



Effects of Lotus (*Nelumbo nucifera* Gaertn.) Stamen Extract on Growth Performance, Feed Utilization and Intestinal Morphology of Catfish (*Clarias gariepinus*)

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Abstract

The effects of dietary supplementation of lotus (*Nelumbo nucifera* Gaertn.) stamen extract (NNSE) on growth performance, feed utilization efficiency and intestinal morphology of Catfish (*Clarias gariepinus*) were evaluated experimentally in a 7-week feeding trial. Fish with an initial weight of 12.54 ± 0.64 g were completely randomized into 4 groups with 3 replications and reared in circular concrete tanks. Group 1, fish were fed the basal diets and served as the control group. Groups 2-4, fish were fed the diets supplemented with 0.1, 0.5 and 1% of NNSE, respectively. At the end of experiment, it was found that fish fed the diets containing NNSE at different levels significantly increased weight gain, specific growth rate and feed conversion ratio compared to the control ($P < 0.05$). No significant differences were observed in the survival rate and the relative weights of internal organs between the groups ($P > 0.05$). Villi heights and widths, muscularis thickness and goblet cell number in anterior and posterior portion of fish intestines observed under the light microscope were significantly increased in the experimental groups compared to the control group ($P < 0.05$). Additionally, feeding behavior, palatability and feed acceptability did not differ among the groups. The optimal level of NNSE observed was 0.1%. Taken together, these findings reveal that NNSE could be applied in fish feed as a natural feed additive without negative side-effects to improve growth, feed utilization and intestinal morphology in Catfish.

Keywords : *Nelumbo nucifera* Gaertn., *Clarias gariepinus*, Growth performance, Feed utilization, Intestinal morphology.

1. Introduction

Aquaculture products are served as an important source of protein for human consumption (1,2). In addition to the increased demand for aquaculture products worldwide, an intensive aquaculture production in the modern farming systems has been performed around the globe (1,2). The basic requirements for this practice are to promote fish growth and health. Antibiotics and growth promoting agents are useful for improving animal health and production (3). However, synthetic drugs and their residues could produce the high risk to animals and consumers. In addition, the emergence of antibiotic-resistant microorganisms will be increased in the future (4). Thus, the development of novel natural growth promoting agents are required for aquaculture industry (2).

Medicinal plants are generally served as invaluable materials for the treatment of a variety of health ailments and for the synthesis of the synthetic drugs in modern pharmaceutical industries (5). In animal production section, botanical products are used as the main sources of veterinary medicines to control various diseases and to improve the immune functions (1-4). Furthermore, the application of herbal preparations to the diets as natural feed additives can enhance growth performance and feed utilization efficiency without side effects when compared to the synthetic drugs (1-4).

Lotus (*Nelumbo nucifera* Gaertn.) is generally cultivated throughout Southeast Asia. All parts of lotus are useful for the treatment of several diseases by local people for a long time (6). Seeds and rhizomes of lotus have been reported to have anti-inflammatory, antioxidant, antiviral,

antiproliferative, anti-fertility and immunomodulatory activities (7). Lotus stamens also possess antioxidant, antibacterial, aldose reductase inhibitory and aphrodisiac properties (8). Leaves are useful for hematemesis, metrorrhagia, epistaxis and fever (6-8). Several phytochemical compounds such as alkaloids, flavonoids, triterpenoids, saponins, carotenoids, tannins, phenolics and essential oils have been isolated from lotus (6-8).

Sivagurunathan et al. (9) found that *Cirrhinus mrigala* fed the diets supplemented with 0, 1 and 3% of the lotus extract significantly feed conversion ratio and specific growth rate compared to control diet. In addition, they also found an increase in lymphocytes, neutrophils and monocytes in *C. mrigala* fed with the lotus extract and infected with *Pseudomonas aeruginosa* (9). Recent reports indicated that the diets containing *N. nucifera* peduncle extracts significantly enhanced the growth performance of Nile tilapia (*Oreochromis niloticus*) without side effects (10). These results indicated that *N. nucifera* can be used to improve fish growth and enhance the role of fish immune system (10).

