Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Effects of Dietary Lasia (*Lasia spinosa* (L.) Thwaites) Extract on Growth Performance and Physiological Indices of Common Lowland Frog (*Rana rugulosa*)

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Article info

Article history:
Received : 10 February 2021
Revised : 25 April 2021
Accepted : 29 April 2021

Keywords:
Raniculture, Natural feed, Additives, Growth promoters, Intestinal histology

Abstract

A wide range of biological and pharmacological activities of lasia (*Lasia spinosa* (L.) Thwaites) are well established. However, little attention has been paid to the growth-promoting effects of lasia on aquatic animals. This study was aimed at elucidating the effects of dietary lasia extract (LE) on growth performance and physiological indices of common lowland frog (*Rana rugulosa*). Lasia leaf extract was prepared and phytochemical screening showed the presence of flavonoids, terpenoids, phenolic compounds, tannins, steroids, glycosides, and anthraquinones. Four treatments of male frog (initial weights of 14.00±1.00 g) were fed for 8 weeks with diets supplemented with 0 (control), 1, 3, and 5 g LE/kg diets. The results revealed that growth performance parameters were significantly improved in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). The survival rate, hepatosomatic index, intestinosomatic index, splenosomatic index, cardiosomatic index, and renosomatic index did not change among the treatments ($P > 0.05$). An increase in intraperitoneal fat weight was noticed in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). Dietary LE significantly improved villi height, villi width, and the absorptive surface area of the frog intestine compared to control ($P < 0.05$). Hemoglobin was markedly increased in frog fed with 5 g LE/kg diet compared with the other treatments ($P < 0.05$). There were no significant differences in hematocrit, white blood cells, and red blood cells among all treatments ($P > 0.05$). Dietary LE did not affect the levels of serum total protein, albumin, alkaline phosphatase, aspartate transaminase, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol ($P > 0.05$). Decreased cholesterol levels were detected in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). Glucose value was significantly enhanced in frog fed with 5 g LE/kg diet compared with the other treatments ($P < 0.05$). Taken together, these findings support the beneficial effects of dietary LE on the growth and physiological indices of the frog. The suitable level of LE observed by using the second-order polynomial regression analysis was 3.60 g LE/kg diet.

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Introduction

The role and importance of aquaculture and fisheries are to enhance world food and nutrition security for all (Awad & Awaad, 2017). Additionally, products from aquaculture provide income and livelihoods for many locations around the globe (Citarasu, 2010; Rico et al., 2013). An increase in consumption and demand for food sources has led to improved cultivation of several species of aquatic animals (FAO, 2020). Thailand is well-known as one of the producers and exporters of aquaculture products in the world. The main internationally traded products from Thailand are shrimp, tilapia, catfish, and carp. Interestingly, common lowland frog (*Rana rugulosa*) is also one export aquatic species with a high tendency in the global market because of the presence of the high nutritional value of its meat (Somsueb & Boonyaratpalin, 2001). Department of Fishery of Thailand reported that frog production rose from 1537 tonnes in 2007 to 3898 tonnes in 2017 (Department of Fishery, 2020). Muanmueangsong et al. (2014) reported the data from Taksin (2005) that he said the markets of frog products of Thailand are Hong Kong, China, Singapore, the European Union, and USA. Due to a decrease in frog population in nature, intensive and semi-intensive farming systems have been developed to enhance production output per unit (Pariyanonth & Daorerk, 1994). However, these production procedures are now faced with many problems including farmer expertise and lack of knowledge regarding the frog culture, low water quality, several diseases and pathogens, malnutrition, low growth rate, and cannibalism (Kamatit et al., 2016; Saman & Thiammueang, 2021). Therefore, good management and husbandry practices are required.

Nowadays, the application of drugs and chemicals in aquaculture operations has dramatically increased worldwide and plays a key role in the world food supply chain (Citarasu, 2010; Okocha et al., 2018). The use of these drugs is aimed to improve production performance, disease resistance, and the general well-being of animals (Rico et al., 2013; Van Hai, 2015). However, the public has been concerned about the benefits and risks associated with the use of synthetic drugs in animal feeds for a long time (Bulfon et al., 2015; Reverter et al., 2014). Residual drugs detected in aquaculture products may harm people who consume them (Awad & Awaad, 2017). Additionally, the uncontrolled administration of antimicrobials in cultivation practices may result in the emergence of antimicrobial-resistant strains (Bilen et al., 2018; Hoseinifar et al., 2020; Van Doan et al., 2019). To reduce the potential risks associated with the application of chemicals, medicinal plants and their novel compounds are continually developed and verified (Amin et al., 2019; Chakraborty & Hancz, 2011; Dawood et al., 2020; Jankham et al., 2020). In raniculture, many plant species such as *Butea superba, Curcuma longa, Pueraria mirifica, Nelumbo nucifera* Gaertn., and *Limnophila aromatica* have been evaluated in the laboratory conditions and shown as potential substitutes for the use of antibiotics to enhance growth indices and health status in heterogeneous farm conditions (Kaewtapee et al., 2011; Thainum & Chitmanat, 2019; Srinuansom et al., 2019; Thummek et al., 2016; Wongtha et al., 2019).

