Effects of *Bauhinia strychnifolia* Craib leaf extract on growth parameters and intestinal morphology of catfish (*Clarias gariepinus*)

Phukphon Munglue[1,*], Kajohnpong Dasri[2]

[1] *Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University, 2 Ratchathani Road, Muang District, Ubon Ratchathani, 34000, Thailand*
[2] *Program of Microbiology, Faculty of Science, Ubon Ratchathani Rajabhat University, 2 Ratchathani Road, Muang District, Ubon Ratchathani, 34000, Thailand*

*Corresponding Author: phukphon.m@ubru.ac.th

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ABSTRACT

The leaves, stem and root of *Bauhinia strychnifolia* Craib have long been used for the treatment of several ailments in Thai traditional medicine. However, the use of this plant as a natural feed additive in aquaculture is still limited. The aims of this research were, therefore, to examine the effects of *B. strychnifolia* leaf extract (BSLE) on growth performance and intestinal morphology of catfish (*Clarias gariepinus*). Fish were reared in circle cement tanks and fed with the aquadiets containing different levels of BSLE (0, 0.1, 0.5 and 1%) for up to 4 weeks. We found that fish fed the diets supplemented with BSLE significantly increased in weight gain and specific growth rate when compared to the control diet. The diets supplemented with 0.1% BSLE produced the highest weight gain (205.16±11.08%), followed by 1% (167.98±11.57%), 0.5% (165.92±22.93%) and the control diet (120.83±1.37%). Survival rate, general appearance and feed acceptability of the experimental groups (95.49±2.38 to 100.00±0.00%) were similar to the control group (97.29±1.56%). Observations on intestinal histology exhibited that the height of intestinal villi and goblet cell numbers of catfish fed the experimental diets were significantly higher than those of the control diet ($P<0.05$). Our findings support the use of BSLE as a natural feed additive in aquafeeds for improving growth and intestinal morphology in catfish.

Keywords: *Bauhinia strychnifolia* Craib, Catfish, *Clarias gariepinus*, growth performance, intestinal morphology.

INTRODUCTION

A search for natural feed additives used in aquaculture to enhance growth performances and health status of aquatic animals is now increasing worldwide, due to their higher safety and efficacy (Chakraborty *et al*. 2013; Heidarieh *et al*. 2012). Such feed additives are usually derived and isolated from medicinal plants which could be substituted synthetic compounds (Francis *et al*. 2002; Francis *et al*. 2005; Serrano, 2013). However, active ingredients, optimal concentrations, application procedures and modes of action of herbs are required to completely understand pharmacological and biological effects in animals (Chakraborty *et al*. 2013).

Bauhinia strychnifolia Craib is the plant belonging to the family of Leguminosae-

Caesalpiniaceae. Leaves and stems of this plant have been used to alleviate alcoholic toxication, fever, cancer and allergy in Thai traditional medicine (Bunluepuech *et al*. 2013; Kaewpiboon *et al*. 2012; Yuenyongsawad *et al*. 2013). A decoction of *B. strychnifolia* leaves was found to be useful for the treatment of breast, colon and stomach cancers (Yuenyongsawad *et al*. 2013). Biological investigations indicated that *Bauhinia* sp. are very potent against some bacterial strains, virus, free radicals and diabetes (Ahmed *et al*. 2012; Kaewpiboon *et al*. 2012; Pepato *et al*. 2002). Phytochemical studies revealed that isolated active compounds obtained from different parts of the genus *Bauhinia* can be classified into alkaloids, steroids, triterpenes, terpenoids, flavonoids, saponins, phenolic compounds and volatile oils (Duarte-Almeida *et al*. 2004; Modh *et al*. 2011; Pandey, 2015). Base on several scientific reports, there appears to be no such examinations of growth promoting effects of *B. strychnifolia* on any species of aquatic animals.

As aquaculture producers are required to find novel drugs to replace synthetic chemicals, and some compounds are successfully applied to animal feed components as growth enhancers (Chakraborty *et al*. 2013; Heidarieh *et al*. 2012), the aims of this research were to examine the effects of *B. strychnifolia* leaf extract (BSLE) on growth performance and intestinal morphology of catfish (*Clarias gariepinus*).

MATERIALS AND METHOD

Plant Preparation and Extraction

B. strychnifolia leaves were collected from Muang District, Ubon Ratchathani, Thailand. They were cleaned by using tab water and dried in a hot air oven at 60^{o}C for 6 days. Dried leaves (50 g) were powered by a household blender and macerated in 70% ethanol (500 mL) for 1 weeks. The extract was filtered through a filter paper, Whatman No 1, and evaporated in rotary evaporator. The residual extract was then lyophilized by a lyophilizer. Percentage yield of the plant extract was 12.30%.

