ผลของสารสกัดจากฝักบัวหลวง (*Nelumbo nucifera* Gaertn.) ต่อสมรรถนะการเจริญเติบโตของปลานิล (*Oreochromis niloticus*)

Effect of Lotus (*Nelumbo nucifera* Gaertn.) Fruit Extract on Growth Performances of Nile Tilapia (*Oreochromis niloticus*)

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บทคัดย่อ

บัวหลวง *(Nelumbo nucifera* Gaertn.) ถูกนำมาใช้ในการแพทย์พื้นบ้านเพื่อการรักษาโรคต่างๆ มาแต่อดีต อย่างไรก็ตามการใช้บัวหลวงเพื่อเป็นวัตถุธรรมชาติเสริมในอาหารปลายังมีน้อย ดังนั้น การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดฝักบัวหลวงเป็นวัตถุเสริมในอาหารต่อการเจริญเติบโตของปลานิล (*Oreochromis niloticus*) ลูกปลานิล (น้ำหนัก 9.43±0.89 กรัม) ถูกแบ่งออกเป็น 5 กลุ่ม โดยลูกปลาแต่ละกลุ่มได้รับอาหารผสมฝักบัวหลวงที่สกัดด้วยเอธิลแอลกอฮอล์ร้อยละ 70 ที่ความเข้มข้นแตกต่างกัน ดังนี้ กลุ่มที่ 1 ถึงกลุ่มที่ 4 ปลาได้รับอาหารผสมสารสกัดฝักบัวหลวงความเข้มข้นร้อยละ 0 0.05 0.1 และ 1 ตามลำดับ และกลุ่มที่ 5 ปลาได้รับอาหารผสมยาปฏิชีวนะออกซิเตตราซัยคลินความเข้มข้นร้อยละ 0.05 และจัดให้เป็นกลุ่มควบคุมเชิงบวก ปลาได้รับอาหารผสมสารสกัดฝักบัวหลวงหรือยาปฏิชีวนะ 2 ครั้งต่อวัน เป็นเวลา 60 วัน การปฏิบัติต่อสัตว์ทดลองได้กระทำตามหลักปฏิบัติที่เห็นชอบจากคณะกรรมการการใช้สัตว์ทดลอง มหาวิทยาลัยราชภัฏอุบลราชธานี ผลการศึกษาพบว่าการเสริมสารสกัดฝักบัวหลวงลงในอาหารมีผลเพิ่มน้ำหนักตัวและอัตราการเจริญเติบโตจำเพาะและมีผลลดค่าอัตราการเปลี่ยนอาหารอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม ($P<0.05$) อาหารเสริมสารสกัดฝักบัวหลวงไม่มีผลต่ออัตราการตายสะสม ค่าดัชนีตับ และค่าดัชนีลำไส้ต่อตัวเมื่อเปรียบเทียบกับกลุ่มควบคุมและกลุ่มควบคุมเชิงบวก ($P>0.05$) ระดับความเข้มข้นที่เหมาะสมของสารสกัดฝักบัวหลวงจากการศึกษาครั้งนี้ คือ 1% นอกจากนี้ในปลาทุกกลุ่มที่ได้รับสารสกัดไม่พบความผิดปกติของสุขภาพและพฤติกรรมการกินอาหาร ดังนั้น ผลการศึกษาครั้งนี้จึงยืนยันได้ว่าสารสกัดฝักบัวหลวงสามารถใช้เป็นวัตถุธรรมชาติเสริมในอาหารปลาเพื่อกระตุ้นการเจริญเติบโตและประสิทธิภาพการใช้อาหารในปลานิล

คำสำคัญ: บัวหลวง, *Nelumbo nucifera* Gaertn., การเจริญเติบโต, ปลานิล, *Oreochromis niloticus*, สมุนไพร