Lasia (*Lasia spinosa* (L.) Thwaites) is a perennial herb that belongs to the family Araceae. It is a native plant of Southeast Asia, where it occurs in shaded areas along the river, wet forests, and wetlands. Phytochemical analysis indicated the presence of flavonoids, terpenoids, phenolic compounds, steroids, saponins, coumarins, glycosides, and anthraquinones (Hong Van et al., 2006; Napiroon et al., 2013). Phytoandrogens and phytoestrogens were also detected in rhizomes, leaves, and roots of this plant (Suthikrai et al., 2007). Interestingly, lasia has been reported to possess a variety of pharmacological effects including antioxidant, antinociceptive, anti-inflammatory, anti-diarrheal, antimicrobial, and cytotoxic properties (Alam et al., 2011; Deb et al., 2010; Goshwami et al., 2012; Nanasombat & Teckchuen, 2009). Recently, Kaewamatawong et al. (2013) evaluated acute and subacute toxicity and reproductive effects of lasia extract in male rats and they found the extract of lasia did not show any signs of acute and subacute toxicity in rats. Blood biochemical values and the weight of the body and internal organs of the experimental groups were similar to the control group. However, testicular weight and sperm count significantly increased in the treated rats. These findings indicate the safe use of lasia in traditional medicines and home remedies. As mentioned above, there are very few reports indicated the application of lasia as a natural feed additive in animal diets (Munglue et al., 2019; Suthikrai et al., 2005; Suthikrai et al., 2007). Available evidence indicated that dietary supplementation with lasia improved growth and nutrient utilization efficiency in large ruminants (Suthikrai et al., 2007). Additionally, hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fed with the diet supplemented with 3% lasia

extract for 8 weeks showed a significant improvement of growth rate and healthy intestinal villi (Munglue et al., 2019). Based on these scientific reports, lasia may be effectively used to replace the synthetic feed additives in aquaculture industries (Chakraborty & Hancz, 2011; Reverter et al., 2014; Suthikrai et al., 2007). To our knowledge, there is no information regarding the beneficial effects of lasia in the frog culture. The objective of this study was to evaluate the effects of lasia extract (LE) on growth performance parameters and physiological indices of common lowland frog.

Materials and methods

1. Animal ethics and regulation

This research was performed in the Ubon Ratchathani Rajabhat University Fishery Farm. The experimental protocol of this research was approved by the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. The approval number is AN63006.

2. Lasia preparation and extraction

Lasia extract was performed according to the report of Munglue et al. (2019). Arial parts of lasia were harvested from Sirindhorn District, Ubon Ratchathani, Thailand during August and October. The plant specimen (Munglue 006) was kept at the Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University, for further reference. The plant samples were cleaned using tap water, cut into small pieces approximately 1 to 2 cm, and dried in a hot air oven at 45°C for 1 week. Dried lasia samples (100 g) were macerated with 75% ethanol (300 mL) (1:3 w/v) for 1 week and then it was filtered through Whatman paper No.1. The resultant mixture was evaporated to remove the solvent using rotary evaporator (Buchi, R-200, Switzerland) under reduced pressure and low temperature. The solution was subsequently transferred to lyophilizer (Labconco Corporation, Missouri, USA). The crude extract (10.15 g) was collected and kept in a refrigerator (-20°C) until use.

3. Phytochemical screening

Phytochemical constituents including alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, steroids, saponins, coumarins, glycosides, and anthraquinones were determined using colorimetric methods of Evans et al. (2002) and Kar (2007). The results were reported as (+) presence or (-) absence to indicate the active compounds in the plant extract.

4. Experimental diet preparation

A commercial diet containing 42% crude protein, 5% crude fat, and 5% fiber (Charoen Pokphan Food PCL, Samutsakorn, Thailand) was obtained and used as a basal diet. The dietary protein level in this study was in the range optimal for frog rearing (Coppo et al., 2001; Marschall, 1978). In this research, the levels of LE (0 (control), 1, 3, and 5 g/kg diets) were estimated according to the previous study (Munglue et al., 2019). The diets were mixed with LE using cassava starch as a binder and 200 mL distilled water added. The mixture was pelleted by using a meat mincer (2 mm). Pellets were then dried in a hot air oven at 45°C for 24 h and kept in sealed plastic bags at 4°C in a refrigerator until use.

5. Experimental frog and frog culture

Male frogs with the mean initial weight of 14.00 ± 1.00 g were obtained from Ubon Ratchathani Fishery Cooperative, Ubon Ratchathani, Thailand. A total of 240 frogs were transferred to the Ubon Ratchathani Rajabhat University fishery farm. They were randomly distributed into 12 circular cement tanks (0.70 m in diameter and 0.45 m in height) filled with cleaned water and covered with black shade nets. Water quality parameters were checked daily using ExStik® EC500 (Extech Instrument Corporation, U.S.A.) and maintained in standard levels for frog farming (temperature 29.0 ± 1.0°C, pH 7.5 ± 0.3, alkalinity 100 - 200 mg/L, and dissolved oxygen 6.00 ± 0.10 g/L). After a 2-week acclimatization period, three cement tanks of frogs were randomly determined as one of four experimental treatments containing different levels of LE (0 (control), 1, 3, and 5 g LE/kg diets). Frogs were fed with the experimental diets on Styrofoam *ad libitum* two times a day (08.00 and 16.00 h) for 8 weeks.

