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RESEARCH PAPER

Effects of Dietary Katuk Leaf Extract on Growth Performance, Feeding Behavior and Water Quality of Grouper *Epinephelus coioides*

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Abstract - Plant-derived materials are believed as potential nutrient sources to be applied in aquaculture. Although many studies to assess the benefits of plant extracts have been conducted, however effects of dietary katuk (*Sauropus androgynus* L. Merr.) on growth performances, feeding behavior and water quality of grouper (*Epinephelus coioides*) are not well known. In this study, 25 grouper juveniles weighing 11.27 ± 2.53 g were reared into 100-L tank (60 x 50 x 35 cm) and cultivated for 70 days. The fish were divided into four groups in triplicate, and were offered diet without katuk extract (control), diet supplemented with 1% katuk extract (SAA); diet supplemented with 2.5% katuk extract (SAB) and diet supplemented with 5.0% katuk extract (SAC) twice daily. Statistical analyses showed that dietary katuk extract caused a significant ($P < 0.05$) increase in growth rate and feed intake. The efficiency of feed was also significant when fish offered diets supplemented with 1% of katuk extract which indicated by a lower feed conversion ratio. However, no statistical differences were observed on the survival rate, condition factor, viscerosomatic index and hepatosomatic index. Observation on feeding behavior found that all treated fish consumed compounded diet just after provided into their tank and there were no abnormal behavior or any healthy problems during experimental periods. It demonstrated that application of katuk extract in fish diets is acceptable and can stimulate the fish appetites. In conclusion, our studies indicated that dietary katuk (*Sauropus androgynus*) extract can be applied in aquaculture to stimulate the growth and improve feed utilization.

Keywords: Feed conversion ratio, feed utilization, plant extract, *Sauropus androgynus*.

Introduction

Fisheries development has been greatly intensified in recent years and a rapid expansion of aquaculture activities has been practiced in many countries including: Indonesia, Thailand, China, Japan and Taiwan (Rimmer *et al.*, 2004; Sadovy, 2000; Liao *et al.*, 2001). To support good quality seeds and to improve productivity, some marine species such as: grouper, sea bass, mullet and sea bream are cultured in intensive system (Sorgeloos *et al.*, 1995; Liao *et al.*, 2001). However, in intensive system, most aquaculturists are commonly dependent on trash fish to feed grouper (Shahaama and Adam, 2005). This method is considered as a non economic practice because of the inconstant quality and insufficient supply (Eusebio *et al.*, 2004a). In addition, low feeding efficiency, poor feed conversion ratio and competition with human in utilizing the fisheries product should be taken into consideration (Rimmer *et al.*, 2004). Furthermore, in such conditions, trash fish often negatively affect water pollution (Phillips, 1998). Therefore, issues regarding compounded diet to replace trash fish are widely discussed in order to improve feed utilization, sustainability, high efficiency and minimizing water deterioration.

The use of compounded diets in grouper culture has been carried out in different species for example: *Cromileptes altivelis* (Laining *et al.*, 2004; Williams *et al.*, 2004), *Epinephelus fuscoguttatus* (Giri *et al.*, 2004), *Epinephelus coioides* (Millamena, 2002; Eusebio *et al.*, 2004a; Eusebio *et al.*, 2004b), *Epinephelus malabaricus* (Chen and Tsai, 1994; Lin and Shiau, 2003), and *Epinephelus areolatus* (Chu *et al.*, 1996). Moreover, some studies have also been conducted to examine the effect of plant extracts in fish diets. Application of plant extracts as a dietary supplement is acceptable and it can affect fish growth (Ji *et al.*, 2007; Galina *et al.*, 2009). Several experiments reported on beneficial effects of dietary plant materials on fish, for instant: *Achyranthes aspera* (Chakrabarti *et al.*, 2012), *Vitex negundo* and *Allium sativum* (Nargis *et al.*, 2011), *Cynodon dactylon* (Kaleeswaran *et al.*, 2011), *Massa medicate, Crataegi fructus, Artemisia capillaries, Cnidium officinale,* (Ji *et al.*, 2007) and *Viscum album* and *Zingiber officinale* (Dugenci *et al.*, 2003).