The utilization of *N. nucifera* as a natural feed additive in aquatic diets has been successful in many fish species (9,11,12). However, there was no research regarding its use in the case of Catfish (*Clarias gariepinus*). Thus, the aims of this experiment were to investigate the effects of dietary supplementation of lotus stamen extract (NNSE) on growth performance, feed utilization efficiency and intestinal histology of Catfish.

In Thailand, Catfish production is ranked second next only to Nile tilapia and this species is primarily cultivated for

domestic consumption. In 2014, Thai farmers produced Catfish approximately 137,000 ton and this rate tends to increase annually (13). It has been reported that Catfish farming is now challenged with the impact of increasing feed prices and several pathogenic agents. Thus, more research are required to find novel feed ingredients for enhancing growth and production of fish.

2. Materials and Methods

2.1 Plant preparation and extraction

Fresh lotus flowers were harvested from Sirindhorn District, Ubon Ratchathani. They were cleaned using tap water. The stamens were manually collected, dried in hot air oven at 60°C for 8 h and powered using a household electric grinder. The powder samples were macerated with ethanol 70% for 14 days at room temperature. The extract was filtered using Whatman No.1 paper and the filtrate was evaporated by rotary evaporator which was maintained at 50°C and 90 rpm. The extract was dried by lyophilizer and stored in a refrigerator until used.

2.2 Experimental diets

The basal fish diet was obtained from the commercial Catfish feed containing not less than 30% Protein and 3% Lipid and not more than 12% Moisture and 6% Fiber. The diet was sprayed with different levels of NNSE (0.1, 0.5 and 1%) and gently mixed. Diets were then dried at 30°C for 24 h in hot air oven.

2.3 Fish and experimental design

A total of 500 fish with an initial weight of 12.54±0.64 g were obtained from Ubon Ratchathani Fishery Cooperatives, Ubon Ratchathani. During the 1 week of acclimatization period, 360 fish were fed with the basal diet and distributed

randomly into 4 groups, with 3 replicates each (30 fish per replication) and reared in circular concrete tanks (90 cm in diameter and 50 cm in height). Then, fish were fed the experimental diets for 7 weeks as follows.

Group I: fed the basal diet and served as the control group.

Group II: fed the diet containing 0.1% NNSE.

Group III: fed the diet containing 0.5% NNSE.

Group IV: fed the diet containing 1% NNSE.

Water quality was maintained in the optimal conditions for Catfish (temperature, 28±2°C, pH, 7.2±0.5 and dissolved oxygen 7.0±0.05 mg/L). All tanks were cleaned once every 5 days. Fish were fed *ad libitum* two times a day with a rearing rate of 3% of live weight and weighted every one week. Dead fish were recorded and removed (9,11).

2.4 Effect on growth performance

At the end of the experimental period, fish were fasted for 24 hours before study. The parameters of fish growth and feed utilization efficiency were calculated using the following equations (9,11).

Weight gain (WG, g) = final fish weight (g) – initial fish weight (g).

Specific growth rate (SGR, % d⁻¹) = 100 × [ln final wet weight (g) – ln 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).

2.5 Effect on intestinal morphology

At the end of the feeding period, the intestines from three fish in each replication were removed, cleared from adherent tissues and divided into anterior

and posterior parts. Each part of the intestine (approximately 1 cm) was fixed in 10% neutral buffered formalin and subsequently dehydrated, embedded in paraffin. The samples were cut transversely into 5 μ m thickness and stained with hematoxylin and eosin (H&E). The sections were observed for the measurement of heights and widths of the intestinal villi and the number of goblet cells as described by Pirarat et al. (14) and Heidarieh et al. (15) under the microscope connected to a computer running DinoCapture 2.0 software.

2.6 Adverse effects

During the experimental period, fish were observed daily for feeding behavior, feed acceptance, palatability, general behavior and external characteristics (9,11,16). Relative organ weights of heart, stomach, liver, spleen, kidney and gills were measured by using the following equation.

Relative organ weight (%) = $100 \times$ (weight of organ (g)/weight of fish (g)).

2.7 Data Analysis

All data are expressed as mean \pm standard error of the mean (SEM). The significant differences among the various parameters were evaluated by using one-way ANOVA followed by Duncan's multiple range test. If P value <0.05 was considered statistically significant.