Diet Preparation

The basal diets obtained from a commercial fish feed manufacturer containing Protein 32% and Lipid 4% were mixed BSLE at different levels of 0, 0.1, 0.5 and 1%, coated with white egg yolk and dried in a hot air oven at 30^{o}C for 1 day. Dried diet samples were kept in a labeled zipper locking bag to prevent humidity until used.

Fish Preparation

Catfish were obtained from Ubon Ratchathani Fisheries Cooperatives, Ubon Ratchathani, Thailand. At the end of the acclimatization period for 1 week, fish were randomly allocated into 4 treatments with 3 replication each and reared in circular cement tanks. Fish were fed the diets containing the plant extract at various levels of 0, 0.1, 0.5 and 1% for 4 weeks. General behavior, palatability and feed acceptability as well as external appearance of fish were observed daily. Water was changed every 5 days by siphoning. Water qualities were maintained in the standard conditions for fish cultivation. Dead fish were removed and recorded daily for 4 weeks.

Effects on Growth Performance and Survival Rate

At the end of the feeding period of 4 weeks, catfish in each tank were weighted. Parameters of growth performance were calculated as follows:

Weight gain (WG, %) = 100 × (final fish weight (g) – initial fish weight (g))/ initial fish weight (g)

Specific growth rate (SGR, % d-1) = 100× [*In* final wet weight (g) – *In* initial wet weight (g)]/experimental days.

Feed conversion ratio (FCR) = feed intake (g)/ weight gain (g).

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

Effects on Intestinal Morphology

At the end of feeding trial, fish were fasted for 24 h before being weighed. Three fish from each treatment were sampled and anesthetized with clove oil. Fish intestines were collected and weighed to calculate the intestinosomatic index. Fish intestines were cut and subsequently divided into anterior and posterior part and each part was cut into 0.5 cm long carefully. Specimens were fixed in 10% neutral buffered formalin for 24 h, dehydrated and embedded in paraffin. The tissue samples were then strained with haematoxylin and eosin (H&E) for the histological analysis (Pirarat *et al*. 2015; Vechklang *et al*. 2011).

Statistical Analysis

Data are presented as means ± standard error of the mean (SEM). Significant differences were determined by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. If $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effects on Growth Performance and Survival Rate

Growth parameters and survival rate of catfish fed diets supplemented with different levels of BSLE for 4 weeks are shown in Table 1. Our results exhibited that catfish fed the diets containing BSLE significantly increased in final wet weight and SGR ($P<0.05$). FCR values of the experimental groups decreased significantly when compared to the control group ($P<0.05$). Increased intestinosomatic index values of the plant extract-treated fish were observed, but they did not reach statistical differences ($P>0.05$). Survivals showed no significant differences among the treatments ($P>0.05$). During the treatment period, fish fed the diets containing the plant extract did not show any signs of toxicity. Feeding response, feed acceptability and general behavior as well as external appearance of treated fish were similar to untreated fish.

TABLE 1: Growth performance and survival rate of catfish fed the diets containing BSLE for 4 weeks.

Growth parameters	Treatments			
	Control	0.1% BSLE	0.5% BSLE	1% BSLE
Initial weight (g)	12.56±0.21[a]	12.54±0.12[a]	12.53±0.15[a]	12.51±0.38[a]
Final weight (g)	27.83±1.01[a]	38.03±1.98[c]	34.33±1.25[b]	35.66±3.52[b]
Weight gain (%)	120.83±1.37[a]	205.16±11.08[c]	165.92±22.93[b]	167.98±11.57[b]
SGR	2.09±0.21[a]	3.85±0.14[b]	3.47±0.31[b]	3.23±0.13[b]
FCR	2.50±0.06[a]	1.52±0.14[c]	1.73±0.08[b]	1.65±0.02[b]
Survival rate (%)	97.29±1.56[a]	97.29±2.70[a]	95.49±2.38[a]	100.00±0.00[a]
ISI	2.21±0.13[a]	2.62±0.24[a]	2.95±0.19[a]	2.76±0.27[a]

BSLE = *B. strychnifolia* leaf extract, ISI = intestinalsomatic index, Values are expressed as mean ± SEM. n = 6. One-way analysis of variance (ANOVA) was used. Means with different superscripts [(a-c)] at the same row are significantly different (P<0.05).

TABLE 2: Intestinal morphology of catfish fed the diets containing *B. strychnifolia* leaf extract for 4 weeks.

Treatments	Anterior Intestine		Posterior Intestine	
	Villus Height (µm)	No. of Goblet Cells	Villus Height (µm)	No. of Goblet Cells
Control	1285.13±87.70[a]	35.66±7.83[a]	1011.22±57.93[a]	16.66±3.92[a]
0.1% BSLE	2697.06±310.96[b]	77.00±12.50[b]	1828.01±53.24[b]	37.66±2.66[b]
0.5% BSLE	2124.20±167.89[b]	81.66±10.98[b]	1252.69±57.50[b]	34.00±5.03[b]
1% BSLE	1659.20±40.65[b]	68.64±8.29[b]	1249.25±12.97[b]	35.66±5.20[b]

BSLE = *B. strychnifolia* leaf extract, Values are expressed as mean ± SEM. n = 3. One-way analysis of variance (ANOVA) was used. Means with different superscripts [(a-b)] at the same column are significantly different (P<0.05).

