APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Technology Transfer Affairs Division,
Khon Kaen University, Thailand

Growth performance and intestinal morphology of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fed diet supplemented with rice paddy herb (*Limnophila aromatica*) extract

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Received 5 February 2018
Revised 23 January 2019
Accepted 1 February 2019

Abstract

Rice paddy herb (*Limnophila aromatica*) has long been used in Thai traditional medicine for the treatments of inflammation, digestion and fever. However, the use of *L. aromatica* in aquaculture diets as a natural growth stimulant has not yet been investigated. Therefore, the aims of this research were conducted to examine the effects of dietary supplementation of rice paddy herb extract (LAE) on growth performance and intestinal morphology of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*). Fish (6.55±0.20 g) were fed with the diets containing 0, 1, 3 and 5% (based on dry basis) of LAE for 8 weeks. The results demonstrated that dietary LAE produced a significant increase in weight gain, specific growth rate and feed utilization efficiency when compared to the control diet ($P<0.05$). In addition, intestinal villi width, intestinal villi length, goblet cell number and the thickness of intestinal smooth muscle observed in the intestines of fish fed the diets incorporated with LAE were significantly higher than those of the control ($P<0.05$). There were no changes in survival rate, feeding behavior, palatability, feed acceptability and external characteristics of dietary LAE supplementation compared to the control. The optimal level of LAE observed in this present study was 1%. Thus, it is concluded that LAE may be effectively used as a natural feed supplement in aquaculture diets for improving growth performance and feed utilization of cultured fish species.

Keywords: rice paddy herb, growth performance, intestinal morphology, hybrid catfish

1. Introduction

Aquaculture industries have been recognized as the important sources for protein, minerals and vitamins in many developing countries [1]. In addition, the consumer demand for fish products has been dramatically increasing for decades, leading to an increase in overfishing and intensive fish culture around the globe. In fish farms, several new approaches have been developed to promote fish growth and productivity [2&3]. Feed additives have long been used in aquaculture industries in order to enhance growth rates, feed utilization efficiency, health status as well as general well-being of cultured aquatic animals [2&3]. Due to people's awareness about the uses of synthetic compounds such as hormones, antibiotics and growth enhancers in animal feeds, natural products have been received much attention as rich sources of phytochemicals that individually or in combination [3&4]. Bioactive ingredients found in plants including alkaloids, terpenoids, flavonoids and essential oils have been reported to have antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory properties [2-4]. Interestingly, such plants have tested for their various pharmacological effects [2-4]. It was found that plant materials showed growth promoting, appetite-enhancing and tunic properties in fish cultivations when they were used in appropriate manners [2-4].

Various herbs and spices have been reported to have beneficial effects on growth of fish [2-4]. Talpur [5] demonstrated that dietary peppermint (*Mentha piperita*) at different doses significantly enhanced growth, feed efficiency and disease resistance of Asian seabass (*Lates calcarifer* (Bloch)) infected with *Vibrio harveyi* due to the improvement of phagocytic activity, respiratory burst and lysozyme observed in tested fish. In addition, Brum et al. [6] indicated that growth parameters of Nile tilapia (*Oreochromis niloticus*) fed the diets containing essential oils of clove basil and ginger were better than the control and increased immune responses were observed in treated fish which infected with *Streptococcus agalactiae*. Moreover, Nile tilapia fed dietary curcumin supplementation for 84 days significantly increased growth performance, nutrient utilization, immune status and resistance to *Aeromonas hydrophila* infection [7].

Rice paddy herb (*Limnophila aromatica*) is a tropical flowering plant that has long been used as medicinal plant and spicy flavoring agent [8-10]. Many experiments revealed that rice paddy herb possesses antioxidant, antibacterial, anti-proliferative, anti-cancer and anti-inflammatory properties [8-12]. Several active ingredients found in this plant are terpenoids, phenolics, flavonoids and essential oils [8-13]. Chowdhury et al. [10] and Sribusarakum et al. [11] reported that the main constituents found in *L. aromatica* analyzed by GC-MS were Z-ocimene, terpinolene, camphor, limonene, +-trans-isolimonene, α-pinene and α-caryophyllene, which might be useful for aquaculture production [2-4].

As there is an urgent need to find novel natural additives in order to improve fish growth and productivity, the aims of this research were conducted to examine the effects of rice paddy herb extract on growth and intestinal morphology of hybrid catfish (*C. macrocephalus* × *C. gariepinus*).

2. Materials and methods

2.1 Plant preparation and extraction

Arial parts of *L. aromatica* were collected from the local garden in Warinchumrap District, Ubon Ratchathani during rainy season. Plant materials were cleaned by using tab water, dried by using hot air oven at 50^{o}C for 24 h and blended to a find powder by using a home electric blender [14]. Powdered plants (500 g) were macerated with 70% ethanol (2000 mL) for 7 days at room temperature. The extract was filtered using Whatman paper No. 1 and dried by rotary evaporator which was maintained at 50^{o}C and 90 rpm. The yield of the extract was 9.46% based on dried plant weight. The extract was kept at 4^{o}C in a refrigerator until use as feeds or samples for proximate composition analysis.