6. Growth parameters

At the end of the feeding trial, four frogs from each tank were individually weighed. Additionally, all frogs in each tank were counted to calculate the survival rate (SR). Growth parameters including weight gain (WG), specific growth rate (SGR), average daily gain (ADG), and feed conversion ratio (FCR) were evaluated as follows:

WG (g) = final weight (g) – initial weight (g);
SGR (%/day) = 100 × [(*In* final weight (g) – *In* initial weight (g))/number of days in the experimental period];
ADG (g/day) = weight gain (g)/ number of days in the experimental period;
FCR = feed intake (g)/weight gain (g);
SR (%) = 100 × (final number of frog/initial number of frog).

7. Relative organ weights

Four frogs from each replicate were randomly collected and double-pithed. Liver, intestine, spleen, heart, kidney, and intraperitoneal fat samples were collected, cleared from the blood and connective tissues, and weighed to calculate the relative organ weight using the following equation:

$$\text{Relative organ weight (\%)} = 100 \times (\text{organ weight (g)}/\text{frog body weight (g)}).$$

8. Histological analysis

The intestines were divided into 3 parts including the anterior, middle, and posterior and cut transversally. The contents in the intestinal lumen were cleared using 0.90% sodium chloride solution and fixed in phosphate-buffered formalin 10% (v/v), pH 7.4, for 24 h. After dehydration, the samples were embedded in paraffin wax, sectioned at 5 µm thick, and mounted on glass slides. The tissues were then stained routinely with Hematoxylin & Eosin (H & E) as the method reported by Escaffre et al. (2007). Observations on intestinal histology were performed using a light microscope connected with a computer using Dino Capture 2.0 software. The six longest intact villi were selected for the measurement of villi height and villi width according to the report of Jankham et al. (2020). The absorptive surface area of the intestine was also estimated using the following equation (Abdel-Tawwab et al., 2018):

$$\text{The absorptive surface area of the intestine } (\mu m^2) = \text{villi height } (\mu m) \times \text{villi width } (\mu m).$$

9. Blood collection

At the end of the feeding period, the frog fasted for 24 h. Four frogs from each tank were randomly collected, weighed, and double-pithed with a needle. The abdominal wall was opened and frog blood was carefully collected by cardiac puncture using a sterile syringe and divided into 2 parts. One part was transferred to 3 ml tube containing 10% ethylenediaminetetraacetic acid (EDTA), which was used as an anticoagulant, for hematological analysis. Another part was gently transferred to an Eppendorf tube, left to clot at room temperature for 3 h, and centrifuged at 3000 rpm for 10 min. The collected serums were kept in microtubes and stored at -20°C for biochemical analysis (Campbell & Ellis, 2007).

10. Hematological analysis

Red blood cells (RBC) and white blood cells (WBC) were measured by using a Neubauer hemocytometer (Campbell & Ellis, 2007). Hematocrit (Ht) was evaluated using the microhematocrit method (Ramezanzadeh et al., 2020). The level of hemoglobin (Hb) was measured using the cyanomethemoglobin method (Abdel-Tawwab et al., 2018).

11. Biochemical analysis

Aspartate transaminase (AST) was determined by the method reported by Schumann et al. (2002). Serum alkaline phosphatase (ALP) was evaluated according to Tietz et al. (1983). Serum glucose was analyzed using Trinder's method (Barham & Trinder, 1972). Cholesterol level was tested using the cholesterol oxidase-phenol+aminophenazone (CHOD-PAP) method (Flegg, 1973). Triglyceride was measured by following Cole et al. (1997). Commercial reagent kits obtained from Erba Lachema s.r.o. (Czech Republic) were used to detect high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol ether (PEGME) coupled classic precipitation method (PVS/PEGME).

12. Data analysis

All data were tested for the normal distribution and the homogeneity of variances by using Kolmogorov-Sminov test and Levene's test, respectively. If data were non-normal distribution, the arcsin square-root transformation was used. All data were subsequently subjected to one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to test significant differences among the treatments when $P < 0.05$. The optimal level of LE for the frog culture was estimated using the second-order polynomial regression analysis. Results were represented as mean ± standard error of the mean (SEM).

Results and discussion

1. Phytochemical screening

Phytochemical screening indicated that LE contains flavonoids, terpenoids, phenolic compounds, tannins, steroids, glycosides, and anthraquinone. However, the plant extract showed the absence of alkaloids, saponins, and coumarins (Table 1). These findings are similar to the report of Napiroon et al. (2013) who showed that lasia consists of organic compounds, terpenoids, phenolic compounds, coumarins, and alkaloids. Moreover, lasia leaf extract has been reported to possess flavonoids, terpenoids, phenolic compounds, steroids, saponins, coumarins, glycosides, and anthraquinones

(Munglue et al., 2019). When compared with the other reports, a difference in chemical compounds found in lasia in this present study may be due to extraction methods, geographical sources, soil compositions, harvesting seasons, and weather conditions (Evan et al., 2002; Kar, 2007).