In the present study, we use katuk (*Sauropus androgynus* L. Merr.) as a feed additive in fish diets. Katuk (family: *Euphorbiaceae*) is widely known as a high nutritive-value vegetable, contained important materials such as: multi-vitamin

(Nahak and Sahu, 2010), metabolic compounds (Agustal *et al.*, 1997), essential oils (Malik, 1997), alkaloids and non alkaloids (Santoso and Sartini, 2001; Santoso *et al.*, 2009) and active compounds (Suprayogi, 1993). This plant is mostly grown in South Asia and Southeast Asia. Additionally, it is commonly used as traditional medicine to purifying the blood, fever, ulcer, urinary problem, earache and frambesia (Astuti *et al.*, 1997; Benjapak *et al.*, 2008). Recent studies showed that supplementing *S. androgynus* in terrestrial animals' diets could affect the growth performance, stimulate body weight, feeding efficiency and feed conversion ratio (Santoso *et al.*, 2009, Suprayogi and Meulen, 2006). Even though several trials have been applied in terrestrial animal as a result of beneficial substances contain in katuk leaves, the efficacy of katuk in aquaculture is still unknown. Thus, this study is conducted to investigate the effects of dietary katuk leaf extract in grouper diet on growth performance, feeding behavior and water quality.

Materials and Methods

Experimental fish preparation

Grouper (*Epinephelus coioides*) weighed 11.27±2.53 g body weight were obtained from Aquatic Animal Center, National Taiwan Ocean University and then acclimated in the hatchery of the Department of Aquaculture for 2 weeks prior to experimentation. Fish were reared and fed twice a day by feeding commercial diet. Fish of each group were distributed into 100-L total water volume (60 cm length, 50 cm wide, 35 cm height) at 25 fish/tank. Well aerated water was provided from a storage fiberglass. Water quality parameters were maintained at temperature 28.0±1°C; pH 8.0±1 and salinity 34±1 ppt.

Dietary Preparation

Fresh katuk leaves (*Sauropus androgynus*) have been collected from Bengkulu, Indonesia. The leaves were cleaned and shadow dried at room temperature for three days. After drying, all specimens were ground using an electrical blender and then filtered using 20 μ mesh to obtain the powder. Then, the powder was extracted using 70% ethanol using soxhlet apparatus. This extraction was operated with gently shaking at room temperature for 24 h (Chopra *et al.*, 1992). The obtained extract was then filtered and condensed using rotary vacuum evaporator under reduced pressure at 45 ^{o}C. *S. androgynus* extract was prepared in three different concentrations (1.0%, 2.5% and 5.0% of total ingredients) before mixed into experimental diets (Table 1). The ingredients of each diet were mixed together for 30 min for pelletization. Control diet was treated similarly with the supplemented diets, but no extract was added. The diets were then dried in a forced-air drier at room temperature for 24 h. After drying, the pellets were stored in plastic bags and stored at 4 ^{o}C for further use.

During experimentation, fish were hand fed twice daily (08:00 and 17:00) at 3% of the total biomass. Consequently, the total number and feed size changed as a result of mouth gape and fish growth. Uneaten pellets were collected and measured after each feeding time. For feed utilization, the amount of food consumed was calculated using the following formula: Feed intake (FI, g/fish/days) = [dry diet given (g) – dry diet remained (g)] / no. of fish; and feed conversion ratio (FCR) = dry feed intake (g) / [final body weight (g) – initial body weight (g)] (Stickney, 2005)

Experimental design

The experimental facility was composed of 12 tanks (100-L each). Water was provided from storage tanks, filtered and supplied to the system. The water system was equipped with mechanical filter (spongy), UV light, automatic heater and supplied with compressed air via air-stones from air pumps. Water flows in experimental aquaria were measured and adjusted before the experiment in order to be proportional to the fish density and to ensure sufficient water circulation.

Grouper juveniles were randomly distributed into cultivating system in triplicate at a density of 25 fish/tank per dietary treatment. The fish were divided into four groups, and were offered diet without katuk extract (control), diet supplemented with 1% katuk extract (SAA); diet supplemented with 2.5% katuk extract (SAB) and diet supplemented with 5.0% katuk extract (SAC). All juveniles were received their respective diets twice daily for 70 days.