2.2 Diet preparation

A commercial catfish diet containing not less than 30% protein and 4% lipid was used. The basal diet was mixed with different levels (0, 1, 3 and 5%) of LAE by distilled water (1 ml/g) to produce the homogenous mixtures. Each mixture was mixed by using a meat mincer to obtain extruded string forms, dried in hot air oven at 50^{o}C for 24 h and kept in the zip-lock bag [14]. Nutrient compositions of basal and experimental diets were analyzed by using the standard methods as previously reported by the Association of Official Analytical Chemists (AOAC International 2012) including moisture (%), ash (%), crude protein (%) and crude lipid (%) [15]. Proximate composition of the diets is presented in Table 1.

Table 1 Proximate composition of the experimental diets.

Proximate composition (%)	LAE levels in the experimental diets (%)			
	0	1	3	5
Moisture	3.18	3.92	3.38	3.91
Ash	12.52	12.71	12.91	12.95
Crude protein	33.35	33.34	32.44	32.41
Crude lipid	7.55	6.39	7.20	7.25

LAE, *L. aromatica* extract.

2.3 Fish and rearing conditions

Catfish with an average of 6.55±0.20 g were obtained from Ubon Ratchathani Fishery Cooperatives, Ubon Ratchathani, Thailand. Fish were acclimatized to the experimental conditions for 2 weeks. Healthy fish were randomly divided into 4 treatments with 3 replications. Each replication was reared in 255 L circular concrete tanks (30 fish/tank). Treatment 1 was fed with the basal diet and served as a control group. Treatments 2-4 were fed with the basal diets supplemented with 1, 3 and 5% LAE (based on dry basis), respectively, daily for 8 weeks. Water quality parameters were maintained in the standard conditions for catfish rearing (temperature, 29.00±2.00ºC, pH, 7.20±0.50 and dissolved oxygen 7.00±0.05 mg/L) [14]. All tanks were cleaned once every 5 days. The fish were hand-fed *ad libitum* two times a day at the rate of 5% body weight and weighted every week. Dead fish were noted and removed. All procedures used in this present research were conducted according to the guideline for the care and use of laboratory animals (National Research Council of Thailand).

2.4 Effect on growth

At the end of the trail, fish were fasted for 24 h before study. Growth parameters were evaluated using the following formulae.

Weight gain (WG, g) = $W_f - W_i$ (1)

Specific growth rate (SGR, %/day) = $100 \times [\mathit{In}\ W_f - \mathit{In}\ W_i]/T$ (2)

Average daily growth (ADG, g/day) = $(W_f - W_i)/T$ (3)

Feed conversion ratio (FCR) = $FI/(W_f - W_i)$ (4)

Condition factor (K-factor) = $100 \times (FW/FL^3)$ (5)

Survival rate (SR, %) = $100 \times (N_f/N_i)$ (6)

Where W_i is the initial weight of fish (g), W_f is the final weight of fish (g), T is the feeding days, FI is the feed intake, FW is the final weight of fish (g), FL is the final length of fish (cm), N_i is initial number of fish and N_f is the final number of fish.

In addition, three fish from each replication were collected to study the effects of dietary LAE on the relative organ weights of liver (hepatosomatic index, HSI) and intestine (intestinosomatic index, ISI) by using the following equation.

Relative organ weight (%) = 100× (weight of organ (g)/weight of fish (g)).

2.5 Effect on intestinal morphology

At the end of the experimental trail, fish were fasted for 24 h and then weighed. Three fish collected from each tank were anesthetized by using clove oil (5 ppm). The abdominal cavity was opened and the gastrointestinal tract removed. Fish intestines were cleared from other organs and weighted. Proximal, middle and distal portions of the intestines were dissected and fixed in 10% neutral buffered formalin for 24 h and processed for haematoxylin and eosin (H&E) [14].

In order to study the effects of dietary LAE on the macromorphological parameters, villi height, villi width and muscular thickness were examined as previous reports [14,16&17]. The micromorphological parameters including enterocyte height, supranucleus height, subnucleus height, enterocyte nucleus height and enterocyte nucleus width were also evaluated as described elsewhere [16 & 17] using DinoCapture 2.0 software [14].

2.6 Statistical analysis

The experimental data were analyzed using one-way ANOVA and expressed as mean±standard error of the mean (SEM). The significant differences among the treatments were assessed by Duncan's multiple-range test and set at $P<0.05$.

3. Results

3.1 Effect of growth

Table 2 demonstrates the growth parameters of catfish fed different diets. It was found that fish fed the diets incorporated with LAE significantly increased in final weight, WG, SGR and ADG compared to the basal diet ($P<0.05$). K-factor values tended to increase in fish fed LAE supplementation ($P>0.05$). In addition, there was no difference observed in the SR between or within the experimental and control groups ($P>0.05$), ranging from 97 to 100%. HSI and ISI of fish fed the diets containing various levels of LAE were significantly higher than that of the control diet ($P<0.05$). Throughout the experimental trail, it was observed that catfish fed the experimental diets did not generate any side-effects on external appearance, feed consumption behavior and general health compared to the control group.