Table 1 Phytochemical screening of LE

Phytochemicals	Tests performed	Results
Alkaloids	Dragendorff's Tests	-
	Mayer's Test	-
Flavonoids	NaOH Tests	+
Terpenoids	Salkowski Test	+
	Liebermann-Burchard Test	+
Phenolic compounds	Ellagic Acid Test	+
	Phenol Tests	+
Tannins	Gelatin Test	+
	Lead acetate Test	+
Steroids	Salkowski Test	+
Saponins	Foam Test	-
Coumarins	Test for coumarins	-
Glycosides	Liebermann's Test	+
	Molisch's Test	+
	Keller-Kiliani Test	+
Anthraquinones	Borntrager's Test	+
	Modified Borntrager's Test	+

Remark: + = presence; - = absence; LE = lasia extract

2. Growth parameters

Growth parameters, feed utilization, and the rate of survival of frog fed dietary LE are presented in Table 2. At the end of the feeding trial, it was found that FW, WG, SGR, and ADG of frog fed with 3 and 5 g LE/kg diets were significantly higher than those of frog fed with 1 g LE/kg diet and the basal diet ($P < 0.05$). Additionally, FCR values significantly decreased in frog fed with LE-supplemented diets compared with the control diet ($P < 0.05$). No significant difference in the SR was noted among the treatments ($P > 0.05$). By using the second-order polynomial regression analysis on the FW ($y = -2.824x^2 + 20.355x + 64.348$, $R^2 = 0.876$, $p = 0.000$), the optimal level of LE was found to be 3.60 g/kg diet (Fig. 1).

Medicinal plants and their chemical compounds have long been used in aquaculture industries as growth enhancers, appetizers, anti-stressor, and immune stimulants (Awad & Awaad, 2017; Citarasu, 2010). However, more research is required to clarify their diverse beneficial physiological effects on various species of aquatic animals. It was postulated that lasia has long been used in folk medicine to treat various diseases (Deb et al., 2010; Goshwami et al., 2012). Besides, it has been

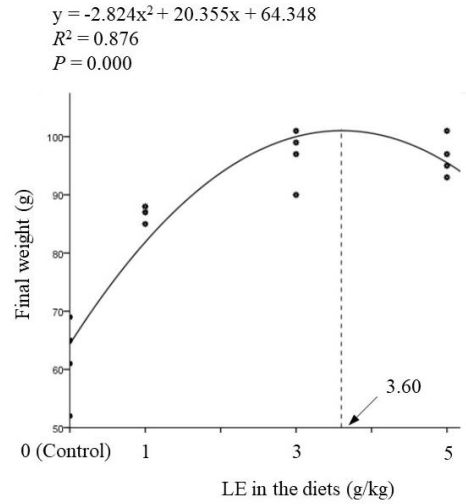

Fig. 1 The second-order polynomial regression analysis on the final weight of frog fed LE-supplemented diets for 8 weeks

reported to have beneficial effects on the growth of fish and terrestrial animals such as buffalos (Munglue et al., 2019; Suthikrai et al., 2005; Suthikrai et al., 2007). However, there is no information up to now on the effects of dietary LE on raniculture. This work has aimed to use lasia as a growth promoter in frog diets.

Table 2 Growth parameters and survival rate of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
IW (g)^{ns}	14.50 ± 0.28	15.50 ± 0.28	14.00 ± 0.70	14.75 ± 0.25
FW (g)	61.75 ± 3.63^{c}	68.75 ± 0.62^{b}	96.75 ± 2.39^{a}	95.50 ± 1.70^{a}
WG (g)	46.75 ± 1.22^{c}	72.25 ± 0.47^{b}	82.75 ± 2.49^{a}	81.75 ± 1.54^{a}
SGR (%/day)	2.51 ± 0.02^{c}	2.66 ± 0.03^{b}	3.33 ± 0.00^{a}	3.37 ± 0.01^{a}
ADG (g/d)	0.83 ± 0.06^{c}	1.29 ± 0.03^{b}	1.47 ± 0.04^{a}	1.46 ±0.02^{a}
FCR	3.78 ± 0.32^{a}	2.40 ± 0.01^{b}	2.10 ± 0.06^{b}	2.12 ± 0.03^{b}
SR (%)^{ns}	95.00 ± 2.88	95.00 ± 2.84	96.66 ± 1.62	96.66 ± 1.63

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). Superscript ^{ns} indicates no statistical difference ($P > 0.05$); LE = lasia extract; IW = initial weight (g); FW = final weight (g); WG = weight gain (g); SGR = specific growth rate (%/day); ADG = average daily gain (g/day); FCR = feed conversion ratio; SR = survival rate (%)

This present study demonstrated that growth parameters were significantly improved in frog fed with LE containing diets. No significant difference was observed in the SR among the treatments. These results suggested growth-stimulatory activity of LE in frogs without a negative effect. Similar results have been reported by Munglue et al. (2019) who found that hybrid catfish fed with LE-supplemented diets for 8 weeks showed a significant increase in growth performance

parameters compared with the basal diet. A study by Wongtha et al. (2019) reported that incorporating *L. aromatica* in frog diets significantly improved feed utilization and growth performance. The underlying mechanism of LE on the growth of frogs remains unknown. It is established that herbal plants could act as growth promoters by improving feed palatability, feed intake, nutrient metabolism, digestive enzyme activity, and the molecular pathways of protein synthesis in various cell types (Awad & Awaad, 2017; Citarasu, 2010). In addition, a variety of micronutrients from plants such as steroids, alkaloids, flavonoids, glycosides, phenolics, saponins, terpenoids, anthraquinones, and coumarins have been reported to have a marked enhancement in the growth of frog (Wongtha et al., 2019). Taken together, an increase of growth parameters of frog fed with LE-supplemented diets may be due to phytonutrients that found in *L. spinosa* such as flavonoids, terpenoids, and phenolic compounds (Bulfon et al., 2015; Chakraborty et al., 2014; Reverter et al., 2014).