Data on growth rate was recorded regularly every 2 weeks. On each sampling day, each of individual fish was caught from tank using a small net. Then the fish were quickly weighed and measured. The body wet weight was measured using an analytical balance (Ohaus Navigator, no.4120, Canada) and the total length using digital caliper (Mitutoyo, Absolute Digimatic, Japan). Immediately after measurements, the fish were carefully returned to its original tank. Growth performances were calculated as following: weight gain (WG, %) = 100 x [(final weight (g) – initial weight (g)] / initial weight (g); specific growth rate (SGR, %/day) = 100 x ($\ln W_2 - \ln W_1$) / T; where: W_1 and W_2 are initial weight and final weight, respectively and T is the number of days in the feeding periods (Hepher, 1988; Zhao *et al.,* 2012); and condition factor (K) = [(10^5 x weight of fish (g) / (length of fish)^3(cm)] (Fulton, 1911). In experiment on survival rate, all treatments were observed daily and the data was calculated by the following formula: survival rate (SR, %) = (final no. of fish / initial no. of fish) x 100 (Khan *et al.,* 1994). Viscerosomatic index (VSI) was examined by sacrificing five experimental fish from each replication to weigh the visceral. VSI was measured using formula: (VSI, %) = 100 x [weight of visceral (g) / weight of fish (g)]. Hepatosomatic index (HSI) was carried out by dissecting five experimental fish from

each replication to weigh the liver. HSI was calculated using formula: (HSI, %) = 100 x [weight of liver (g) / weight of fish] (Hopskin, 1992; Ghanawi, 2011).

Water quality measurement

Water parameters including dissolved oxygen (DO), temperature, pH and salinity were sampled every 5 days. DO and temperature were measured *in situ* using DO meter (DO600: Waterproof ExStick, Extech Instument Corp. USA), pH with a pH meter (PH100: ExStick, Extech Instument Corp. USA), salinity using refractometer (ATAGO S/Mill-E, ATAGO CO. LTD, Japan). Data collection was conducted by placed the detector (at the tip of equipments) into the water surface. All displayed number was recorded as water quality parameters. During the whole experiments, sea water was changed around 10% daily.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA). When the differences were significant at $P<0.05$ level, Tukey's test was used to compare the means between individual treatments. Statistical analysis was performed using the SAS software (SAS Inc. Cary, NC, USA).

Results and Discussions

Results

The fish growth rate was significantly affected by feeding experimental fish using katuk (*Sauropus androgynus*) extract mixed diets. A summary of growth responses, viscerosomatic index (VSI) and hepatosomatic index (HSI) in each trial is provided in Table 2. The highest growth performances were observed in fish offered 1% of katuk diets (SAA). It showed a significantly different ($P<0.05$) compared to control and fish offered 5% katuk diets (SAC), with an average final body weight 89.52 ± 6.49 g and final length 17.25 ± 0.55 cm. Among treatment groups, the lowest fish growth was occurred at control group. However, it found that there is no significant different between control group and SAC in their final weight and length. During the whole experimental periods, all groups increase the weight and length steadily every week. All groups showed the greatest weight increments, with fish received katuk extract tended to have a better growth performances compare with control fish (Figure 1a and Figure 1b). No statistical differences were observed on the VSI and HSI during the 10-weeks rearing. However, the mean values of VSI and HSI in group received katuk extract (SAC) were lower than that of the control group.

The greatest improvements in weight gain (WG) was observed in group of fish fed with 1% katuk diets (SAA), maintaining a significantly higher WG than other groups. The poorest fish growth was obtained in control group, with the percentage of weight gain and specific growth rate (SGR) was 440 ± 42 and 2.41 ± 0.11, respectively. There was a decrease of SGR in all groups; in contrast, the increased in WG was observed during the experimental time. Result on the WG_s and SGR_s indicated that fish fed with katuk extract could enhance overall relative growth rate compare with control fish. However, statistical analyses showed that there were no significant differences between control group and SAC in WG and SGR (Figure 2).

Feeding parameters such as feed intake (FI), feed conversion ratio (FCR) and condition factor (K) were significantly affected by feeding experimental fish using test diets. The best feed utilization was obtained by SAA, with FCR showed a significant different ($P<0.05$) compared to SAC, however, no meaningful significant different compare with control group and SAB (Table 3). This study found that, fish offered katuk extract tended to consume more feed compared with control fish (Figure 3). There was no significant different in condition factor (K) among treatments, however it showed that K value was higher in SAA compare with other treated groups.