3. Results

3.1 Effect on growth performance

The results revealed that the application of NNSE to the diets significantly increased WG, SGR and FCR when compared with the basal diet (P<0.05). No significant difference was observed in the survival rate among the experimental groups (P>0.05). The optimal level of NNSE observed in this present investigation was 0.1%. The effects of diets supplemented with NNSE on growth performance of Catfish are summarized in Table 1.

Table 1. Effects of dietary NNSE on growth performance and feed utilization of Catfish.

Parameters	Treatments			
	Control	0.1%NNSE	0.5%NNSE	1%NNSE
Initial weight (g)	12.57 \pm 0.24	12.53 \pm 0.47	12.50 \pm 0.99	12.56 \pm 0.87
Final weight (g)	27.83 \pm 1.01 ^a	38.00 \pm 2.26 ^b	36.83 \pm 3.27 ^b	34.00 \pm 2.38 ^b
WG (g)	15.26 \pm 1.37 ^a	25.16 \pm 1.85 ^b	24.32 \pm 2.29 ^b	21.83 \pm 2.02 ^b
SGR (%d ⁻¹)	2.06 \pm 0.22 ^a	3.28 \pm 0.24 ^b	3.69 \pm 0.13 ^b	3.43 \pm 0.22 ^b
FCR	1.48 \pm 0.02 ^a	1.87 \pm 0.09 ^b	1.61 \pm 0.08 ^b	1.70 \pm 0.08 ^b
Survival rate (%)	93.33 \pm 6.66	86.66 \pm 13.33	90.00 \pm 10.00	100.00 \pm 0.00
Gill somatic index (%)	5.56 \pm 0.13	5.87 \pm 0.25	5.24 \pm 0.25	5.53 \pm 0.17
Cardiacsomatic index (%)	0.14 \pm 0.10	0.22 \pm 0.08	0.15 \pm 0.01	0.15 \pm 0.01
Hepatosomatic index (%)	2.28 \pm 0.31	2.22 \pm 0.23	2.24 \pm 0.26	2.26 \pm 0.14
Splenosomatic index (%)	0.10 \pm 0.01	0.13 \pm 0.02	0.11 \pm 0.06	0.12 \pm 0.01
Gastrosomatic index (%)	1.77 \pm 0.23	1.89 \pm 0.20	1.79 \pm 0.07	2.02 \pm 0.18
Intestinosomatic index (%)	2.28 \pm 0.27	2.83 \pm 0.47	2.56 \pm 0.11	2.69 \pm 0.14

Remarks: NNSE = *N. nucifera* stamen extract, Values are expressed as mean \pm SEM. One-way analysis of variance (ANOVA) was used. Means with different superscripts (^{a-b}) at the same row are significantly different (P<0.05).

3.2 Effect on intestinal morphology

As shown in Table 2 and Figures 1 and 2, the heights of intestinal villi and goblet cell number observed both in anterior and posterior parts of intestines under the light microscope were significantly increased in the experimental groups compared to the control group (P<0.05). In addition, the thicknesses of longitudinal and circular muscularis as well as villi widths of anterior intestine were significantly increased in fish fed the diet

incorporated with NNSE compared to the control diet (P<0.05). In posterior intestine, the thickness of circular smooth muscle of fed the fed 1% NNSE diet was also significantly higher than that of fish fed the basal diet (P<0.05). However, no significant difference in the longitudinal muscularis thickness of posterior intestine was observed when compared to the control group (P>0.05).

Table 2. Intestinal morphology of Catfish fed the diets containing NNSE for 7 weeks.