Table 2 Effects of dietary LAE on growth and the relative organ weights of catfish.

Variables	LAE levels in the experimental diets (%)			
	0	1	3	5
IW (g)	6.22±0.14	6.55±0.24	6.77±0.22	6.66±0.23
FW (g)	32.22±1.19[a]	48.00±2.94[b]	44.11±4.12[b]	45.77±2.43[b]
IL (cm)	8.55±0.24	8.88±0.26	9.11±0.26	9.22±0.32
FL (cm)	17.11±0.35	18.11±0.45	17.22±0.57	17.72±0.46
WG (g)	26.77±1.15[a]	42.77±2.81[b]	38.77±3.96[b]	39.11±2.34[b]
SGR (%/day)	3.08±0.06[a]	3.55±0.10[b]	3.34±0.15[b]	3.44±0.07[b]
ADG (g/day)	0.51±0.02[a]	0.74±0.05[b]	0.66±0.07[b]	0.68±0.04[b]
FCR	2.73±0.08[a]	2.12±0.04[b]	2.13±0.08[b]	2.19±0.08[b]
K-factor	0.70±0.03	0.84±0.09	0.88±0.10	0.87±0.11
SR (%)	97.77±2.22	97.77±2.22	97.77±2.22	100.00±0.00
HSI (%)	1.59±0.19[a]	1.79±0.13[b]	1.22±0.18[a]	2.05±0.22[b]
ISI (%)	1.07±0.17[a]	2.47±0.16[c]	1.75±0.14[b]	1.95±0.19[b]

Remarks: Data are expressed as mean±SEM (*n* = 9); means followed by different letters [(a-c)] in each row are significantly different (P<0.05).
LAE, *L. aromatica* extract; IW, initial weight (g); FW, final weight (g); IL, initial length (cm); FL, final length (cm); WG, weight gain (g); SGR, specific growth rate (%/day); ADG, average daily gain (g/day); FCR, feed conversion ratio; K-factor, condition factor; SR, survival rate (%); HSI, hepatosomatic index (%); and ISI, intestinosomatic index (%).

3.2 Effect on intestinal morphology

3.2.1 Effect on macromorphology

Histological studies of catfish intestines are shown in Figure 1 to 3 and the data are demonstrated in Table 3. It was found that villi heights and villi widths in all parts of the intestines of fish fed diets supplemented with 3 and 5% LAE were significantly higher than fish fed the control diet (P<0.05). Total muscular thickness and the thicknesses of inner circulatory muscle and outer longitudinal muscle in the proximal parts were significantly increased in fish fed 3% LAE diet compared to the control (P<0.05). Intestinal villi of fish fed dietary LAE did not show any marked degradation, damage or tissue disruption.

3.2.2 Effect on micromorphology

Micromorphology of the intestines obtained from catfish fed the experimental diets is presented in Figure 4 and the data are summarized in Table 4. The results revealed that enterocyte heights and supranucleus heights of the proximal and middle parts of the intestines of catfish fed the diets containing LAE at different levels were significantly enhanced compared to the basal diet (P<0.05), whereas microvilli heights of fish fed the experimental diets were significantly increased in all parts of the intestines (P<0.05) (Figure 4A). Goblet cell number was significantly increased by the diets supplemented with 3 and 5% LAE in both middle and distal parts of the intestines (P<0.05) (Figure 4B). Moreover, subnucleus heights, enterocyte nucleus heights and widths of fish fed the diets incorporated with 5% LAE in the distal tract were significantly increased when compared to the basal diet (P<0.05). Subnucleus height, enterocyte nucleus width and goblet cell number in the proximal part of the intestines were not affected by LAE diets (P>0.05). Various levels of cytoplasmic supranuclear vacuolation in the epithelial cells were observed. Enterocyte arrangement and villi structure of treated fish did not exhibit marked cell damage or rupture.

4. Discussion

Several feed additive types have generally been used in aquaculture industries in order to improve growth performance and general health status of cultivated aquatic animals. Medicinal plants containing many invaluable phytochemical compounds have been demonstrated to have growth enhancer and appetite promoter properties in different species of fish. An achievement of these aims may be due to dosages, growth stage and species of the animals. Thus, the purposes of this research were conducted to investigate the effects of dietary LAE on growth and intestinal morphology of catfish.