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Abstract

Lotus (*Nelumbo nucifera* Gaertn.) has been therapeutically utilized as a folk medicine to treat various ailments. However, its use in fish culture as a natural feed additive in fish diets is still limited. Therefore, the aim of this present study was to investigate the effects of *N. nucifera* fruit extract (NNFE) on growth performances of Nile tilapia (*Oreochromis niloticus*). Fingerlings (weighing 9.43±0.89 g) were divided into 5 groups. Group 1-4, fish were fed diets containing 0, 0.05, 0.1 and 1% of *N. nucifera* fruit extracted with 70% ethanol, respectively. Group 5 received 0.05% of oxytetracycline mixed diet and served as a positive control group. Fish were fed two times a day for 60 days. Fish were raised in accordance with the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. The results demonstrated that the diets supplemented with NNFE caused a significant increase in the growth performances such as body weight gain, specific growth rate, and a significant reduction of feed conversion ratio compared to the control diet ($P<0.05$). In addition, no significant changes in the cumulative mortality, behavior, hepatosomatic index and intestinalsomatic index were observed among all the groups ($P>0.05$). The optimum level of NNFE observed in this research was 1%. Thus, these results revealed that NNFE may be used as a natural feed additive in the fish diet to improve growth and feed utilization efficiency of Nile tilapia.

Keywords: Lotus, *Nelumbo nucifera* Gaertn., Growth parameters, Nile tilapia, *Oreochromis niloticus*, Medicinal plants

Introduction

Medicinal herbs and their products have long been used since ancient time for the treatment of various ailments in man (Mukherjee et al., 2009; Citarasu, 2010). However, their uses as growth promoters, immunostimulants and other purposes in aquaculture operations are scant (Citarasu, 2010). Several synthetic compounds such as hormones and antibiotics have led to the development of residual effects in aquaculture products and the creation of resistant strains of bacteria (Francis et al., 2002; Citarasu, 2010). Thus, natural plant products seem to be more attractive alternatives to enhance growth, productivity, and healthiness of fish (Munglue, 2014; 2015).

Many herbal plants with antistress property used in aquaculture have been reported, such as growth promotion, appetite activation, and antibacterial activity, while the success is still questionable (Francis et al., 2002; Citarasu, 2010; Mahdavi et al., 2013; Prutra et al., 2013). It was found that grouper juveniles (*Epinephelus coioides*) received dietary katuk (*Sauropus androgynous* L. Merr.) extract produced a significant increase in growth rate and feed intake and caused a significant decrease in feed conversion ratio when compared to the control fish (Prutra et al., 2013). In addition, diets supplemented

with *Aloe vera* extract at different levels had a positive effect on final length, final weight and growth parameters in common carp (*Cyprinus carpio*) (Mahdavi et al., 2013).

N. nucifera Gaertn. is a perennial aquatic herb belonging to the family of Nelumbonaceae and is widely cultivated throughout Asia and Australia. For being traditional medicine, it has been used to treat obesity, poor digestion, insomnia, palpitations, chronic diarrhoea, spermatorrhoea, tissue inflammation and cardiovascular diseases (Mukherjee et al., 2009; Wethangkaboworn and Munglue, 2014). Phytochemical studies indicated that *N. nucifera* contains alkaloids, steroids, triterpenoids, flavonoids, glycosides, saponins and polyphenols (Mukherjee et al., 2009). Betulinic acid, a steroidal pentacyclic triterpenoids, has been isolated from *N. nucifera* and has been reported to have several pharmacological properties including anti-tumor, anti-inflammatory and anti-cancer (Mukherjee et al., 2010). As the ethnopharmacological relevance described to *N. nucifera* is widely based on scientific data, more research is required to experimentally confirm its safety and efficacy. In addition, the use of this plant in fish culture as a natural feed additive in fish diets to enhance growth is still limited (Munglue, 2014; 2015). Thus, this present study aimed to investigate the effects of *N. nucifera* fruit extract (NNFE) on growth performances of Nile tilapia (*Oreochromis niloticus*).

Materials and Methods

Plant Preparation and Extraction

N. nucifera fruits were collected locally from Ubon Ratchathani, Thailand. The plant was identified by a herbal specialist at the Program of Biology, Ubon Ratchathani Rajabhat University, Thailand. The fruits were then cleaned using tab water, cut into small pieces and dried in a hot air oven at 60°C for 7 days. Dried fruits samples (50 g) were macerated with 70% ethanol (500 mL) for 7 days. The extract was filtered through Whatman paper No. 1 and dried by rotary evaporator. The yield was expressed as 8.33 mg/g based on dried fruit weight.

