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Original Article

Cosmos caudatus as a dietary supplement for Bagrid Catfish, *Mystus nemurus*

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Abstract

The optimal level of *Cosmos caudatus* supplementation in fish formulation was conducted by using *Mystus nemurus* fingerlings in two initial sizes *(*3.05±0.39 g and 4.45±0.71 g*)*. For Experiment 1, crude leaves incorporated were 0.00, 0.50, 1.50, and 2.00*%* with 60 days of feeding trial. For Experiment 2, the level of leave extract was 0.00, 0.01, 0.05, 0.10 and 0.20*%* with an experimental duration of 90 days. Both experiments showed significant differences *(*P<0.05*)* for growth performance and condition factor. The highest weight gain for Experiments 1 and 2 were 13.28±3.07 g and 18.51±4.21 g, respectively. There were no significant differences in the whole body content and survival rate for both experiments *(*P>0.05*)*. The protein level was in the range of 15.70±0.79 to 16.74±1.90*% (*Experiment 1*)* and 14.96±1.90 to 15.67±0.55*% (*Experiment 2*)*. The optimal level of crude leaves and extracts was considered at 0.50*%* and 0.05*%*, respectively, for *M. nemurus* fingerlings.

Keywords: *Cosmos caudatus*, crude leaves, leaves extract, specific growth rate, fish body composition

1. Introduction

Many researchers have conducted studies on the application of herbs in fish feed (Acar, Kesbiç, Yilmaz, Gültepe & Türker, 2015; Ardó *et al.*, 2008; Gabriel *et al.*, 2015; Harikrishnan, Balasundram & Heo, 2011; Ji *et al.*, 2009; Talpur & Ikhwanuddin, 2013). This is because herbs have various properties and can act as anti-stress medication, growth promoters, appetite boosters, tonic, and immunostimulants for aquatic animals such as shrimp and fish. The World Health Organization (WHO) also encourages people to use medicinal herbs and plants to reduce chemical usage (Kaur & Shah, 2017). Applications of these phytobiotics sometimes deemed impractical for farmers because of the unavailability of sophisticated machinery to extracts herb compounds. Moreover, the supply of herb extractions in the market is scarce except for certain commercial natural products such as garlic, turmeric, clove oil, and a few others. Therefore, some farmers substitute the herb extracts in feed with fresh or dried herbs because they are less expensive.

Malaysians are highly familiar with 'ulam raja' or *Cosmos caudatus* and commonly include in their diet. This herb is usually eaten raw or boiled. *C. caudatus* grows easily and takes only a few months to harvest. It contains high levels of calcium which reported to be able to strengthen the bone. It has been used as an alternative treatment for post-menopausal women, appetite stimulant, insect repellent, and helps in blood circulation (Andarwulan *et al.*, 2012; Cheng, Nisak, Anthony, & Ismail, 2015; Mohamed, Sahuggi, Ramli, & Muhamad, 2013; Shui, Leong, & Wong, 2005). The methanol extract of this herb has been proven to have antioxidant and antifungal

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activity (Mohamed *et al.*, 2013). It was proved to give positive sign in the inhibition of some bacteria. The study of *C. caudatus* reviewed in various fields by Moshawih, Cheema, Ahmad, Zakaria, and Hakim (2017), however the application of *C. caudatus* in fish culture is not well documented.

Mystus nemurus has tender, whitish and firm flesh texture. In Malaysia, it is known as highly priced freshwater fish (Hamid, Mahayat, & Hasim, 2011) and demanding in market compared to other type of catfish. The habitat for this fish includes rivers, lakes, and damps. Mostly, farmers culture the fish in small scale cages. The bagrid catfish require longer culture period which about 10 to 15 months to achieve market size compared to tilapia and African catfish which around 3 to 6 months. This long grow-out period (Liao *et al.*, 2008) and increasing price of feed due to high protein diet requirement for fish (Miller, Davis, & Phelps, 2005; Saillant *et al.*, 2013) had reduced the popularity of certain species to be cultured including *M. nemurus*.

Hence, this study aims to address the issue of growth performance of *M. nemurus* by using *C. caudatus* supplementation, in order to favour a shorter growth span. In this study, two different herb preparations were applied; crude dried leaves and crude extract. It is necessary to determine the growth effect of fish using different preparing methods of *C. caudatus* supplementation in feed so that we can provide guidelines for farmers and fish nutritionist. Besides that, we can determine the nutritional potential of this herb to be used in aquafeed in future.

2. Materials and Methods

Herb preparation*: C. caudatus* leaves were bought from the local market. The herb samples were cleaned and dried in an oven for 2-3 days at 35-40 °C until they reached a stable weight. The samples were then weighed and ground to a powder form (size about 0.70 mm) before incorporated in diet or extracted. For Experiment 1, the dried crude herb was directly incorporated into the diet. In Experiment 2, extraction of *C. caudatus* leaves was prepared using a rotary evaporator (Buchi, Switzerland) and then freeze dried into powder form. Both crude leaves and extract were stored at -20 °C until used. This herb preparation was done by referring to Mediani, Abas, Ping, Khatib and Lajis (2012).