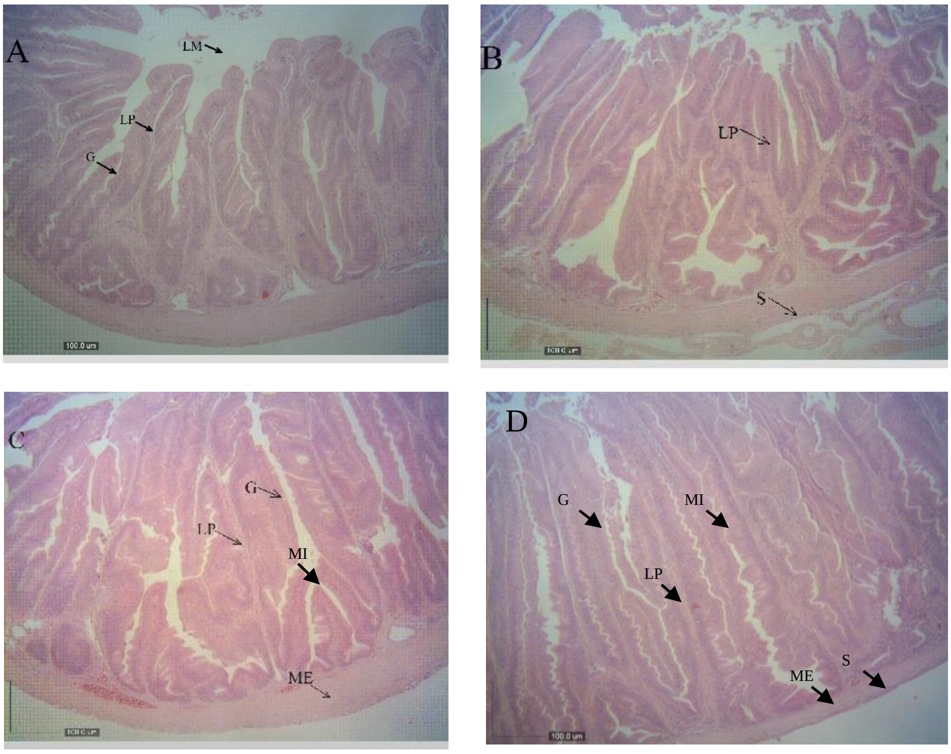

Figure 1. The effects of diets supplemented with LAE different concentrations of 0 (A), 1 (B), 3 (C) and 5% (D) on the proximal part of the intestines. Scale bar = 100 µm. S = serosa, ME = muscularis externa, LP = lamina propria, Lm = lumen, G = goblet cell, MI = microvilli.

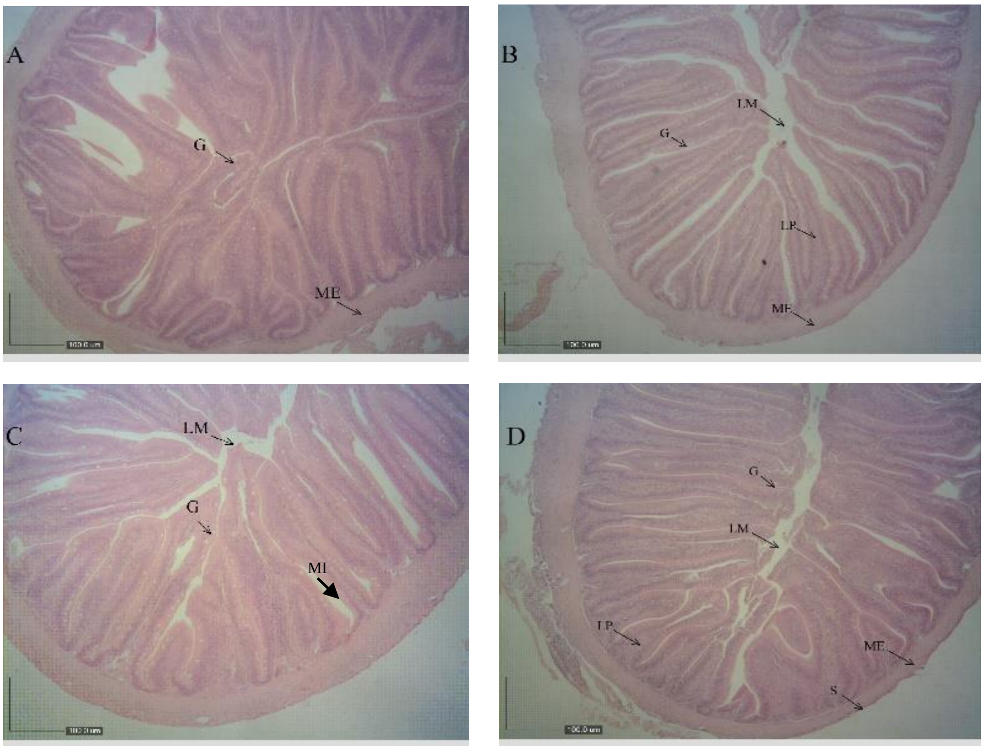

Figure 2. The effects of diets supplemented with LAE different concentrations of 0 (A), 1 (B), 3 (C) and 5% (D) on the middle part of the intestines. Scale bar = 100 µm. S = serosa, ME = muscularis externa, LP = lamina propria, LM = lumen, G = goblet cell, MI = microvilli.