Survival rate was great in all treatment groups. There was no mortality during the whole experimental period. Moreover, it found that all fish consumed compounded diet just after provided into their tank. Since all pellets were eaten as quickly as drop into water, it demonstrated that application of katuk extract in fish diets is acceptable and can stimulate the fish appetites. Data showed that the highest FI was obtained by SAB (62.38 ± 6.93 g.), while the lowest one was at control (43.45±4.31 g). Observation on fish behavior exposed that there were no abnormal behavior or any healthy problems until the end of experiment.

A summary of water quality parameters in each trial is provided in Table 4. Water quality parameters such as: salinity, dissolved oxygen, pH and temperature were checked regularly every 5 days. During the experiment, temperature ranged between 29.42 ─ 29.46 ^{o}C, with the highest temperature was found in SAB. Dissolved oxygen levels were between 5.71 – 5.87 ppm. The data indicated that no effect of supplementing plant extract in fish diets to the water quality, whereas no statistical significant difference was observed in all groups. Mean water salinity was dependent on the daily supply of seawater. During 70 days of experiment, the range of salinity was 34.46 – 34.50 ppt. These ranges are considered within optimal values for fish culture (Boyd, 2000).

Table 1. Composition of basal diets

Materials	Formulation (g/100g diets)			
	Control	SAA	SAB	SAC
Fish meal	67.2	67.2	67.2	67.2
Oil	10.0	10.0	10.0	10.0
α-starch	6.64	6.64	6.64	6.64
Vitamin mix	2.00	2.00	2.00	2.00
Mineral mix	3.00	3.00	3.00	3.00
Vitamin C	0.05	0.05	0.05	0.05
Choline chloride	0.50	0.50	0.50	0.50
Katuk meal	0.00	1.00	2.50	5.00
Carboxymethylcellulose, CMC	2.00	2.00	2.00	2.00
Cellulose	8.57	7.57	6.07	3.57

Abbreviations: SAA: feed supplemented with 1% katuk extract; SAB: feed supplemented with 2.5% katuk extract; SAC: feed supplemented with 5% katuk extract.

Table 2. Mean initial, final length and final body weight, weight gain, specific growth rate, viscerosomatic index and hepatosomatic index

Treatments	Length (cm)		Weight (g)		WG (%)	SGR (%)	VSI (%)	HSI (%)
	Initial	Final	Initial	Final				
Control	9.67±0.33^{a}	15.31±0.37^{c}	10.97±1.99^{a}	59.27±4.61^{b}	440±42^{c}	2.41±0.11^{b}	11.19±0.49^{a}	3.18±0.37^{a}
SAA	9.67±0.33^{a}	17.25±0.55^{a}	10.97±1.99^{a}	89.52±6.49^{a}	716±59^{a}	3.00±0.11^{a}	11.25±0.47^{a}	2.85±0.23^{a}
SAB	9.67±0.33^{a}	16.58±0.31^{ab}	10.97±1.99^{a}	75.01±5.29^{ab}	584±48^{b}	2.74±0.10^{a}	11.85±0.60^{a}	3.25±0.32^{a}
SAC	9.67±0.33^{a}	15.53±0.57^{bc}	10.97±1.99^{a}	59.99±5.23^{b}	447±48^{c}	2.42±0.12^{b}	10.99±0.48^{a}	2.85±0.30^{a}

Data in the same column with different letter are significantly different (p<0.05) among treatments. Values are means of triplicate groups± S.D.

Table 3. Mean feed intake, feed conversion ratio, condition factor and survival rate

Treatments	FI	FCR	K	SR
Control	43.45 ± 4.31^{b}	1.76 ± 0.07^{ab}	1.65 ± 0.10^{a}	100^{a}
SAA	53.71 ± 6.80^{ab}	1.51 ± 0.04^{b}	1.75 ± 0.10^{a}	100^{a}
SAB	62.38 ± 6.93^{a}	1.95 ± 0.21^{ab}	1.65 ± 0.05^{a}	100^{a}
SAC	49.01 ± 3.77^{b}	2.06 ± 0.27^{a}	1.60 ± 0.04^{a}	100^{a}

Data in the same column with different letter are significantly different (p<0.05) among treatments. Values are means of triplicate groups± S.D.

