead of the same amount of water.

2.3. Chemical analysis of the experimental diets and fish

Ten fish at the initiation and five fish from each tank at the termination of the feeding trial were sampled and sacrificed for proximate analysis. Crude protein was determined by the Kjeldahl method (Kjeltec 2100 Distillation Unit, Foss Tecator, Hoganas, Sweden), crude lipid was determined using an ether-extraction method, moisture was determined by oven drying at 105°C for 24 h, fiber was determined using an automatic analyzer (Fibertec, Tecator, Hoganas, Sweden) and ash was determined using a muffle furnace at 550°C for 4 h, all methods were according to standard AOAC (1990).

2.4. Chemical analysis of blood

At the end of the 8-week feeding trial, blood samples were obtained from the caudal vein of five randomly chosen fish from each tank. Fish were starved for 24 h prior to bleeding. Serum was collected after centrifugation (3000 rpm for 10 min), pooled by tank, stored freezer at -70°C as separate aliquots for analysis of total protein, glucose, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and triglyceride, and analyzed by using automatic chemistry system (Vitros DT60 II, Vitros DTE II, DTSC II Chemistry System, Johnson and Johnson Clinical Diagnostics Inc., New York, USA).

2.5. Challenge test

Ten externally normal fish from each tank were chosen at the end of the 8-week feeding trial, shown to be free from bacterial infection and stocked into 24, 60 L static plastic tank (water volume 45 L). One third amount of water in each tank was replaced in a 5-day interval throughout challenge test. The bacteria used for challenge test were obtained as a reference pathogenic strain of gram negative, *Vibrio anguillarum* (FP5208) and gram positive, *Streptococcus iniae* (FP5228) isolated from olive flounder *Paralichthys olivaceus*.

The culture suspension of *V. anguillarum* and *S. iniae* were grown agar for 24 h, collected, washed and suspended in a sterile 0.85% saline solution and counted. Then, fish were artificially infected by intraperitoneal injection with 0.1 mL of culture suspension of pathogenic *V. anguillarum* and *S. iniae* containing 3.9×10^8 cells/mL. Fish were monitored for the next 20 and 30 days after *V. anguillarum* and *S. iniae* infection, respectively and dead fish were removed every 6 h for the first 10 days and 12 h for the rest days of the study.

2.6. Statistical analysis

One-way ANOVA and Duncan`s multiple range test (Duncan 1955) with SAS version 9.1 (SAS Institute, Cary, NC, USA) were used to analyze the significance of the difference among the means of treatments.

3. Results and discussion

Survival (%), weight gain (g/fish) and specific growth rate (SGR) of far eastern catfish fed the experimental diets with the various concentrations of SB and commercial product of immune enhancer for 8 weeks are given in Table 2. Survival was over 93% for all experimental diets and not significantly ($P > 0.05$) affected by the experimental diets. Weight gain and SGR of fish fed the experimental diets containing the various concentrations of SB slightly, but not significantly ($P > 0.05$), improved. This probably indicated that dietary inclusion of SB seemed to be not in effect for growth promoter of far eastern catfish.

Table 2. Survival (%), weight gain (g/fish) and specific growth rate (SGR) of far eastern catfish (*Silurus asotus*) fed the experimental diets containing the various concentrations of *S. baicalensis* and commercial product of immune enhancer (CP) for 8 weeks