Treatments	Parameters									
	Anterior part of intestines					Posterior part of intestines				
	Villus height (µm)	Villus width (µm)	Longitudinal muscularis thickness (µm)	Circular muscularis thickness (µm)	Goblet cells	Villus height (µm)	Villus width (µm)	Longitudinal muscularis thickness (µm)	Circular muscularis thickness (µm)	Goblet cells
Control	1873.57±39.24 ^a	549.167±30.65 ^a	36.00±2.94 ^a	52.92±15.62 ^a	27.66±8.96 ^a	1431.19±24.80 ^a	585.05±30.40 ^a	44.87±7.43	91.86±2.39 ^a	28.66±5.48 ^a
0.1% NNSE	2579.18±53.89 ^c	871.245±38.28 ^b	39.68±3.59 ^a	75.17±3.00 ^{ab}	49.00±1.73 ^b	1798.44±29.20 ^b	660.32±55.70 ^{ab}	48.16±7.14	112.77±14.44 ^b	38.62±3.28 ^{ab}
0.5% NNSE	2650.41±50.54 ^c	1044.80±70.15 ^b	51.14±9.29 ^{ab}	101.05±13.23 ^b	54.33±1.83 ^b	2001.52±82.48 ^b	734.60±77.10 ^a	47.26±2.88	97.09±8.74 ^a	91.68±5.36 ^c
1% NNSE	2369.89±92.85 ^b	764.98±52.16 ^c	65.99±9.59 ^b	157.98±213.55 ^c	49.33±2.33 ^b	1952.95±17.09 ^b	791.90±24.20 ^b	57.40±7.95	123.66±8.93 ^b	89.64±5.89 ^c

Remarks: NNSE = *N. nucifera* stamen extract, Values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used. Means with different superscripts^(a-c) at the same column are significantly different (P<0.05).

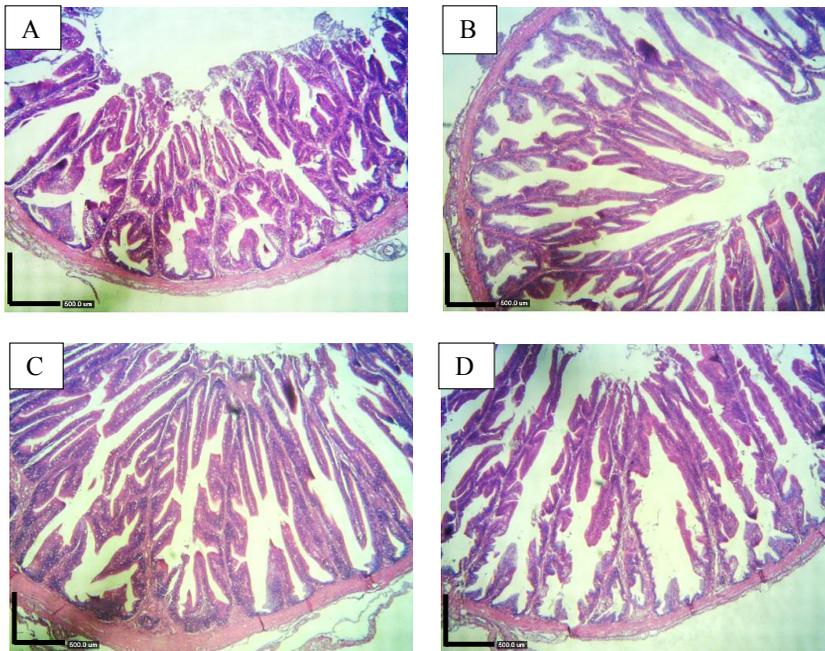


Figure 1. Effects of the diets containing NNSE at different levels of 0 (A), 0.1 (B), 0.5 (C) and 1% (D) on anterior intestinal morphology of Catfish fed for 7 weeks. Scale bar = 500 μ m.