Diet preparation*:* Ten experimental diets were used in the two different experiments. For Experiment 1 (crude leaves), the levels were 0.00%, 0.50%, 1.00%, 1.50% and 2.00%. Meanwhile for Experiment 2 (leaves extract), the levels were 0.00%, 0.01%, 0.05%, 0.10% and 0.20%. These diets were formulated in dry matter basis as described in Table 1. The *C. caudatus* was incorporated in the diets by replacing the α-cellulose. Firstly, the *C. caudatus* was mixed with the binder, then added with the dry ingredients. Lastly, the liquid ingredients were added and mixed well. After the mixture became homogeneous, they were pressed through die using a pelleting machine. The prepared pellets were dried in a drying cabinet for 24 hours at 40 °C or until the moisture dropped to approximately 10%. The pellets were stored in sterile plastic bags at -20 °C until used.

Fish and feeding trial*:* Juvenile catfish in two size groups were used. Experiment 1 used catfish in 2-3 inches long while Experiment 2 used catfish 3-4 in inches long. The fish were obtained from a supplier in northern Peninsular Malaysia and then transferred to Underwater World Langkawi (UWL). For acclimatization, the fish were placed in 24 hours aerated tank (150 L) for a few weeks. Initially fish were fed with commercial feed, then introduced with control diet (40% crude protein, 12% lipid) for two weeks before the experiment commenced. The initial average weight of the fish was 3.05±0.39 and 4.45±0.71 g in Experiment 1 and 2, respectively.

Table 1. Feed composition for juvenile bagrid catfish, *M. nemurus* (%)

Ingredients	Control	Experiment 1				Experiment 2			
		Crude leaves-CL				Crude extract-CE			
	0.00%CL/CE	0.50%CL	1.00%CL	1.50%CL	2.00%CL	0.05%CE	0.10%CE	0.15%CE	0.20%CE
Fish Meal[1]	40	40	40	40	40	40	40	40	40
Soybean meal[1]	15	15	15	15	15	15	15	15	15
Shrimp meal[2]	3	3	3	3	3	3	3	3	3
Wheat flour[3]	20	20	20	20	20	20	20	20	20
Fish oil[1]	4	4	4	4	4	4	4	4	4
Soybean lecithin[1]	3	3	3	3	3	3	3	3	3
Vitamin Mixture[4]	3	3	3	3	3	3	3	3	3
Mineral Mixture[5]	3	3	3	3	3	3	3	3	3
Vitamin C[1]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CMC[6]	2	2	2	2	2	2	2	2	2
C. caudatus[7]	0	0.5	1	1.5	2	0.05	0.1	0.15	0.2
α-Cellulose[1]	6.8	6.3	5.8	5.3	4.8	6.75	6.7	6.65	6.6
Total	100	100	100	100	100	100	100	100	100

[1]Sri Purta Trading, Alor Setar, Kedah. [2]Local market, dried, grounded and sieved in laboratory. [3]Local market. [4]Rovithai, DSM Nutritional Products Ltd. Scotland; composition (IU/g/mg per kg): vitamin A 50 IU, vitamin D_3 10 IU, vitamin E_1 30 g, vitamin B_1 10 g, vitamin B_2 25g, vitamin B_6 16 g, vitamin B_{12} 100mg, biotin 500 mg, panthothenic acid 56 g, folic acid 8 g, niacin 200 g, anticake 20 g, antioxidant 0.2 g and vitamin K_3 10g. [5]Rovithai, DSM Nutritional Products Ltd. Scotland; composition (g per kg): copper 7.50 g, iron 125.0 g, manganese 25.0 g, zinc 125.0 g, cobalt 0.50 g, iodine 0.175 g, selenium 0.300 g and anticake 10.0 g. [6]CMC: carboxymethyl cellulose from Sri Purta Trading, Alor Setar, Kedah. [7]*C. caudatus* leaves from local market, dried, grounded, sieved and with or without extraction.

To increase the precision of the result among treatments, the fish were stocked in quadruplicate per treatment with 25 fish per tank. Every treatment tank shared a simple filter system which consisted of bio-balls and a filter. Dechlorinated tap water was used, with continuous aeration supplied into the tanks. Water temperature was maintained at 28.5±0.04 °C with a photoperiod of 12 hours light and 12 hours dark. Every alternate day, 50% water was exchanged. The fish were fed ad libitum in twice a day for 60 days (Experiment 1) and 90 days (Experiment 2). Bulk sampling was conducted every fortnight to adjust their daily feed. Water quality such as temperature and dissolved oxygen were monitored throughout the experiment and maintained at 28.5±0.04 °C and 5.90±0,24 mg L^{-1}.