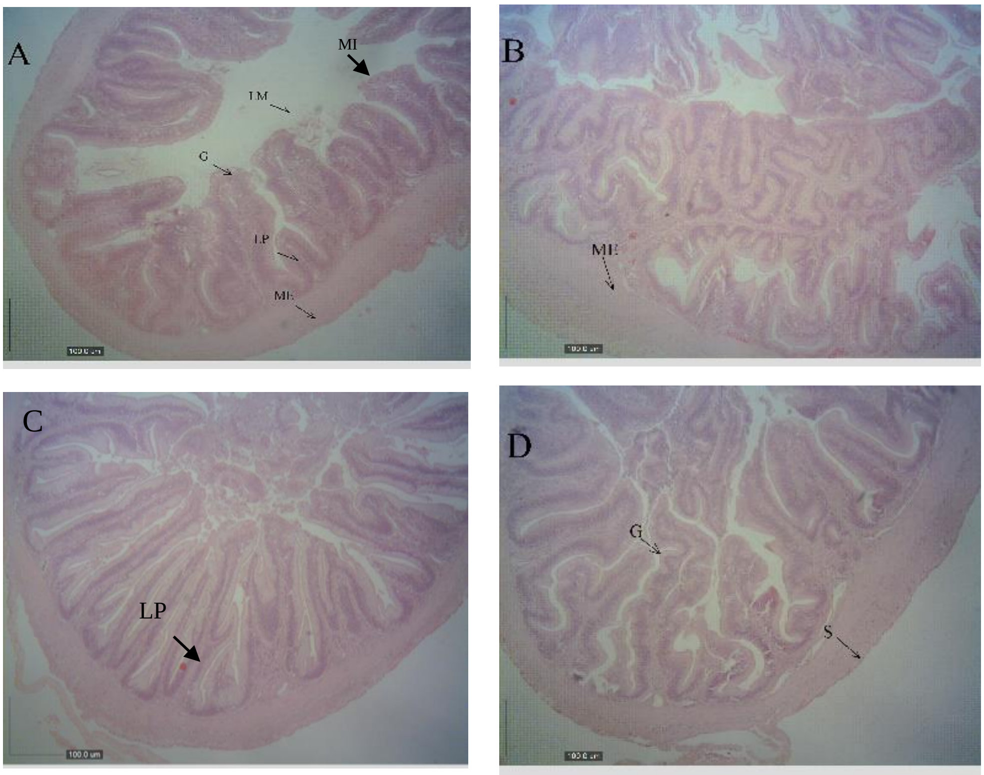

Figure 3. The effects of diets supplemented with LAE different concentrations of 0 (A), 1 (B), 3 (C) and 5% (D) on the distal part of the intestines. Scale bar = 100 µm. S = serosa, ME = muscularis externa, LP = lamina propria, LM = lumen, G = goblet cell, MI = microvilli.

4.1 Effect on growth

At the end of experimental evaluation, fish fed the LAE- supplemented diets had significantly higher growth parameters and feed utilization than the control diet. It has been reported that dietary supplementation with many plant extracts can improve digestion, feed efficiency, immunological responses and disease resistance of fishes [3-7].

It has been reported that fish fed the diets mixed with medicinal plants significantly enhanced growth and feed utilization efficiency [3-5]. It was postulated that herbal plants may improve feed palatability, feed acceptability and the functions of digestive enzymes in the gastrointestinal tract [3-5]. Jiang et al. [18] showed that dietary curcumin enhanced digestive enzyme activities including lipases, alkaline phosphatase, trypsin, gamma-glutamyl transpeptidase and creatine kinase in crucian carp (*Carassius auratus*) and this could be due to an increase of the gene transcription of digestive enzymes as well as brush border enzymes. El-Dakar et al. [19] stated that dietary supplementation of basil leaves and seeds significantly increased growth and feed utilization indices of Gilthead sea bream (*Sparus aurata*) due to the increase of the levels of amylase and lipase. Thus, increased growth and feed utilization of fish observed in this present research might be attributed to the enhancement of digestive enzyme activities by LAE. Interestingly, it was observed that growth performance of fish fed the diets mixed with 3 and 5% LAE tended to decrease the values of growth parameters when compared to fish fed the diet supplemented 1% LAE, although these decreases were not statistically significant. Regarding these findings, it might be due to toxicity and supplementation period of LAE [4]. However, there are no previous reports to indicate deleterious effects of LAE on the growth and health condition of fish. Therefore, it is suggested that the optimal amount of LAE observed in this present study was 1%.

Table 3 Effects of dietary LAE on macromorphology of catfish intestines.