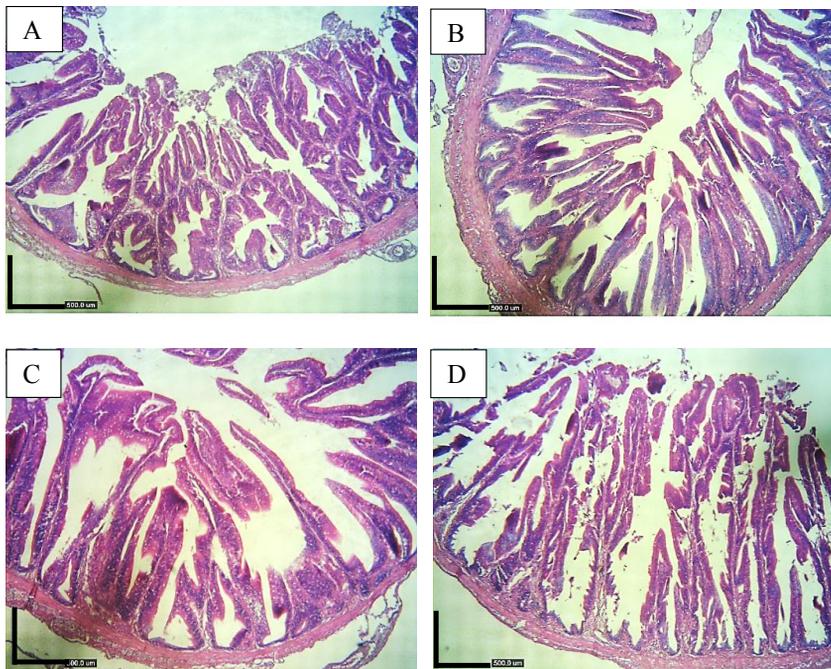


Figure 2. Effects of the diets containing NNSE at different levels of 0 (A), 0.1 (B), 0.5 (C) and 1% (D) on posterior intestinal morphology of Catfish fed for 7 weeks. Scale bar = 500 μ m.

3.3 Adverse effects

No adverse effects of NNSE were observed during the feeding trial. Feeding behavior, feed acceptability and palatability of the experimental groups were similar to the control group. Relative weights of vital organs including heart, stomach, liver, spleen, kidney and gills were no statistically significant difference between among the treatments ($P > \text{Table 1}$).

4. Discussion

Herbal plants are served as invaluable sources of phytochemical compounds which can be used to synthesis the synthetic drugs for the treatment of emerging infectious diseases (1-4). In aquaculture industry, plants are submitted to prevent the infectious diseases and to improve general aquatic animal health (1-4). In this study, NNSE significantly enhanced WG, SGR, the heights of intestinal villi and the number of goblet cells of Catfish, suggesting that NNSE could be useful as natural feed additive in fish rearing (9,11). The optimal level of NNSE observed was 0.1%.

4.1 Effect on growth performance

It has been reported that several plant additives can increase growth and health of fish. Babahydari et al. (17) found that fish fed the diets supplemented with different levels of *Stachys lavandulifolia* extract (0, 2, 4 and 8%) for 10 weeks significantly increased in final weight, WG, SGR and ADG in a dose-dependent manner. Talpur (4) revealed that the application of peppermint (*Mentha piperita*) leaf extract (0, 1, 2, 3, 4 and 5 g/kg) to the diet for 28 days significantly improved growth performance, SR and disease resistance of Asian seabass (*Lates calcarifer* Bloch) against the infection of *Vibrio harveyi*.

In addition, dietary supplementation of garlic bulbs (*Allium sativum*) (0, 5, 10, 15 and 20 g/kg) for 4 weeks significantly enhanced SGR, WG and FCR of Asian seabass (3). African catfish (*Clarias gariepinus*), Common carp (*Cyprinus carpio*) and Tilapia (*Oreochromis aureus*) received the diets containing red clover (*Trifolium pratense*) were significantly improved growth rate and feed utilization efficiency as well as protein efficiency ratio of fish (18-20). Such phytochemical compounds like flavonoids, alkaloids, saponins, phenolics and essential oils are found to be effective alternatives to synthetic drugs used in aquaculture industry (1-4). Zhai and Liu (21) exhibited that tilapia fed the diets supplemented with quercetin, a plant flavonoid, increased average values for final body weight, SGR and condition factor compared to the control diet. In addition, quercetin also significantly decreased the concentrations of triglyceride and low-density lipoprotein cholesterol in serum (21). Francis et al. (16) found that Common carp fed diets containing *Quillaja* saponins, triterpenoid saponins found in *Quillaja saponaria*, showed increased growth and feed efficiency of those fish compared to the control diet. It has been found that plant secondary metabolites should promote growth and feed utilization efficiency of fish through the improvement of feed intake, digestive enzyme activities and beneficial gastrointestinal bacteria (1-4).