Fish and Feeding Preparations

The animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. Fingerlings of *O. niloticus* (weighing 9.43±0.89g) were divided into five treatments, with four replicates each group. Each aquarium (20 fish per aquarium) was aerated using aquarium air pumps. Fish wastes were removed by siphoning one half of aquarium's water and replaced by aerated tap water from storage tank. The fish diet containing 18% protein and 13% lipid was obtained from a commercial fish feed and mixed at different doses (0, 0.05, 0.1 and 1%) of the plant extract. Fish were fed *ad libitum* two times a day for 60 days. Every week, fish in each aquarium were weighed. Dead fish once appeared in aquaria were noted and removed. Water quality was maintained at pH of 6.5-7.5, a dissolved oxygen of 7 mg/L and a temperature of 25-30°C.

Growth Parameters

At the end of the experiment, the fish were fasted for 24 h before study. The mean body weight and growth parameters were calculated as follows (Francis et al., 2002; Mukherjee et al., 2010; Mahdavi et al., 2013; Zhai and Liu, 2013):

Weight gain (%) = 100 × (final fish weight (g) – initial fish weight (g))/ initial fish weight (g)
Specific growth rate = 100× [*In* final wet weight (g) – *In* initial wet weight(g)]/experimental days. (SGR, % d^{-1})
Feed conversion ratio (FCR) = feed intake (g)/ weight gain (g).
Survival rate (SR, %) = 100× (final number of fish/initial number of fish).
Hepatosomatic index (HSI, %) = 100× (weight of liver (g)/weight of fish (g))
Intestinalsomatic index (ISI, %) = 100× (weight of intestine (g)/weight of fish (g))

Adverse Effects of Plant Extract

Fish were observed daily for signs of toxicity. Also, feeding behavior, palatability and acceptability of feed were noted (Munglue, 2014 and Munglue, 2015).

Statistical Analysis

Data are expressed as mean±standard error of the mean (SEM). The significance of difference was analyzed using one-way analysis of variance (ANOVA). *P* value <0.05 was considered statistically significant (Munglue, 2014 and Munglue, 2015).

Results and Discussion

Medicinal plants serve as the great sources of the novel compounds not only for the treatment of various ailments but also for the synthesis of the conventional drugs (Francis et al., 2005; Citarasu, 2010; Dada, 2012; Serrano, 2013). In addition, herbal biomedicines have also been used in the aquaculture production as growth promoters, immunostimulants, antimicrobial agents, anti-stress agents and appetite stimulators (Citarasu, 2010; Munglue, 2014; 2015). However, the effect of NNFE on growth of fish has not yet been elucidated. Thus, the aims of this present study were therefore, to investigate the effect of the diet supplemented with NNFE on survival rate, growth performance and adverse effects of the plant extract in Nile tilapia. It has been accepted that oral administration is a non-stressful procedure to study the diet containing medicinal herbs or their products in aquaculture (Citarasu, 2010; Munglue, 2014; Munglue, 2015).

The survival rates of all Nile tilapia fed with the experimental diets were determined at the end of 60 days. The results showed that the survival rates of Nile tilapia fed diets containing the plant extracts were not significant when compared to the control group ($P>0.05$) (Table 1). Fish fed diet with NNFE produced a significant increase in both weight gain and SGR ($P<0.05$) and caused a significant decrease in FCR when compared to the control animals ($P<0.05$) (Table 1). No significant negative effects ($P>0.05$) on the HSI and the ISI were observed in the fish fed all experimental diets and diet containing oxytetracycline when compared to those fed the control diet .

This present study demonstrated that dietary NNFE supplementation produced a significant increase in weight gain and SGR and caused a significant decrease in FCR when compared to the control diet. Furthermore, the plant extract did not significantly alter the survival rate, HSI and ISI when compared to control and positive control animals. In this study, dietary of 1% NNFE is the optimal levels because it exhibited significantly the

highest values for weight gain and specific growth rate when compared to fish fed diets containing 0.05 and 0.1% of NNFE.