Table 4. Mean Salinity, dissolved oxygen, pH and temperature of grouper

Treatments	Salinity (ppt)	Dissolved oxygen (ppm)	pH	Temperature (^{o}C)
Control	34.50 ± 0.52	5.82 ± 0.29	7.40 ± 0.34	29.43 ± 0.71
SAA	34.50 ± 0.52	5.87 ± 0.37	7.26 ± 0.19	29.42 ± 0.63
SAB	34.46 ± 0.51	5.71 ± 0.37	7.17 ± 0.08	29.46 ± 0.74
SAC	34.48 ± 0.52	5.80 ± 0.46	7.18 ± 0.11	29.42 ± 0.56

Data in the same column with the absence of letters indicate not significantly different between treatments. Values are means of triplicate groups±S.D.

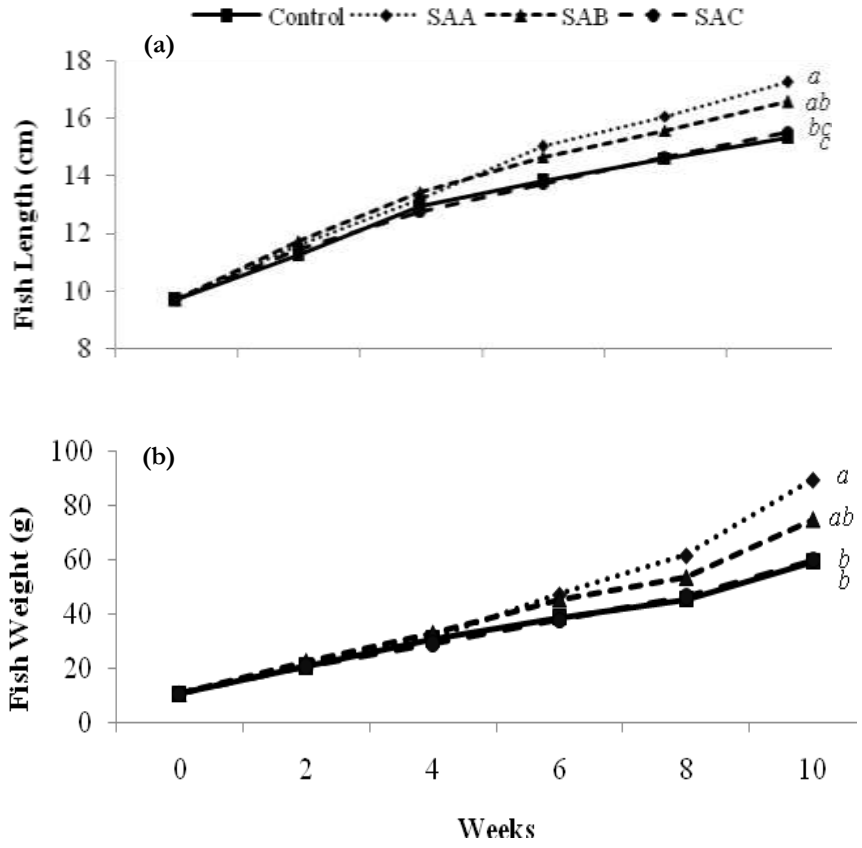

Figure. 1. Fish length (a) and fish weight (b) after feeding experimental diets during 10 weeks.

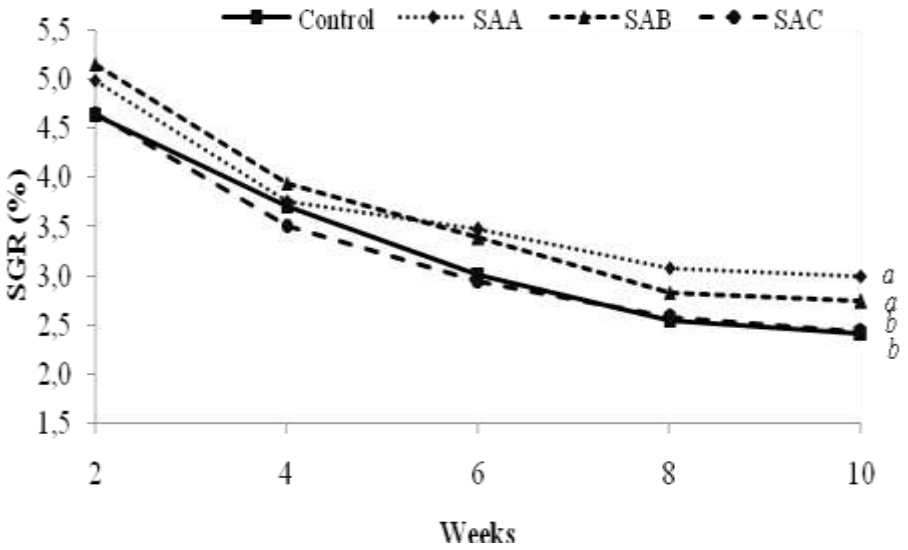

Figure. 2. Specific growth rate of grouper after feeding experimental diets during 10 weeks.