Variables	LAE levels in the experimental diets (%)			
	0	1	3	5
Proximal part of the intestines				
Villi height (μm)	321.11±3.12[a]	338.11±5.17[b]	374±5.90[c]	366.33±5.89[c]
Villi width (μm)	112.79±7.71[a]	118.06±8.39[a]	127.98±8.15[b]	131.86±7.60[b]
Total muscular thickness (μm)	129.23±9.86	123.94±6.59	128.16±8.43	126.83±7.31
Inner circulatory smooth muscle thickness (μm)	65.51±4.82[a]	57.56±6.65[a]	98.69±11.87[b]	66.06±9.96[a]
Outer longitudinal smooth muscle thickness (μm)	56.70±4.21[a]	66.46±11.31[a]	74.46±5.94[b]	64.09±7.63[a]
Middle part of the intestines				
Villi height (μm)	408.22±2.06[a]	420.11±3.64[b]	438.77±9.48[c]	432.55±5.84[bc]
Villi width (μm)	94.83±2.60[a]	112.92±4.73[b]	131.34±8.17[bc]	147.70±7.87[c]
Total muscular thickness (μm)	94.87±8.83[a]	122.63±7.86[ab]	100.71±11.25[ab]	130.67±13.69[b]
Inner circulatory smooth muscle thickness (μm)	51.08±1.90	54.09±3.61	58.06±6.52	63.51±5.95
Outer longitudinal smooth muscle thickness (μm)	55.92±3.49	62.79±4.86	72.22±4.29	64.18±6.82
Distal part of the intestines				
Villi height (μm)	627.55±6.06[a]	653.44±8.65[b]	663.55±5.24[bc]	672.77±5.61[c]
Villi width (μm)	99.76±2.97[a]	111.25±3.70[b]	127.79±8.68[b]	147.96±7.52[c]
Total muscular thickness (μm)	119.66±8.26	119.06±7.89	133.10±8.99	118.58±7.43
Inner circulatory smooth muscle thickness (μm)	68.42±8.89	66.91±5.97	70.69±8.37	63.22±4.39
Outer longitudinal smooth muscle thickness (μm)	51.26±6.57	52.10±4.33	62.14±7.58	55.36±4.71

Remarks: Data are expressed as mean±SEM (*n*=3); means followed by different letters (a-c) in each row are significantly different (P<0.05). LAE, *L. aromatica* extract.

Many reports revealed that medicinal plants contain several active compounds such as alkaloids, phenolics, flavonoids, terpenoids and essential oils and all of these compounds found to have growth promoting properties in various species of fish [3 & 4]. Turan and Akyurt [20] studied the effects of red cover, a plant containing high levels of isoflavones, on growth of African catfish (*C. gariepinus*) and they found that fish fed dietary red cover extract for 120 days significantly increased WG and SGR and significantly decreased FCR compared to the control. Tilapia (*O. mossambicus*) fed the diets supplemented with 0.1% essential oil obtained from sweet orange peel (*Citrus sinensis*) for 90 days had significantly higher WG and SGR as well as significantly lower FCR when compared to the control group [21]. In addition, Nile tilapia fed the diets incorporated with 0.5% essential oil extracted from clove basil for 55 days significantly increased WG and SGR compared with fish fed a diet without essential oil [6]. Therefore, growth promotion of LAE observed in this research may be attributed to the present of phytochemical substances like flavonoids and essential oils [3&4,20&21].

This study showed that the relative weights of liver and intestines were significantly higher than those of the control. It is hypothesized that dietary LAE might enhance cell turnover rate [22]. Additionally, an increase in the digesta viscosity by the diet supplemented with LAE would subsequently induce the hypertrophy of the intestinal tract [22].

Biological activities such as antioxidant, anti-inflammatory and antimicrobial properties of *L. aromatica* have been reported and all these properties could lead to support stress tolerance and general health of fish [3 & 4]. During the experimental period, treated fish did not generate any side-effects on external appearance, feed consumption behavior and general health compared to the control group. These findings indicated that LAE could be useful as a natural feed additive for improving growth rates and utilization of dietary nutrient without negative effects.

Table 4 Effects of dietary LAE on micromorphology of catfish intestines.

Variables	LAE levels in the experimental diets (%)			
	0	1	3	5
Proximal part of the intestines				
Enterocyte height (µm)	37.38±2.45[a]	46.53±1.98[b]	54.69±2.20[c]	54.02±2.57[bc]
Supranucleus height (µm)	16.74±1.71[a]	21.31±3.96[b]	21.98±1.10[b]	20.80±0.67[b]
Subnucleus height (µm)	12.43±1.21	13.05±1.01	11.59±2.39	14.20±0.86
Enterocyte nucleus height (µm)	5.33±0.33[a]	6.24±0.58[ab]	6.30±0.49[ab]	7.60±0.68[b]
Enterocyte nucleus width (µm)	4.20±0.36	4.84±0.34	4.80±0.47	3.61±0.42
Middle part of the intestines				
Enterocyte height (µm)	38.80±1.81[a]	44.94±3.13[ab]	41.14±2.68[ab]	48.14±2.33[b]
Supranucleus height (µm)	15.40±0.74[a]	20.28±2.19[b]	19.98±0.77[b]	21.51±1.26[b]
Subnucleus height (µm)	11.84±0.80[a]	16.55±1.02[b]	13.68±0.62[ab]	16.49±1.33[b]
Enterocyte nucleus height (µm)	4.92±0.28[a]	6.78±0.58[b]	6.23±1.13[ab]	4.97±1.04[b]
Enterocyte nucleus width (µm)	3.54±0.15	4.78±0.53	4.24±0.64	4.46±0.25
Distal part of the intestines				
Enterocyte height (µm)	42.45±2.99[a]	47.95±1.82[ab]	54.13±2.56[b]	45.38±1.89[a]
Supranucleus height (µm)	16.16±1.72[a]	21.68±1.96[b]	18.34±1.06[ab]	18.34±0.79[ab]
Subnucleus height (µm)	11.85±1.02[ab]	14.16±1.07[b]	10.32±1.19[a]	14.67±1.19[b]
Enterocyte nucleus height (µm)	5.32±0.54[a]	5.39±0.75[a]	4.00±0.25[a]	8.47±1.22[b]
Enterocyte nucleus width (µm)	3.62±0.27[ab]	4.39±0.39[b]	2.89±0.16[a]	4.12±0.43[b]