It has been documented that herbal plants offer new sources of the natural molecules which could be applied in aquafeeds to enhance growth performance and the health status of cultured aquatic animals (Chakraborty *et al*. 2013; Francis *et al*. 2002; Francis *et al*. 2005; Heidarieh *et al*. 2012; Serrano, 2013). *B. strychnifolia* is an interesting medicinal plant for investigations, due to its biological activities (Bunluepuech *et al*. 2013; Kaewpiboon *et al*. 2012). This present work was aimed at determining the growth promoting effects of dietary supplementation with BSLE on growth of catfish. Our findings indicate that catfish fed the diets incorporated with the plant extract at different levels significantly improved various parameters of growth including final weight, FCR and SGR compared with control. No significant differences were found in the survival rate among the groups. Previous report has shown that oral administration of diet supplemented with wood betony (*Stachys lavandulifolia*) extract to common carp (*Cyprinus carpio*) significantly enhanced final weight, mean weight gain, SGR and average daily gain in comparison with the control diet (Bahrami Babahydari *et al*. 2014). Rainbow trout (*Oncorhynchus mykiss* Walbaum) fed the diets containing garlic significantly increased growth performance, protein efficiency and FCR (Nya & Austin, 2009). The mechanisms contributing to growth promoting property of medicinal plants in aquatic animals are unclear in the present. However, it has been speculated that herbs could increase the growth of fish through: 1) the improvement of feed intake and palatability (Francis *et al*. 2002; Francis *et al*. 2005; Li *et al*. 2012; Serrano, 2013); 2) the activation of specific digestive enzymes in the digestive tract to enhance the digestion and absorption of nutrients (Francis *et al*. 2002; Francis *et al*. 2005; Heidarieh *et al*. 2012; Serrano, 2013; Zahran *et al*. 2014); 3) the improvement of intestinal microflora (Acar *et al*. 2015; Nya & Austin, 2009; Reda & Selim, 2015; Zahran *et al*. 2014); 4) the modulation of non-specific and specific immune responses as well as disease resistant (Talpur, 2014; Talpur & Ikhwanuddin, 2012) and; 5) the biological activities of plants like anti-oxidant, anti-fungal, anti-viral and anti-bacterial properties which may help to promote general well-beings and immune functions of cultured fish (Li *et al*. 2012; Talpur, 2014; Talpur & Ikhwanuddin, 2012; Zahran *et al*. 2014).

Effects on Intestinal Morphology

Effects of dietary BSLE on intestinal morphology of catfish are summarized in Table 2. The height of intestinal villi and goblet cell numbers observed in anterior and posterior intestine of fish fed dietary supplementation with BSLE were significantly higher than fish fed the diet without the plant extract ($P<0.05$).

The length of intestinal villi and goblet cell numbers are associated with digestive and absorptive functions of fish intestines (Li *et al*. 2012; Manzanilla *et al*. 2006; Reda & Selim, 2015; Specian & Oliver, 1991). It is well known that goblet cells synthesize and secret mucus containing high molecular weight glycoproteins named mucins to protect the mucosal layer of intestines from dehydration, mechanical or chemical damage and invading pathogens (Kamali Sangani *et al*. 2014; Pirarat *et al*. 2015; Specian & Oliver, 1991). Our study indicated that dietary supplementation with BSLE enhanced villi and goblet cell count which would indicate the mode of action of this plant on fish digestibility and nutrient utilization efficiency (Chakraborty *et al*. 2013; Heidarieh *et al*. 2012; Reda & Selim, 2015; Vechklang *et al*. 2011). The mechanism associated with increased villus height and goblet cells produced by BSLE is still questioned. It is hypothesized that herbal plants and their compounds may be involved in the modulation of various cell functions in fish intestines including DNA, RNA and protein synthesis (Chakraborty *et al*. 2013; Citarasu, 2010; Kamali Sangani *et al*. 2014). In addition, natural products could act as modulators to promote cell proliferation and cell mitotic division of goblet cells in order to increase in nutrient digestion and absorption as well as improve the growth of fish (Antushevich *et al*. 2014; Chakraborty *et al*. 2013; Citarasu, 2010; Kamali Sangani *et al*. 2014).

CONCLUSION

We found that fish diets containing BSLE significantly increased final weight, WG and SGR and decreased FCR values significantly.

Our study also showed that the diets supplemented with the plant extract significantly enhanced the height of intestinal villi and the number of goblet cells. Thus, our results support the use of BSLE as a natural feed additive in aquafeeds for improving growth and intestinal morphology in catfish.

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