3. Relative organ weights

The data on the relative weights of internal organs are summarized in Table 3. There were no significant differences in the values of HSI, ISI, SSI, CSI, and RSI ($P > 0.05$). However, a marked increase of IPF values was noted in frog fed with LE-supplemented diets compared with the control ($P < 0.05$).

Table 3 Relative organ weights of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
HSI (%)^{ns}	5.00±0.15	4.62±0.27	5.32±0.14	4.75±0.42
ISI (%)^{ns}	1.61±0.07	1.57±0.06	1.68±0.07	1.65±0.07
SSI (%)^{ns}	0.04±0.01	0.05±0.01	0.04±0.01	0.04±0.01
CSI (%)^{ns}	0.63±0.04	0.56±0.02	0.52±0.02	0.55±0.02
RSI (%)^{ns}	0.53±0.03	0.47±0.04	0.46±0.02	0.54±0.03
IPF (%)	4.79±0.27^{c}	5.47±0.16^{ab}	5.27±0.21^{bc}	6.15±0.26^{a}

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). Superscript ^{ns} indicates no statistical difference ($P > 0.05$); LE = lasia extract; HSI = hepatosomatic index (%); ISI = intestinosomatic index (%); SSI = spleen somatic index (%); CSI = cardiosomatic index (%); RSI = renosomatic index (%); IPF = intraperitoneal fat (%)

The relative organ weight is an important index of physiological and pathological status in experimental animals (Fisher & Myers, 2000; Gupta et al., 2017). The previous studies have proved that changes in the weight of internal organs may influence by a variety of biological, chemical, and physical factors (Brodeur et al., 2020; Dekić et al., 2016; Penn et al., 2011). Therefore, studies on the relative weights of organs may provide information on dosage regimens, the mode of action, and the toxic effects of natural products in aquatic organisms (Porwal et al., 2017; Smith et al., 2017). In this research, relative organ weights of the liver, intestine, spleen, heart, and kidney in the experimental groups did not differ significantly when compared with the control group. Thus, this finding suggested that lasia can be submitted as a supplement in frog feed without negligible side effects. Interestingly, a significant increase of the fat body mass was recorded in frog fed with LE-supplemented diets. It is well known that the physiological significance of the fat body is related to the modulation of reproductive processes, metamorphosis, hibernation, and energy homeostasis in amphibians (Zancanaro et al., 1996; Zhu et al., 2019). The results of this work were consistent with those of Klahan & Pimpimol (2018) who found that frogs fed with the diets supplemented with crude papain extract for 90 days displayed high levels of protein and lipid in the edible flesh compared with the control diet. Additionally, hybrid catfish that were fed diets containing *Euphorbia hirta* plant leaf extract for 90 days showed a significant increase in IPF values compared with the control (Panase et al., 2018). To date, there is no information available on the effect of dietary LE on a fat depot in frogs. However, it may speculate that some phytochemicals could improve feed utilization and enhance protein and lipid accumulation in frogs that were fed with LE-supplemented diets (Panase et al., 2018; Turan & Akyurt, 2005; Sun et al., 2018). Therefore, further researches are necessary to elucidate the mechanism of the effect of dietary LE on the fat storage in frogs.

4. Histological analysis

The intestinal histology of frog fed with LE-supplemented diets is given in Table 4 and Fig. 2. In the anterior intestine (2A – 2D), significantly increased villi height and absorptive area were observed in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). Enhanced villi width was detected in frog fed with 5 g LE/kg diet compared with the other treatments ($P < 0.05$). In the middle intestine (2E – 2H), the frog that were fed with 1 g LE/kg diet showed a significant increase in villi height compared with the other treatments ($P < 0.05$). Significantly improved villi width and the absorptive surface area were found in frog fed with LE-supplemented diets compared with the control ($P < 0.05$). In the posterior intestine (2I – 2L), a significant increase in villi height and the absorptive area