Remarks: Data are expressed as mean±SEM (*n*=3); means followed by different letters (a-c) in each row are significantly different (P<0.05). LAE, *L. aromatica* extract.

4.2 Effect on intestinal morphology

The functional activities of fish intestine are digestion, absorption and osmoregulation [23]. In addition, the epithelium lining the intestine are known to be important for preventing several pathogen invasion and harmful materials presented in the environments by non-specific and specific mechanisms [24]. Thus, study on intestinal histology would be helped to fully understand the underlying mechanisms of cellular responses to different ingredients that are used to formulate the fish diets [23-25].

4.2.1 Effect on macromorphology

Macromorphological evaluations of the intestines of fish fed the dietary LAE showed significant increases in villi height, villi width, muscular layers, fold height and fold depth when compared to the control.

The thicknesses of intestinal wall and muscular layers are associated with the movement of the tract to support general functions of the intestines including the digestion of micronutrients, reabsorption of moisture and the defecation [23]. Increased intestinal muscular thickness is generally due to cell proliferation and cell division [23]. However, few scientific evidences are available for the underlying mechanisms of effects of herbal plants and phytochemical compounds on the thickness of the gastrointestinal tract. Munglue [14] found that catfish (*C. gariepinus*) fed dietary supplementation of lotus (*Nelumbo nucifera* Gaertn.) for 8 weeks showed a significant increase in the muscular thicknesses of the anterior and posterior parts of the intestines. In addition, Kamatit et al. [26] indicated that common lowland frog (*Rana rugulosa*) fed dietary waterlily (*Nymphaea pubescens*) stamen extract for 11 weeks had significantly increased the thicknesses of longitudinal muscularis and circular muscularis of the posterior intestine when compared to the control diet. Previous reports speculated that medicinal plants and their phytochemicals such as phenolic compounds could modulate cell proliferation, cell division and cell differentiation as well as decrease cell apoptosis in intestinal smooth muscles, resulting in an increase in the thickness of muscular layer [3 & 4]. Therefore, these reasons could lead to support the use of LAE as natural feed additive in order to improve digestive ability and intestinal integrity in fish.

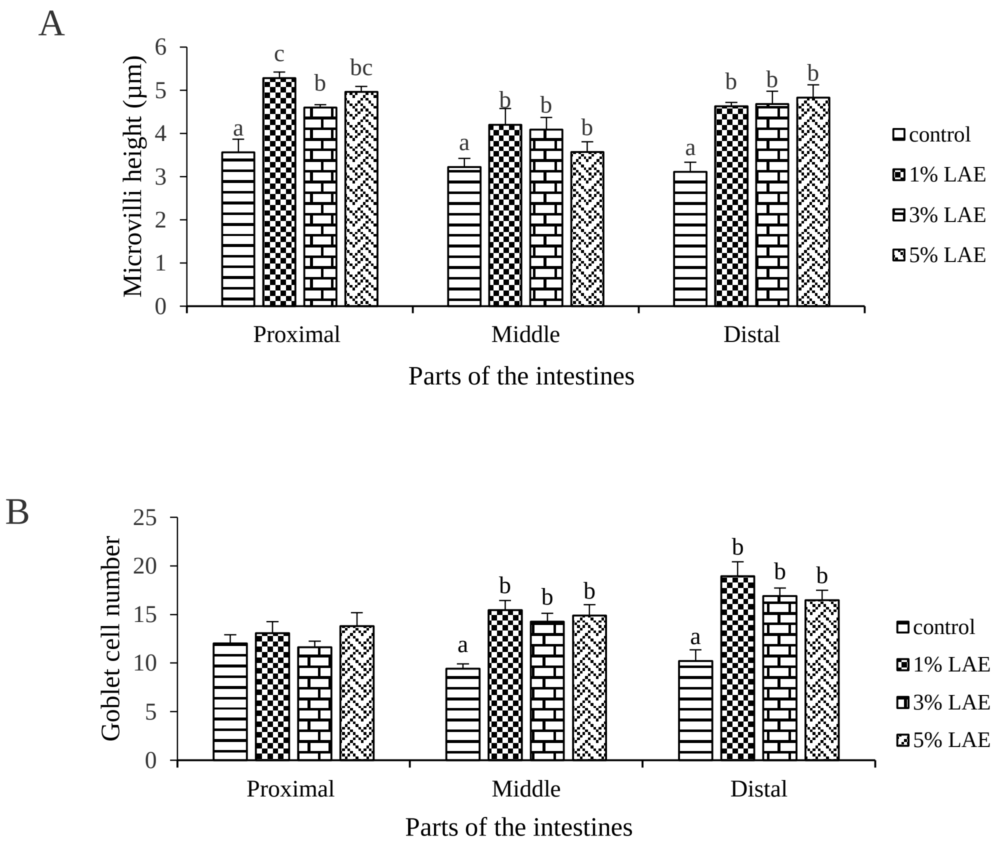

Figure 4 Effects of dietary LAE on microvilli height (A) and goblet cell number (B) in the different parts of the intestines. Data are expressed as mean±SEM (*n*=3); different letters ($^{a\text{-}b}$) are significantly different at P<0.05.