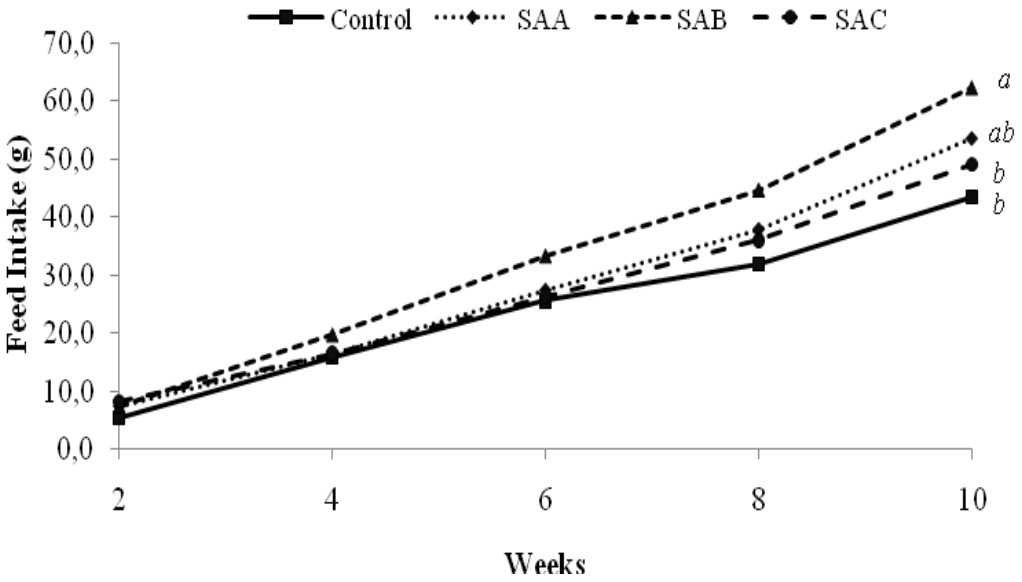

Figure 3. Feed intake of grouper after feeding experimental diets during 10 weeks.

Discussion

This present study indicated that supplementing plant extract in fish diets had greater effects on fish growth performances and feed utilization. Growth rate and feed utilization were higher in fish received *Sauropus androgynus* extract in their feed. Presumably, this was caused by nutritious substances and high-vitamin contents in katuk leaf such as pro-vitamin A in the form of β-carotene, vitamin C, vegetable oil, protein, carbohidrate and other minerals. Similar results have been reported in terrestrial animal whereas Santoso *et al.* (2009) reported that supplemented katuk extract into broiler feed at 3% body weight showed a better weight gain with a lower feed conversion. It turned out that katuk extract tend to increase weight gain and lower feed conversion. However, Mide (2012) reported that supplemented katuk in chicken feed has resulted in lower growth rate due to tanin and saponin compounds in katuk leaf. Observation found that fish offered katuk mixed diets seemed to consume more feed than control fish and acted aggressively to catch the feed. It is assumed that the respective experimental diets effect on the fish appetite. Citarasu (2010) and Bafna and Mishra (2005) mentioned that herbal biomedicine active in aquaculture has the characteristics of appetite stimulation and improve the immune system and anti stress due to the active compounds such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils. Moreover, Galina *et al.* (2009) stated that herbs are currently used as growth promoting substance, anti-microbial agents, and nutrient sources. In the present study, plants extract had been supplemented in fish diet to know the efficacy of katuk (*S. androgynus*) using oral administration. According to Sakai (1999), oral administration is a non-stressful technique and it allows mass administration in different fish size. Furthermore, National Research Council, NRC (2011) supported that some plants product can be offered orally in aquaculture without complete degradation in the digestive system.