Table 4 Histological analysis of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
Anterior intestine				
Villi height (μm)	2039.18±202.07^{b}	3968.29±232.84^{a}	3251.88±132.27^{a}	3852.22±578.73^{a}
Villi width (μm)	299.71±9.62^{b}	307.86±9.30^{b}	296.84±10.42^{b}	351.13±12.48^{a}
Absorptive area (μm^2)	604073.37±48625.07^{c}	1232175.87±95392.01^{a}	967684.72±42739.13^{b}	1377547.73±218692.28^{a}
Middle intestine				
Villi height (μm)	2654.30±192.16^{b}	4255.35±380.50^{a}	2963.25±222.35^{b}	2940.27±254.33^{b}
Villi width (μm)	237.94±10.13^{b}	273.12±16.06^{a}	279.18±10.58^{a}	275.96±10.87^{a}
Absorptive area (μm^2)	561775.07±25979.12^{b}	1186668.01±145735.58^{a}	828634.73±67594.08^{a}	838348.29±97817.84^{a}
Posterior intestine				
Villi height (μm)	1361.37±95.70^{b}	1224.38±23.63^{b}	1774.38±99.79^{a}	1269.66±78.20^{b}
Villi width (μm)	274.09±22.58^{a}	232.31±8.81^{b}	276.33±5.72^{a}	275.81±9.88^{a}
Absorptive area (μm^2)	290711.72±22541.60^{b}	285714.44±14582.06^{b}	489893.62±29026.25^{a}	351780.93±25718.30^{b}

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). LE = lasia extract

was observed in frog fed with 3 g LE/kg diet compared with the other treatments ($P < 0.05$). Additionally, decreased intestinal villi width was noticed in frog fed with 1 g LE/kg diet compared with the other treatments ($P < 0.05$).

Study on intestinal histology is an important indicator of digestion and absorption processes in animals (Adeshina et al., 2019; Escaffre et al., 2007). Results of this study indicated that frog fed with LE-supplemented diets showed a significant increase in villus height, villi width, and absorptive area in all parts of the intestine. These findings are in agreement with the report of Munglue et al. (2019) who demonstrated that hybrid catfish fed with dietary LE for 8 weeks showed a significant increase in villus height, villi width, and goblet cell number compared with the control diet. Similarly, Thummek et al. (2016) recorded a significant enhancement of villi height, villi width, and muscular thickness of frog fed with diets containing 1, 3, and 5% *N. nucifera* stamen extract for 11 weeks. Also, dietary supplementation of *N. pubescens* stamen extract improved villi height, villi width, and muscular thickness of frog (Kamatit et al., 2016). It was noted that nutrient digestibility and metabolic activity are related to intestinal histomorphology (Amin et al., 2019; Escaffre et al., 2007; Wilson & Castro, 2011). Dietary

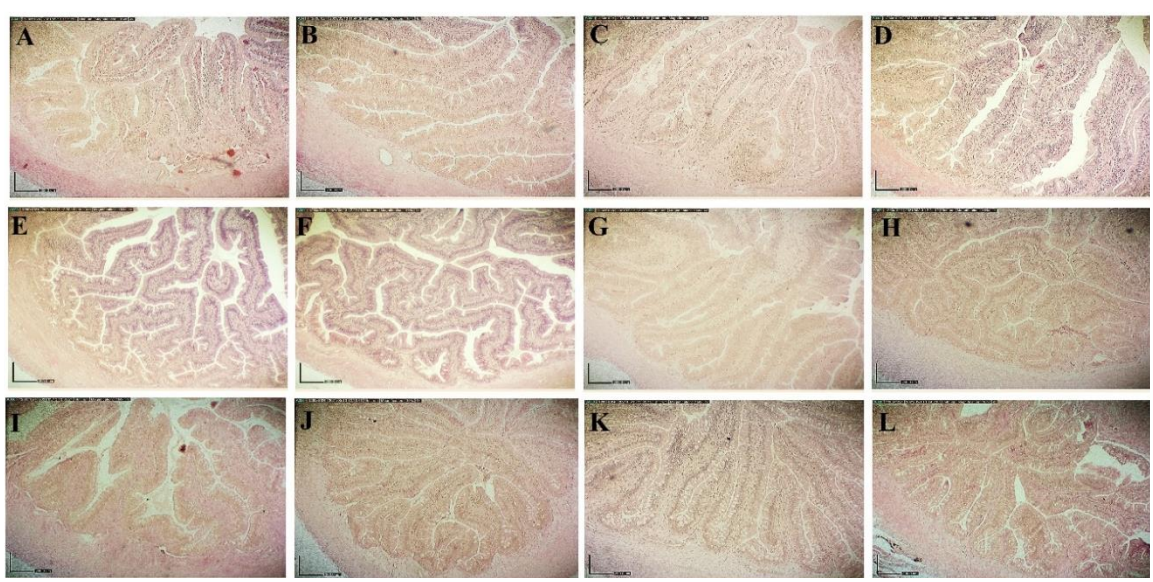

Fig. 2 Light microscopy of the anterior (A-D), middle (E-H), and posterior (I-L) intestine of frog fed the diets supplemented with 0 (A, E, and I), 1 (B, F, and J), 3 (C, G, and K), and 5 (D, H, and L) g LE/kg diets for 8 weeks. Scale bar = 200 μm.

supplementation with medicinal plants produced an increase of villus height, villi width, and the absorptive surface area of the intestine by the modulation of the renewal of the intestinal epithelial cells, resulting in the improvement in nutrient absorption capability, and subsequently the enhancement in feed efficiency and weight gain (Abdel-Tawwab et al., 2018; Antushevich et al., 2014; Crosnier et al., 2006). Moreover, antimicrobial and antioxidant properties of medicinal plants could reduce pathogenic microorganisms in the gut and enhance efficient nutrient digestion and performance (Chakraborty & Hancz, 2011; Nanasombat & Teckchuen, 2009; Tatli Seven et al., 2008). There are limited research studies on the effect of dietary LE on the intestinal histomorphology of frogs. It is hypothesized that the improvement of villi height in frog has been achieved due to such phytochemicals found in LE, which would modulate cell division, cell proliferation, and cell apoptosis in the tract and these could positively affect the growth of frog (Antushevich et al., 2014; Crosnier et al., 2006; Wlodarska et al., 2015).