Some studies have been reported the herbal growth promoting agents (Francis et al., 2005; Citarasu, 2010; Dada, 2012; Munglue, 2014; Munglue, 2015; Serrano, 2013). The herbal active compounds such as phenolics, flavonoids, saponins, polyphenols, terpenoids and alkaloids, play an important role in antistress, growth stimulation, appetite activation and immunomodulation in finfish and shrimp larviculture (Francis et al., 2005; Citarasu, 2010; Dada, 2012; Munglue, 2014; Munglue, 2015). Interestingly, some phytonutrients were also found to be very effective alternatives to antibiotic, hormones and other synthetic agents (Citarasu, 2010; Zhai and Liu, 2013). It is generally accepted that the botanical plants are the great sources of safer and cheaper active constituents (Citarasu, 2010; Munglue, 2014). It was found that the growth parameters such as weight gain, the average feed conversion ratio, the average protein utilization and metabolic growth rate of common carp fed with a diet *Quillaja* saponins were significantly better than those of the control group (Francis et al., 2002). Recent work demonstrated that *Quillaja* saponin may increase the activity of specific enzymes in gastrointestinal tract such as trypsin, amylase, cytochrome *c*-oxidase and lactate dehydrogenase through specific mechanisms to improve digestion and absorption of food nutrients in fish (Francis et al., 2002). It is assumed that dietary saponins may also enhance the intestinal membrane permeability to absorb the digested dietary components (Francis et al., 2002). Zhai and Liu (2013) reported that quercetin, a bioflavonoid, mixed into diets significantly improved the growth performance and significantly decreased serum lipid levels in Nile tilapia. It was hypothesized that the growth promoting effect of quercetin on fish may be due to the activation of digestive enzymes activities, resulting in better growth, productivity and healthiness in animals (Zhai and Liu, 2013). Citarasu (2010) revealed that the mechanisms of action of herbal plants with growth promoting activity seem to be due to an increase of the transcription rate, leading to the production of RNA, amino acids and proteins in virtual animal cells.

Phytochemical studies indicated that *N. nucifera* fruit contain flavonoids, steroids, alkaloids, terpenoids and saponins (Mukherjee et al., 2009; 2010). Betulinic acid was isolated from the methanol extract of *N. nucifera* and was evaluated for its anti-tumor and anti-inflammatory (Mukherjee et al., 2009; 2010). Mukherjee et al. (2010) exhibited that the ethanolic rhizome and seed extracts of *N. nucifera* can improve overall immunological parameters tested by using *in vivo* models. Botanical remedies as immunostimulants can be used to enhance the resistance of fish against various diseases (Francis et al., 2005; Dada, 2012; Serrano, 2013). Thus, the growth promoting effect of NNFE on fish growth and feed utilization might be due to the presence of some secondary metabolites found in the plant extract including alkaloids, flavonoids and saponins (Munglue, 2014; 2015).

The results of this study also showed that the plant extract did not produce unwanted side-effects during the treatment period. General behavior, palatability or acceptability of feed observed in the experimental fish were similar to control and positive groups, suggesting that NNFE could be used for longer time without producing any sign of toxicity or treatment-related adverse effects (Munglue, 2014 and Munglue, 2015).

Table 1: Growth performance, survival rate and feed utilization of Nile tilapia fed the diets containing NNFE at the different levels for 60 days.

Parameters	Treatments				
	Control	0.05% NNFE	0.1% NNFE	1% NNFE	0.05% Oxy
Initial weight (g)	9.43±0.88	9.43±0.88	9.43±0.92	9.44±0.88	9.43±0.15
Final weight (g)	16.03±0.27^{a}	16.33±0.18^{a}	19.02±0.17^{b}	20.03±0.18^{b}	19.06±0.15^{b}
WG (%)	77.78±4.56^{a}	88.02±3.32^{b}	93.28±5.82^{b}	106.82±2.34^{c}	87.08±4.11^{b}
SGR (%)	0.95±0.01^{a}	1.16±0.04^{b}	1.20±0.02^{c}	1.33±0.02^{d}	1.17±0.01^{b}
FCR	1.64±0.01^{a}	1.59±0.02^{b}	1.54±0.03^{b}	1.48±0.03^{c}	1.56±0.02^{b}
HSI (%)	4.71±0.44	4.78±0.36	4.79±1.36	4.70±0.95	4.74±0.42
ISI (%)	6.05±0.45	6.29±0.37	6.33±0.50	6.38±0.21	6.26±0.37
SR (%)	97.09±3.02	95.04±4.80	96.45±5.39	95.09±4.27	93.57±6.34