In the present study, grouper juveniles received 1% katuk diets (SAA) exhibited increased in the WG and reduced in FCR whereas no significant different in the WG and SGR was observed between control and SAC, respectively. This finding showed that supplementing katuk extract in diets meet the maximum value in grouper diet. Analyses of final body weight and weight gain showed that application of katuk extract at 1% of total diets meet the optimal growth of the fish. Similar low dosage requirement of katuk extract was investigated in broilers chicken (Santoso *et al.*, 2009) and lactating sheep (Suprayogi, 1993) finding a low dietary katuk extract in diets for better growth rate and milk production. It is also suggested dietary of 1% katuk is the optimal dosage of this grouper. This may be due to individuals acquiring of nutrition, quantity and feeding frequency. It has been demonstrated that appropriate usage of plant extract depends on species, age, size and any stress factors during cultivation periods (NRC, 2011). Therefore studies on different dosage or percentage of katuk extract need to be determined in each species for management efficiency in increasing productivity and profitability.

When fish received experimental diets with higher dietary katuk, it seemed to reduce growth performances and feed utilization. In this study, fish fed with 2.5% and 5% katuk mixed diets exhibited lower growth and higher FCR compare with fish offered 1% katuk mixed diets. It is assumed that higher percentage of plant extracts has resulted in activation of enzyme inhibitors due to most plants contain inhibitors to protect their major components from unintended degradation. In previous studies revealed that some antinutrients substances found in plant derived materials such as protease inhibitors, lectins, amylase and lipase, tannins, saponins and antivitamins (Francis *et al.*, 2001) may cause disturbance in the gastrointestinal tract (NRC, 2011). This condition has been attributed to disturbance of digestive processes and decrease feed efficiency. As a result, consumed nutrients cannot be utilized for growth maximization. Even though no specific experiment was carried out to determine the antinutrients in katuk leaf extract and the effect of those inhibitors, but observation found that different dosages of katuk extract play a big role for the growth and feeding efficiency in experimental fish. Several plants such as soybean meal, kidney beans, rapeseed beans, pea seed meal, cottonseed meal and sunflower oil cake are commonly used in aquaculture, but those plants reported contain enzyme inhibitors (Francis *et al.*, 2001).

In these trials where different dietary of *Sauropus androgynus* had shown effects on juveniles' growth rate, the feed utilization was also observed. The results exposed that FI and FCR were better fish received dietary katuk compared with control. It is assumed that dietary katuk and long-day photoperiods during this experiment affected the fish appetites and feed intake. Similar studies have been reported on the effects of dietary plant extract and photoperiods in increasing appetite (Citarasu, 2010; Bafna and Mishra, 2005), feed intake (Imsland *et al.*, 1995) and feeding ratio (Nordgarden *et al.*, 2003; Boeuf and Le Bail, 1999). Based on the results, it is alleged that continuously light and schooling behavior seems playing a big role in increasing fish appetence, therefore feed intake can be improved in SAA and SAB in order to gain a maximum growth increment. This finding was complied with studies conducted by Webster *et al.* (2001) suggested that growth may be enhanced by increasing feed consumption to meet the energy demand in maintaining continually growth. In this aspect, we recommended that increasing in feeding percentage can be considered to recently studied species; however, well feeding administration is acquired to avoid FI and FCR values deterioration.

During the experiment, measurements on water quality were examined in the morning before the feeding activity in each sampling time. This practice was similar with the experiment on juvenile cod, Gadus morhua (Bjornsson and Olafsdottir, 2006) with an assumption that all water quality measurements must be checked at minimum values (Burel *et al.*, 1996). The present experiments found that, water quality parameters were not significantly different among treatments. Deterioration seemed not affected by dietary katuk considering all diets were eaten directly after offered to the fish, subsequently no meaningful effect of uneaten feed in the tank. Since the culture system was provided with good controlling water facilities including aeration and filters supported by relatively low water exchange (around 10% daily), caused in constant and stable water during experimental period. Boyd (2000) mentioned that the problems such as oxygen deficiency, ammonia-nitrogen and carbon dioxide accumulation and other organics pollution are frequently occurring in aquaculture, by then aeration is considered as the best way to solve this matter.

Conclusions

In conclusion, our studies indicated that dietary katuk (*Sauropus androgynus*) extract in grouper can affect the growth performance, feeding behavior and water quality. Thus, for grouper juveniles, it is suggested to feed with low percentage of katuk to attain a maximum growth. However, maintaining acceptable water quality and stocking density is required to sustain fish health and to prevent aggressive behavior.

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