5. Hematological analysis

As shown in Table 5, it was demonstrated that the highest level of Hb was found in frogs fed with 5 g LE/kg diet ($P < 0.05$). However, dietary LE did not affect the values of Ht, WBC, and RBC compared with the control ($P > 0.05$).

Hematological indices have long been used to assess the health status and the immune system of aquatic animals (Abdel-Tawwab et al., 2018; Mohammadi et al., 2018; Ramezanzadeh et al., 2020). The levels of RBC, Hb, and Ht are important indicators of erythrocyte status and oxygen transport efficiency (Coppo et al., 2005a). Moreover, WBC level would demonstrate the ability of innate immune response to defend frog from the external environment (Gabriel et al., 2015; Ramezanzadeh et al., 2020). In this report, frog fed with a diet containing 5 g LE/kg showed a significant increase of Hb compared with the other groups. However, no significant differences in WBC, RBC, and Ht were noticed in frog fed with LE-supplemented diets. Hematological values observed in this research were similar to those published on common lowland frog (Wongtha et al., 2019), bullfrog (*Rana catesbeiana*) (Coppo et al., 2005b), and the African clawed frog (*Xenopus laevis*) (Chang et al., 2015). It is speculated that changes in Hb are related to environmental and physiological conditions (Coppo et al., 2005a, 2005b). Previous reports suggested that medicinal plants like Aloe (*Aloe vera*) (Gabriel et al., 2015), purple coneflower (*Echinacea purpurea*) (Oskoii et al., 2012), and barberry (*Berberis vulgaris*) (Ramezanzadeh et al., 2020) had a positive impact on Hb levels by increasing erythropoiesis or hemosynthesis in hematopoietic organs (Gabriel et al., 2015; Iji et al., 2010). Therefore, dietary LE could have erythropoietin effects on hematopoietic stem cells or could increase intestinal absorption and utilization of thiamine, riboflavin, vitamin E, niacin, and folic acid in frogs (Gabriel et al., 2015; Talmadge et al., 2004).

Table 5 Hematological analysis of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
Hb (g/dL)	9.90 ± 92^{b}	9.80 ± 0.39^{b}	9.75 ± 0.67^{b}	11.76 ± 0.43^{a}
Ht (%)^{ns}	27.58 ± 2.27	22.66 ± 2.07	30.16 ± 4.08	25.75 ± 1.65
WBC ($\times 10^4$ cell/mm^3)^{ns}	8.44 ± 0.41	8.81 ± 0.25	8.55 ± 0.23	8.73 ± 0.17
RBC ($\times 10^{11}$ cell/L)^{ns}	1.51 ± 0.13	1.20 ± 0.20	1.52 ± 0.12	1.31 ± 0.11

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). Superscript ^{ns} indicates no statistical difference ($P > 0.05$); LE = lasia extract; Hb = hemoglobin (g/dL); Ht = hematocrit (%); WBC = white blood cells ($\times 10^4$ cell/mm^3); RBC = red blood cells ($\times 10^{11}$ cell/L)

6. Biochemical analysis

It was found that glucose level was markedly increased in frog fed with 5 g LE/kg diet compared with the other treatments ($P < 0.05$). The reductions of serum cholesterol levels were observed in frog fed with LE-supplemented diets compared with the control diet ($P < 0.05$). However, dietary LE supplementation had no detectable effects on serum total protein, albumin, AST, ALP, triglyceride, HDL-C, and LDL-C values compared with the control ($P > 0.05$) (Table 6).

Serum biochemical parameters have been used to demonstrate the health condition of aquatic animals (Cathers et al., 1997; Naiel et al., 2020; Wilson et al., 2011). Changes in blood chemical values of frogs may be attributed to both environmental and physiological aspects (Adeshina et al., 2019; Coppo et al., 2001; Coppo et al., 2005a). The present results revealed 5 g LE/kg diet significantly enhanced serum glucose level in the cultured frog. It is well known that changes in serum glucose levels are generally related to carbohydrate metabolism, energy utilization, O_2 consumption, stress, and several clinical illnesses (Adham et al., 2002; Coppo et al., 2005b). The highest glucose content observed in frog fed with 5 g LE/kg diet would indicate physiological

Table 6 Biochemical analysis of common lowland frog fed LE-supplemented diets for 8 weeks