Remark: NNFE = *N. nucifera* fruit extract, Oxy = oxytetracycline, Values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used. Means with different superscripts $^{(a-d)}$ at the same row are significantly different (P<0.05).

Conclusion

The results of this present study indicated that NNFE could be used as a growth promoting agent in fish feed to improve fish growth and feed utilization efficiency. The optimum level of NNFE observed in this research was 1%. Further research is needed to purify and characterize its active compound(s) that would help to obtain a natural growth promoter for sustainable fish productivity.

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References

Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International. 18(3), 403–414.

Dada, A.A. 2012. Effects of herbal growth promoter feed additive in fish meal on the performance of Nile tilapia (*Oreochromis niloticus* (L.). Egyptian Academic Journal of Biological Sciences. 4(1), 111-117.

Francis, G., H.P.S. Makkar and K. Becker. 2002. Dietary supplementation with a *Quillaja* saponin mixture improves growth performance and metabolic efficiency in common carp (*Cyprinus carpio* L.). Aquaculture. 203(3-4), 311-320.

Francis, G., H.P.S. Makkar and K. Becker. 2005. *Quillaja* saponins- a natural growth promoter for fish. Animal Feed Science and Technology. 121(1), 147-157.

Santoso, U., M.C. Lee and F.H. Nan. 2013. Effects of dietary katuk leaf extract on growth performance, feeding behavior and water quality of grouper

Epinephelus coioides. Aceh International Journal of Science and Technology. 2(1), 17-25

Mahdavi, M., A. Hajimoradloo, and R. Ghorbani. 2013. Effect of *Aloe vera* extract on growth parameters of common carp (*Cyprinus carpio*). World Journal of Medical Sciences, 9(1), 55-60.

Mukherjee, D., T. N. Khatua, P. Venkatesh, B. P. Saha and P. K. Mukherjee. 2010. Immunomodulatory potential of rhizome and seed extracts of *Nelumbo nucifera* Gaertn. Journal of Ethnopharmacology. 128(2), 490-494.

Mukherjee, P.K., D. Mukherjee, A.K. Maji, S. Rai, and M. Heinrich. 2009. The sacred lotus (*Nelumbo nucifera*)-phytochemical and therapeutic profile. Journal of Pharmacy and Pharmacology. 61(4), 407–422.

Munglue, P. 2014. Effects of dietary *Nelumbo nucifera* (Lotus) peduncle extract on growth performance of Nile tilapia (*Oreochromis niloticus*). *In* the Proceedings of the 1st Environment and Natural Resources International Conference. 6 – 7 November, 2014. The Sukosol hotel, Bangkok, Thailand. 279-282.

Munglue, P. 2015. Effect of *Nelumbo nucifera* Gaertn. leaf extract on growth perform-ance of Nile tilapia (*Oreochromis niloticus*). SDU Research Journal Sciences and Technology. 8 (1), in press.

Serrano, JR.A.E. 2013. Effects of *Quillaja* saponins on growth, feed efficiency, digestive enzyme activities and metabolism of common carp (*Cyprinus carpio* L.). Aquaculture Nutrition. 19(4), 465-474.

Wethangkaboworn, Y. and P. Munglue. 2014. Effect of ethanolic seed extract of *Nelumbo nucifera* on male rat sexual behavior. KKU Research Journal. 19 (6+), 156-161.

Zhai, S.-W., and S.-L. Liu. 2013. Effects of dietary quercetin on growth performance, serum lipids level and body composition of tilapia (*Oreochromis niloticus*). Italian Journal of Animal Science. 12(4), 523-527.