Experimental diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[1]
Con[2]	0.96 ± 0.01	43.5 ± 1.17	98.1 ± 0.95	42.6 ± 1.17	6.8 ± 0.06
SB-0.25[3]	0.95 ± 0.00	44.7 ± 1.42	98.1 ± 0.95	43.8 ± 1.42	6.87 ± 0.06
SB-0.5[4]	0.96 ± 0.00	44.8 ± 4.06	98.1 ± 0.95	43.8 ± 4.05	6.85 ± 0.16
SB-1[5]	0.96 ± 0.00	46.4 ± 3.40	96.2 ± 1.90	45.4 ± 3.40	6.92 ± 0.12
SB-2[6]	0.96 ± 0.00	45.4 ± 1.73	93.3 ± 5.30	44.5 ± 1.73	6.88 ± 0.07
SB-3[7]	0.95 ± 0.00	44.1 ± 1.43	95.2 ± 2.52	43.2 ± 1.43	6.85 ± 0.07
SB-5[8]	0.95 ± 0.01	45.9 ± 2.60	95.2 ± 0.95	45.0 ± 2.61	6.92 ± 0.11
CP[9]	0.95 ± 0.00	44.0 ± 1.40	99.1 ± 0.95	43.1 ± 1.40	6.85 ± 0.05

Values (means of triplicates ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

[1]SGR = (Ln final weight of fish - Ln initial weight of fish)×100/days of feeding trial.

Con[2], SB-0.25[3], SB-0.5[4], SB-1[5], SB-2[6], SB-3[7], SB-5[8], CP[9] refer to Table 1.

A variety sources of dietary additives as growth promoters such as levamisole (Li, Wang & Gatlin 2006), *Quillaja saponin* (Francis, Makkar & Becker 2002), ginseng herb (Goda 2008), herb (Obosan®) (Kim, Moon, Jeong & Kim 2000a) and *Chlorella ellipsoidea* (Kim, Bai, Koo, Wang & Kim 2002), immunostimulants such as *Spirulina platensis* (Watanuki, Ota, Tassakka, Kato & Sakai 2006), bovine lactoferrin (Welker, Lim, Yildirim-Aksoy & Klesius 2007), aloe (Kim, Hwang & Bai 1999), chitosan (Cha, Lee, Song, Lee & Jeon 2008), green tea *Camellia sinensis* L. (Abdel-Tawwab, Ahmad, Seden & Sakr 2010) and echinacea (*Echinacea purpurea*) and garlic (Aly & Mohamed 2010), probiotics such as Biogen® consisted of *Bacillus lichenifromis* and *B. subtilis* (EL-Haroun, Goda & Ghowdhury 2006) and Organic Green® consisted of *Lactobacillus acidophilus*, *B. subtilis*, *Saccharomyces* and *Aspergillus oryzae* (Aly, Mohamed & John 2008b) and vitamin C (Kumari & Sahoo 2005; Lin & Shiau 2005) efficiently improved growth performance of fish. However, unlike these studies, dietary supplementation of chitin and chitosan depressed growth of hybrid tilapia, *O. niloticus* × *O. aureus* (Shiau & Yu 1999). Therefore, application of dietary additives to improve fish performance should be carefully considered because their favorable roles vary depending on fish species, targeting activities of additives, doses of additives, administration method of additives, dietary nutrient content and physiological and nutritional status of fish.

Feed consumption, feed efficiency ratio (FER) and protein retention (PR) of far eastern catfish was not significantly ($P > 0.05$) affected by the experimental diets (Table 3). However, protein efficiency ratio (PER) of fish fed the SB-5 diet was significantly ($P < 0.05$) higher than that of fish fed the SB-1 and SB-2 diets, but not significantly ($P > 0.05$) different from that of fish fed the Con, SB-0.25, SB-0.5, SB-3 and CP diets. The poorest PER was obtained in fish fed the SB-2 diet. Unlike this study, however, feed efficiency of fish improved with dietary inclusion of additives resulting from an increased weight gain of fish (Kumari & Sahoo 2005; Lin & Shiau 2005; EL-Haroun et al. 2006; Li et al. 2006; Ghosh, Sinha & Shau 2008; Goda 2008; Abdel-Tawwab et al. 2010; Aly & Mohamed 2010).