Parameters	LE in the diets (g/kg)			
	0 (Control)	1	3	5
Total protein (g/dL)^{ns}	4.18±0.59	5.76±0.63	4.86±0.58	5.00±0.26
Albumin (g/dL)^{ns}	1.08±0.30	1.06±0.12	1.81±0.22	1.22±0.52
Glucose (mg/dL)	169.68±9.19^{b}	165.15±7.63^{b}	168.95±5.18^{b}	224.24±6.55^{a}
Cholesterol (mg/dL)	90.05±1.19^{a}	24.66±4.93^{b}	23.24±2.33^{b}	22.83±7.31^{b}
Triglycerides (mg/dL)^{ns}	12.58±0.84	19.47±2.93	19.55±1.04	19.28±9.74
AST (U/L)^{ns}	38.49±6.76	31.65±5.54	39.69±6.24	37.72±3.28
ALP (U/L)^{ns}	128.78±5.20	193.24±4.33	148.76±8.00	175.12±7.54
HDL-C (mg/dL)^{ns}	42.70±4.81	42.85±5.66	50.96±7.53	40.54±5.93
LDL-C (mg/dL)^{ns}	35.03±8.43	33.26±5.64	39.80±3.71	32.35±4.04

Remark: Data are presented as mean ± SEM. Different superscripts in the same row are significantly different ($P < 0.05$). Superscript ^{ns} indicates no statistical difference ($P > 0.05$); LE = lasia extract; AST = aspartate transaminase (U/L); ALP = alkaline phosphatase (U/L); HDL-C = high-density lipoprotein cholesterol (mg/dL); LDL = low-density lipoprotein cholesterol (mg/dL).

responses to stress (Güllü et al., 2016). In the current experiment, serum cholesterol contents were significantly declined in frog fed with LE-supplemented diets. This is in agreement with the results presented by Wongtha et al. (2019) who found that frogs fed for 8 weeks with different doses of *L. aromatica* showed a significant decrease in cholesterol levels compared with the control. Decreased cholesterol contents in this research may clarify a hypocholesterolemic effect of dietary LE, which would contribute to an improvement in cholesterol metabolism by increasing bile acid secretion or decreasing cholesterol absorption in the intestine of the frog (Nguyen et al., 2001; Yang et al., 2013). Also, LE may enhance cholesterol deposit in the fat body of frogs (Kasinathan et al., 1978; Panase et al., 2018). The mechanism by which dietary LE improve cholesterol metabolism in the frog is still questioned. It is hypothesized that some phytochemicals such as flavonoids and phenolic compounds found in LE would encourage the hypocholesterolemic effect (Park et al., 2014; Zou et al., 2005). Further research studies related to this aspect are needed to evaluate the effects of LE on the metabolism of cholesterol in frog. Serum total protein and albumin are useful for the clinical assessment of protein metabolism and the functions of the liver and kidney (Eckersall, 2008). Decreased total protein and albumin contents are correlated with malnutrition, chronic liver and kidney diseases, immunological disorders, and inflammations (Kreutzer et al., 2008). There were no changes in total protein and albumin levels in frog fed with LE-supplemented diets, suggesting that dietary LE did not have harmful effects on protein metabolism in frog. The level of AST is consistent with normal physiological functions of the liver, skeletal muscle, and cardiac muscle (Hoffmann & Solter, 2008). Markedly increased serum AST could suggest cellular injury in the liver or muscle (Campbell, 2012; Kreutzer et al., 2008). Serum ALP is found in bone, liver, kidney, and intestine (Coppo et al., 2001; Sharma et al., 2014). Serum ALP is increased following osteopathies and intestinal diseases (Golub & Boesze-Battaglia, 2007; Sharma et al., 2014). Serum HDL-C and LDL-C levels are correlated with lipoprotein metabolism in the frog (Bruss, 2008). Increased HDL-C and LDL-C may be due to hepatic and renal disorders, malnutrition, and infections (Coppo et al., 2005a). The present work indicated serum total protein, albumin, AST, ALP, HDL-C, and LDL-C levels did not change significantly among the treatments and those levels were similar to the reports of Coppo et al. (2005a) and Coppo et al. (2005b). Therefore, these findings encourage the use of LE as a natural feed additive in frog diet without a detrimental effect.

Conclusion

In conclusion, this research indicated the improvement in growth performance, feed utilization efficiency, and intestinal histology of frog fed LE-supplemented diets without a negative effect on the SR. Dietary LE did not affect the values of HSI, ISI, SSI, CSI, and RSI. Increased IPF was found in frog fed with LE-supplemented diets. There were no changes in Ht, WBC, and RBC levels among the treatments. Serum total protein, albumin, AST, ALP, triglyceride, HDL-C, and LDL-C were not influenced by LE-supplemented diets. However, Hb and serum glucose increased significantly in frog fed with 5 g LE/kg diet compared with the other treatments. Frogs that were fed with LE-supplemented diets showed a significant decrease in cholesterol levels. Preliminary phytochemical evaluation of LE demonstrated the presence of flavonoids, terpenoids, phenolic compounds, tannins, steroids, glycosides, and anthraquinone. The optimal level of LE estimated by using the second-order polynomial regression analysis was found to be 3.60 g LE/kg diet. Taken together, these findings provide valuable scientific evidence supporting the beneficial effects of dietary LE in the culture of frog.

Acknowledgments

The authors would like to acknowledge the Program of Science Education, Faculty of Science, Ubon Ratchathani Rajabhat University for financial support. The authors also thank Miss Narissa Santarn, Miss Sudaporn Janthaket, and Mr. Apisit Pongpeaw for their kind help during the study.

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