Previous reports indicated *N. nucifera* contains alkaloids, essential oils, flavonoids and saponins (6-8). Pharmacological studies revealed that *N. nucifera* produced antioxidative, antimicrobial, antiviral and anti-inflammatory activities as well as immunomodulatory (6-8). Thus, the

growth-promoting property of NNSE could be attributed to, at least in part, phytochemical contents present in the extract including alkaloids, flavonoids, essential oils and saponins (9,11). Furthermore, other factors like antioxidative, antibacterial and immunomodulatory properties of *N. nucifera* could support the growth promoting effects of the plant extract by improving overall health of fish (11).

4.2 Effect on intestinal morphology

The results of this research indicated that the application of NNSE on Catfish diets had positive effects on intestinal villi and goblet cells of the intestines. In addition, the thicknesses of longitudinal muscularis and circular muscularis and the width of villi in anterior intestine of fish fed the diet containing NNSE were significantly higher than that of the control diet. It is well known that intestinal villi play a key role in digestion and absorption of essential nutrients functioned by specific digestive enzymes (12,14). In addition, goblet cells synthesize and secrete gel-forming mucins to coat and protect the lining of intestinal epithelium from both physical and biological damages (14). Thus, the increases of villus height and width as well as the density of the goblet cells of Catfish fed NNSE supplemented diets could lead to support the growth promoting potential of NNSE in this present study. Heidarieh et al. (15) found that dietary Ergosan significantly enhanced intestinal villi, goblet cells and lipase activity of rainbow trout (*Oncorhynchus mykiss*), resulting the improvement of growth performance and body composition of this fish species. Pirarat et al. (14) reported that Nile tilapia fed activated charcoal-supplemented diet significantly increased the foregut and midgut villus

height when compared to the control diet. They hypothesized that activated charcoal could improve the digestion and absorption of food ingredients and deplete some toxins and impurities from the gastrointestinal tract of fish (14). It is generally accepted that increased villus height is associated with increased surface area of the gut (12,14,15). Additionally, an increase in muscularis thickness of posterior intestine would also support defecation and moisture reabsorption (22). Therefore, substances affect intestinal morphology by increasing the villus height and width could be used as a growth promoting agent in fish cultivation (14).

4.3 Adverse effects

Feeding behavior and feeding acceptability of fish observed in the treatment groups were the same as the control group. Also, dietary NNSE had no effect on the relative weights of the vital organs and the survival rate of fish. However, it was observed that intestinosomatic and gastrosomatic indices of the groups treated with NNSE were generally higher than the control group but did not quite reach a statistically significant level. Thus, these results indicated that the application of NNSE as a feed additive to the fish diet in a short period is safe. Such reports indicated that lotus contains tannins; the phytochemical compounds that may decrease growth performance of fish (6-8). It was showed that common carp fed the diets incorporated with 2% quebracho tannin for 84 days did not produce any signs of toxicity but fish fed the diets mixed with 2% tannic acid reduced average metabolic rate and oxygen consumption after day 28 of the treatment period (23). In addition, it was found that Nile tilapia fed the diets containing 15 and 25 g/kg of hydrolysable

tannin significantly decreased WG, SGR and protein efficiency ratio compared to the control (24). Kunanusorn et al. (25) examined acute and subchronic oral toxicity of *N. nucifera* stem extract in both male and female rats and they found that the extract at the concentration of 5000 mg/kg B.W. did not cause clinical signs of toxicity and mortality during the experimental period of 14 days. For 90 consecutive days of subchronic toxicity test, gross pathological examinations of important internal organs of animals and hematological indices in the tested groups which received the extract at the doses of 50, 100 and 200 mg/kg/day were similar to the control group (25). This study supports the safe use of *N. nucifera* stem both in cosmetic products and alternative medicine (25). However, long term use of this plant in aquatic feeds should be evaluated to confirm its safety and efficacy (9,11).

5. Conclusion

To the best of our knowledge, this is the first investigation to indicate dietary NNSE could have positive effects on growth performance, feed utilization and intestinal morphology of Catfish. The optimal level of NNSE observed in this report was 0.1%. Thus, these findings support the use of NNSE as a natural feed additive in aquaculture industry.

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7. References

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