Table 3. Feed consumption (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER) and protein retention (PR) of far eastern catfish (*Silurus asotus*) fed the experimental diets containing the various concentrations of *S. baicalensis* and commercial product of immune enhancer (CP) for 8 weeks

Experimental diets	Feed consumption	FER[1]	PER[2]	PR[3]
Con[4]	46.7 ± 1.20	0.91 ± 0.00	2.02 ± 0.01^{abc}	30.26 ± 0.40
SB-0.25[5]	47.0 ± 1.64	0.93 ± 0.01	2.04 ± 0.01^{abc}	30.27 ± 0.72
SB-0.5[6]	46.7 ± 5.03	0.94 ± 0.02	2.01 ± 0.03^{abc}	29.41 ± 1.44
SB-1[7]	49.7 ± 3.63	0.91 ± 0.02	1.98 ± 0.04^{bc}	29.82 ± 1.11
SB-2[8]	50.2 ± 3.14	0.89 ± 0.03	1.96 ± 0.08^{c}	28.43 ± 0.40
SB-3[9]	47.4 ± 1.98	0.91 ± 0.02	2.02 ± 0.06^{abc}	28.89 ± 0.45
SB-5[10]	48.1 ± 1.98	0.93 ± 0.01	2.12 ± 0.03^{a}	32.43 ± 1.04
CP[11]	45.6 ± 1.73	0.95 ± 0.01	2.10 ± 0.01^{ab}	31.07 ± 0.22

Values (means of triplicates ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

[1]Feed efficiency ratio (FER) = Weight gain of fish/feed consumed.

[2]Protein efficiency ratio (PER) = Weight gain of fish/protein consumed.

[3]Protein retention (PR) = Protein gain×100/protein consumed.

Con[4], SB-0.25[5], SB-0.5[6], SB-1[7], SB-2[8], SB-3[9], SB-5[10], CP[11] refer to Table 1.

None of moisture content ranged from 75.0% to 77.4%, crude protein content ranged from 14.4% to 15.2% and ash content ranged from 2.1% to 2.5% of the whole body excluding liver, and moisture content ranged from 63.3% to 66.5%, crude protein content ranged from 11.8% to 12.6% and crude lipid content ranged from 1.3% to 1.6% of the liver of far eastern catfish was significantly affected by dietary concentrations of SB (Table 4). However, crude lipid content of the whole body excluding liver of fish fed the Con and SB-3 diets was significantly ($P < 0.05$) higher than that of fish fed the SB-0.5, SB-2, SB-5 and CP, which was lowest, but not significantly ($P > 0.05$) different from that of fish fed the SB-0.25 and SB-1 diets. Similarly, dietary inclusion of probiotic and green tea changed body composition of Nile tilapia (EL-Haroun et al. 2006; Abdel-Tawwab et al. 2010). In addition, administration of *C. ellipsoidea* lowered body fat of olive flounder (Kim et al. 2002). However, dietary inclusion of additives did not change body composition of fish (Francis et al. 2002; Park, Kwon, Lee, Shin & Min 2003; Goda 2008).

Table 4. Chemical composition (%, wet weight basis) of the whole body excluding liver and liver of far eastern catfish (*Silurus asotus*) at the end of the 8-week feeding trial