4.2.2 Effect on micromorphology

Also, observations on micromorphology of the intestines exhibited the improvement of enterocyte height, supranucleus height, subnucleus height, enterocyte nucleus height and width, microvilli height and goblet cell number in fish fed the experimental diets compared to the control diet. General intestinal epithelial cell structure and microvilli arrangement of treated fish did not show any changes or notice any signs of enteritis in all parts of fish intestines. Various levels of cytoplasmic supranucleus vacuolation were present in fish fed the experimental diets.

It has been reported that intestinal enterocyte height is related to the metabolic status of the fish intestine [27-29]. In this present investigation, the nucleus of the enterocyte is nearly located on the apical domain of the cell. Increased enterocyte height indicated increased absorptive area to support nutrient digestion and absorption in the intestines [28]. Decreased enterocyte height would display starvation or malnutrition of fish [28]. It has been demonstrated that a short-term starvation in fish may induce a proteolysis of the endothelial cells, resulting in the reduction of intracellular digestion and absorption of nutrients [28]. It was documented that the enterocyte height and supranuclear zone observed in pikeperch (*Sander lucioperca*) fed the diet incorporated with 180 g lipids per kg diet were greater than fish fed the diets containing 60 and 100 g lipids per kg diet [30]. The modes of action of the effects of medicinal plants and their active compounds on the height of enterocyte cells are unclear. Previous report indicated that the changes of intestinal epithelium are associated with different dietary ingredients, environmental factors and various types of pathogens [31]. However, it is hypothesized that phytochemicals could improve cell proliferation rate and movement rate of the cells to the top of intestinal villi, resulting in the enhancement of absorptive capacity in the fish intestine [24&25,27,30]. In this present study, it was observed that fish fed the LAE diets had increased enterocyte height when compared to the control. These results indicate the beneficial effects of LAE on growth of fish via the enhancement of enterocyte height.

Goblet cells are located within the intestinal epithelial cells which are responsible for the production of glycoproteins called mucins to form a boundary mucus layer covering the epithelial surface [32]. This mucus protects the intestines from chemical and mechanical damages during the digestion and the invasion of several pathogenic microorganisms [32]. The mechanisms of increasing intestinal goblet cells produced by herbal plants are not well understood. It was postulated that increasing goblet cell number in ascetic broilers fed the diets supplemented with 5 g/kg of each turmeric, thyme and cinnamon were displayed by Kamali Sangani et al. [33] and this may possibly be associated with the activation of *mucin* 2 gene expression in the intestines by herbal plants [33]. Wlodarska et al. [34] exhibited that rats received eugenol as a phytonutrient diet supplementation for 7 days significantly increased goblet cell-specific gene expression including *TFF-3* and *Muc2*, which are responsible for an increase in the production of mucus. Therefore, the modes of action of LAE on the goblet cell number might be attributed to the activation of some phytochemicals through the expression of genes directly or indirectly as mentioned above.

Microvilli are located at the apical domain of enterocytes that provide surface area available for nutrient digestion and absorption. It is assumed that increased microvilli length is related to: 1) the increase of absorptive surface; 2) the contribution of mucous content in order to provide a physical barrier in the nature and; 3) the enhancement of digestive enzyme activities [35-37]. Zahran et al. [35] showed that the application of *Astragalus* polysaccharides to the diets tended to improve the length of microvilli in Nile tilapia. Dimitroglou et al. [36] reported that gilthead sea bream (*Sparus aurata*) fed dietary mannan oligosaccharide (MOS) showed a significant increase in microvilli height when compared with the control. The results of this study showed a significant increase in microvilli height in catfish fed the diets supplemented with LAE. Thus, it is indicated that growth promoting effect of LAE could be due to the improvement of microvilli height in order to increase absorptive capacity of intestinal mucosa.

5. Conclusion

To the best of our knowledge, this present study is the first report to indicate the use of LAE as a natural feed additive in the diet for the improvement of growth rate, feed efficiency and intestinal morphology of catfish. The present study also displayed that the optimum level of LAE which could be used in the diet was 1%.

6. Acknowledgments

The authors would like to acknowledge Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University for providing the financial support of this present study. The authors are grateful to Miss Kannika Chotinet and Miss Laongdow Insee for their help and support during the trial. The authors would like to thank Asst. Prof. Dr. Makabodee Ruaysap for the manuscript editing.

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