Experimental diets	Whole body excluding liver			
	Moisture	Crude protein	Crude lipid	Ash
Con[1]	75.0 ± 0.19	14.9 ± 0.16	6.8 ± 0.23[a]	2.3 ± 0.02
SB-0.25[2]	75.0 ± 0.13	14.7 ± 0.27	6.3 ± 0.15[ab]	2.5 ± 0.06
SB-0.5[3]	75.7 ± 0.41	14.5 ± 0.84	5.6 ± 0.40[c]	2.3 ± 0.12
SB-1[4]	74.5 ± 0.52	14.9 ± 0.48	6.3 ± 0.20[ab]	2.4 ± 0.09
SB-2[5]	77.4 ± 0.69	14.4 ± 0.39	5.6 ± 0.11[c]	2.4 ± 0.15
SB-3[6]	76.0 ± 0.43	14.7 ± 0.18	6.4 ± 0.11[a]	2.4 ± 0.19
SB-5[7]	75.4 ± 0.59	15.2 ± 0.31	5.7 ± 0.12[bc]	2.4 ± 0.10
CP[8]	76.6 ± 1.18	14.7 ± 0.02	4.7 ± 0.14[d]	2.1 ± 0.10
	Liver			
	Moisture	Crude protein	Crude lipid	
Con[1]	65.9 ± 0.97	12.1 ± 0.27	1.5 ± 0.09	
SB-0.25[2]	66.5 ± 1.04	12.4 ± 0.20	1.6 ± 0.24	
SB-0.5[3]	64.8 ± 0.50	12.3 ± 0.51	1.5 ± 0.16	
SB-1[4]	64.3 ± 1.18	12.5 ± 0.12	1.4 ± 0.10	
SB-2[5]	64.8 ± 1.27	12.4 ± 0.22	1.4 ± 0.05	
SB-3[6]	66.4 ± 0.05	12.6 ± 0.07	1.3 ± 0.15	
SB-5[7]	63.3 ± 0.03	11.8 ± 0.31	1.4 ± 0.30	
CP[8]	65.5 ± 1.48	12.5 ± 0.38	1.3 ± 0.09	

Values (means of triplicates ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

Con[1], SB-0.25[2], SB-0.5[3], SB-1[4], SB-2[5], SB-3[6], SB-5[7], CP[8] refer to Table 1.

Serum total protein level ranged from 2.2 g/dL to 2.7 g/dL, glucose level ranged from 114.7 mg/dL to 244.7 mg/dL, cholesterol level ranged from 93.0 mg/dL to 148.3 mg/dL and triglyceride level ranged from 326.7 mg/dL to 646.0 mg/dL of far eastern catfish was not significantly ($P > 0.05$) affected by the experimental diets (Table 5). However, unlike this study, dietary inclusion of SB extract lowered serum triglycerides and cholesterol of rats (Regulska-llow, Bienat, Grajeta, Llow & Drzewicka 2004). Serum GOT level of fish fed the SB-0.25 diet was significantly ($P < 0.05$) lower than that of fish fed the SB-1 and SB-2 diets, which was highest, but not significantly ($P > 0.05$) different from that of fish fed the Con, SB-0.5, SB-3, SB-5 and CP diets. No significant ($P > 0.05$) difference in serum GPT level of fish was found among the experimental diets except for the SB-2 diet. The lowest GOT and GPT levels were obtained in fish fed the SB-0.25 diet. Similarly, oral administration of green tea extract and lactic acid bacteria cultured in herb extract lowered serum GPT, GPT and low density lipoprotein cholesterol levels, and GOT and GPT levels of olive flounder, respectively (Cho, Lee, Park, Ji, Lee, Bae & Oh 2007; Jhon, Kim, Kim & Heo 2009). In addition, dietary inclusion of Obosan® increased serum total protein and glucose levels, but lowered GOT and GPT levels of fish (Kim et al. 2000a).

Table 5. Serum chemical composition of far eastern catfish (*Silurus asotus*) at the end of the 8-week feeding trial

Experimental diets	Total protein (g/dL)	Glucose (mg/dL)	GOT (IU/L)	GPT (IU/L)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Con[1]	2.3 ± 0.15	195.3 ± 13.98	133.3 ± 12.03^{bc}	12.3 ± 0.88^{b}	102.7 ± 7.69	365.7 ± 45.29
SB-0.25[2]	2.5 ± 0.10	114.7 ± 8.76	46.3 ± 9.02^{c}	10.0 ± 1.15^{b}	117.0 ± 17.21	512.3 ± 184.18
SB-0.5[3]	2.5 ± 0.07	153.7 ± 22.41	134.7 ± 26.77^{bc}	14.0 ± 2.08^{b}	128.7 ± 14.44	646.0 ± 144.78
SB-1[4]	2.3 ± 0.24	244.7 ± 52.41	171.0 ± 6.66^{ab}	16.0 ± 3.79^{b}	95.7 ± 14.52	361.0 ± 80.06
SB-2[5]	2.2 ± 0.18	130.7 ± 36.17	261.7 ± 53.29^{a}	35.5 ± 0.50^{a}	148.3 ± 32.12	452.0 ± 164.12
SB-3[6]	2.5 ± 0.09	151.7 ± 26.44	124.7 ± 52.30^{bc}	13.7 ± 1.45^{b}	110.3 ± 9.60	375.7 ± 66.09
SB-5[7]	2.2 ± 0.03	198.3 ± 12.45	97.0 ± 20.01^{bc}	16.0 ± 1.53^{b}	93.0 ± 6.35	326.7 ± 62.73
CP[8]	2.7 ± 0.17	172.0 ± 58.64	84.7 ± 22.26^{bc}	13.0 ± 2.65^{b}	98.0 ± 1.53	336.0 ± 32.51

Values (means of triplicates ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).
Con[1], SB-0.25[2], SB-0.5[3], SB-1[4], SB-2[5], SB-3[6], SB-5[7], CP[8] refer to Table 1.

Change in cumulative mortality of far eastern catfish fed the experimental diets with the various concentrations of SB and commercially available immune enhancer for 8 weeks and subsequently exposed to *V. anguillarum* and *S. iniae* by intraperitoneal injection are depicted in Figs. 1 and 2, respectively. Fish started to show mortality in 3 and 2 days after *V. anguillarum* and *S. iniae* artificial infection, respectively. Cumulative mortality of fish fed the Con diet was significantly ($P < 0.05$) higher than that of fish fed the all other diets since 10 and 25 days after *V. anguillarum* and *S. iniae* infection. However, no significant difference in cumulative mortality of fish was found among the experimental diets containing the various concentrations of SB or CP diet. This indicated that SB was effective to lower mortality of far eastern catfish after *V. anguillarum* and *S. iniae* infection, but the various concentrations of SB did not affect mortality of fish. Determination of optimum dietary concentrations of SB can be made based on the further economical analysis because dietary administration of the various concentrations of SB slightly affected growth performance and mortality of fish after artificial pathogen infection in the present study.

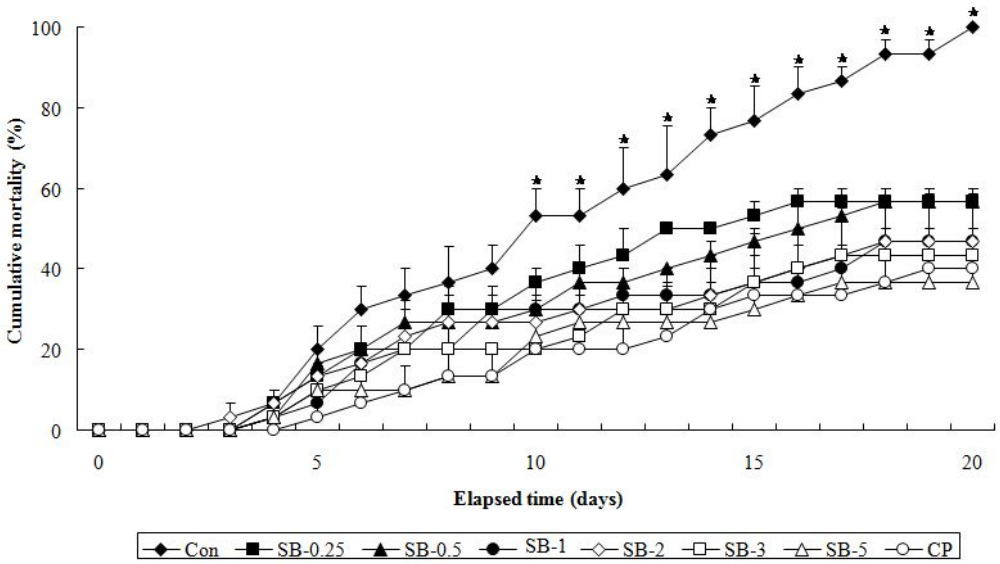

Figure 1. Cumulative mortality (%) of far eastern catfish (*Silurus asotus*) fed the experimental diets containing various concentrations of *S. baicalensis* (SB) and commercial product of immune enhancer (CP) for 8 weeks and subsequently exposed to *V. anguillarum* by intraperitoneal injection (means of triplicates ± SE). * indicates that the cumulative mortality of fish fed the SB-0.25, SB-0.5, SB-1, SB-2, SB-3, SB-5 and CP diets was significantly ($P < 0.05$) lower than that of fish fed the Con diet.

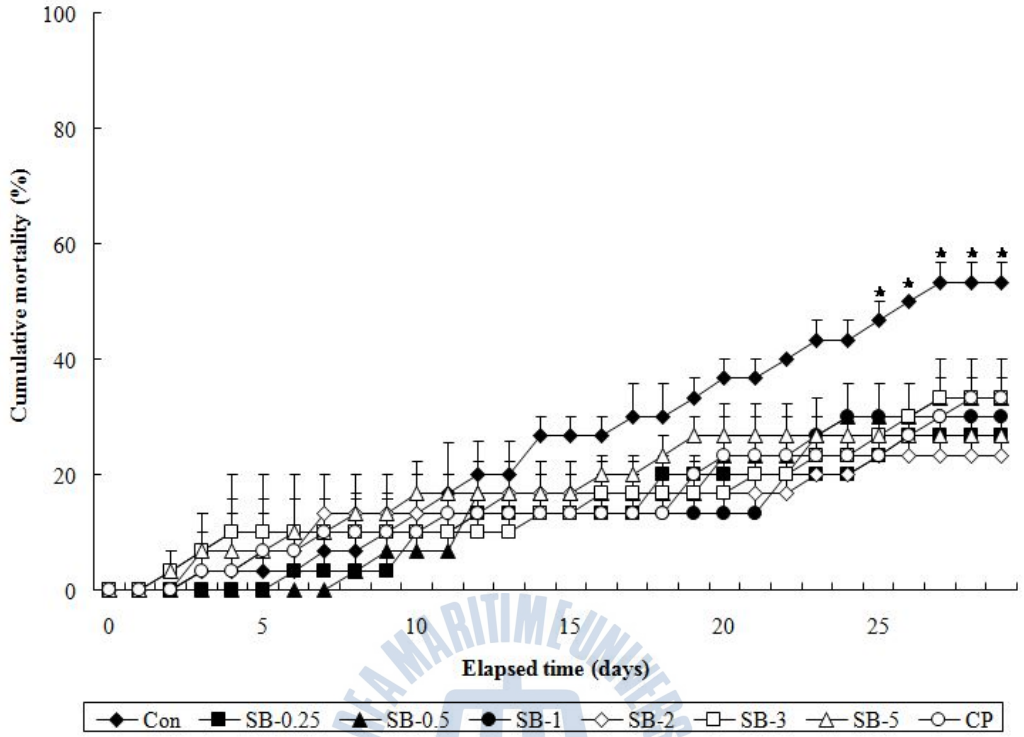

Figure 2. Cumulative mortality (%) of far eastern catfish (*Silurus asotus*) fed the experimental diets containing various concentrations of *S. baicalensis* (SB) and commercial product of immune enhancer (CP) for 8 weeks and subsequently exposed to S. iniae by intraperitoneal injection (means of triplicates ± SE). * indicates that the cumulative mortality of fish fed the CP the SB-0.25, SB-0.5, SB-1, SB-2, SB-3, SB-5 and CP diets was significantly ($P < 0.05$) lower than that of fish fed the Con diet.

SB had a variety of antibacterial activities against staphylococci, cholera, typhoid, paratyphoid, dysentery, diphtheria, hemolytic streptococci, Escherichia coli, pneumococococci and spirochaeta (Chang & But 1986; Huang 1993). In addition, Kim et al. (2000b) reported that *S. radix* had compatible effect with the commercial antibiotics (ampicillin, tetracyclin, chlorampheicol and kanamycin) against *Salmonella typhimurium*, but only acted on pathogenic bacteria rather than normal non-pathogenic bacteria such as *E. coli*. And they explained that *S. radix* is the herb having superior antibiotic function which does not have any possible side-effect over long-term dosage.

No significant improvement in growth performance, but significant decrease in mortality of far eastern catfish after *V. anguillarum* and *S. iniae* infection in this study indicated that SB could be an effective additive for immune enhancer rather than growth promoter of fish. Similarly, administration of Chinese herb mixture *A. radix* and *G. lucidum* for carp and Indian herb, *O. sanctum* or *Solanum trilobatum* for tilapia improved immune response and disease resistance against *Aeromonas hydrophia* (Logambal et al. 2000; Divyagnaneswari et al. 2007; Yin et al. 2009). Furthermore dietary inclusion of Chinese herb mixture, *R. astragalin seu Hedysari* and *R. angelicae sinensis* (Jian & Wu 2003) and aloe (Kim et al. 1999) elevated survival of fish, following challenge with *V. alginolyticus*.

Galina, Yin, Ardo & Jeney (2009) reviewed that use of herbal extracts as immunostimulants of fish and concluded that herbal extracts could be used

in fish culture as alternatives to vaccines, antibiotics or chemotherapeutic agents. An immunstimulatory effect of the leaf extract *O. sanctum* was obvious when administrated through intraperitoneal and oral routes, and dietary intake of *O. sanctum* by tilapia (*O. mossambicus*) enhanced disease resistance against *A. hydrophila* (Logambal et al. 2000). In addition, oral administration of echinacea and garlic extract improved resistance of Nile tilapia against *A. hydrophila* infection (Aly & Mohamed 2010).

When a variety sources of immunostimulants (100 ppm lactoferrin, 0.1% β -1, 3 glucan, 50 ppm levamisole and 500 ppm vitamin C) were orally administrated to Asian catfish (*Clarias batrachus*) before *Aermonas hydrophila* infection, β-1, 3 glucan was found to be the most effective immunostimulant, followed by levamisole, lactorferrin and vitamin C in fish (Kumari & Sahoo 2006). Dietary inclusion of bovine lactoferrin and green tea improved immune functions and survival of fish after *S. iniae* and *A. hydrophila* infection, respectively (Welker et al. 2007; Abdel-Tawwab et al. 2010). In addition, oral administration of probiotics was effective to improve survival of four kinds of ornamental fishes (Ghosh et al. 2008) and Nile tilapia after *A. hydrophila* infection (Aly, Abd-El-Rahman, John & Mohamed 2008a; Aly et al. 2008b). Furthermore dietary inclusion of vitamin C improved survival of grouper *Epinephelus malabaricus* and Asian catfish after *V. carchariae* and *A. hydrophila* infection, respectively (Kumari & Sahoo 2005; Lin & Shiau 2005).

Results of this study indicated that dietary inclusion of SB extract was effective to improve survival of far eastern catfish after *V. anguillarum* and *S. iniae* infection, but the various concentrations of SB did not affect fish performance.

II. Conclusion

In this study, Effects of various concentrations of *Scutellaria baicalensis* (SB) extract in the diets on growth, body composition, serum chemistry and challenge test of far eastern catfish (*Silurus asotus*) were determined.

Survival and weight gain of fish were not affected by dietary concentrations of SB. None of feed efficiency, body composition and serum chemical composition of fish was affected by dietary concentrations of SB. the cumulative mortality of eastern catfish fed the Con diet was higher than that of eastern catfish fed the all other diets since 10 and 25 days after *V. anguillarum* and *S. iniae* infection.

Results of this study indicates that dietary inclusion of SB extract was effective to improve survival of far eastern catfish after *V. anguillarum* and *S. iniae* infection, but the various concentrations of SB did not affect fish performance.

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