Sampling and data collection*:* Fish samples were collected from each treatment and control group at the end of the experiment. The fish was weighed to determine their growth by using the following equations *(*Kader, Koshio, Ishikawa, Yokoyama, & Bulbul, 2010*):*

a) Weight gain (%) = [final fish weight (g) - initial fish weight (g)] x 100

b) Specific growth rate (SGR %, day^{-1}) = [ln final fish weight (g) - ln initial fish weight (g)]/duration (60 - 90 days) x 100

c) Survival (%) = 100 x (final no. of fish/initial number of fish)

d) Feed intake (g $fish^{-1}$ 60 - 90 days) = (dry diet given - dry remaining diet recovered)/no. of fish

e) Feed efficiency ratio (FER) = live weight gain (g)/dry feed intake (g)

f) Condition factor (CF) = Weight (g)/Length $(cm)^3$ x 100

g) Hepatosomatic index (HSI, %) = weight of liver/weight of fish x 100

h) Survival rate (SR, %) = (final total number of fish/initial total number of fish)

Sample collection and composition analysis of feed and fish*:* Before each sampling, fish were starved for one day to empty their stomach content. During stocking, 25 fish was taken as initial sample. At the final sampling, five fish were taken from each replicate as whole body sample and kept at -80 °C for the analysis of whole body proximate composition. The crude protein for both feed and fish samples were determined through the Kjedahl procedure, moisture by drying method, total lipid by Soxtec extraction method and ash by ashing method. The proximate analysis was conducted by following the Association of Official Analytical Chemists (AOAC, 1990) standard methods.

Statistical analysis*:* All the data were expressed as mean±standard deviation, presented from quadruplicate values *(*n=4*)* for feeding trial samples. Data were analysed using one-way analysis of variance using SPSS ver. 21.0 *(*SPSS Inc., Chicago, IL*).* Significance differences between means (P<0.05) were evaluated by Duncan's New Multiple Range Test.

3. Results and Discussion

Nutritional composition in diets*:* Tables 2 and 3 summarize the proximate composition of the diets supplemented with crude leaves and extract, respectively. Based on the results, both experimental diets were formulated nearly isonitrogenous and isolipidic. There were no changes in crude ash with the increase of *C. caudatus* level in each diet.

Growth, feed intake and survival parameters*:* Table 4 and 5 below illustrate the data on the growth performance, feed intake and survival of juvenile *M. nemurus*. In both experiments, there were significant differences recorded for final weight, weight gained and specific growth rate between the treatment groups (P < 0.05). Generally, fish fed the diets supplemented with *C. caudatus* crude leaves or extract showed higher weight gains compared to the control. Significantly higher weight gains (WG%) were found at level 0.50% of herb leaves and 0.05% of leaves extract. However, the values decreased beyond this level.

The survival rate was not significantly different among the treatment groups (P>0.05) for both experiments. The survival rate for Experiment 1 was about 90 to 95% and around 71 to 77% in Experiment 2. However, the feed intake was significantly different (P<0.05) among the treatments supplemented with crude leaves (Experiment 1). But no significant differences (P>0.05) were observed for treatment groups using herb extract (Experiment 2). Therefore, it is evident that supplementation of *C. caudatus* either in crude leaves or extract form helps to improve the growth performance of juvenile bagrid catfish, *M. nemurus* without affecting their survival.

The present study showed two applications of *C. caudatus* incorporated into diets in the form of crude leaves or leaves extract. From the result, there were no significant changes of feed composition in both experiments (Experiments 1 and 2). Therefore, the type of herb did not influence the nutritional value of the feed. This is probably due to the small amount of *C. caudatus* used for both experiments. As a result, the nutrient contents of the formulated feed were similar or only differed slightly especially for crude ash.

Table 2. Proximate composition of diet using crude leaves (Experiment 1) (%)

Parameter	Diet group (crude leave)				
	0.00% CL	0.50% CL	1.00% CL	1.50% CL	2.00% CL
Moisture	7.98	7.28	7.31	7.01	6.67
Crude protein	39.23	38.16	38.67	39.43	39.70
Crude lipid	11.78	13.56	13.07	11.52	12.77
Crude ash	11.60	12.25	12.02	11.28	11.64

Table 3. Proximate composition of diet using crude extract (Experiment 2) (%)

Parameter	Diet group (crude leave)				
	0.00% CE	0.01% CE	0.05% CE	0.10% CE	0.20% CE
Moisture	10.89	10.98	9.15	9.22	10.08
Crude protein	38.79	38.86	39.6	39.8	39.47
Crude lipid	8.72	8.89	9.31	9.61	9.33
Crude ash	10.98	11.08	11.25	11.28	10.98

Table 4. Growth, feed intake, and survival parameters in juvenile bagrid catfish fed with *C. caudatus* leaves supplemented diets for 60 days

Parameters	Diet groups (crude leave)				
	0% CL	0.50% CL	1.00% CL	1.50% CL	2.00% CL
In wt[1]	3.05±0.39	3.05±0.39	3.05±0.39	3.05±0.39	3.05±0.39
Fn wt[2]	14.51±2.51^{a}	16.33±3.07^{c}	15.50±2.47^{bc}	14.95±2.26^{ab}	14.93±2.59^{ab}
WG[3]	11.46±2.51^{a}	13.28±3.07^{c}	12.45±2.47^{bc}	11.90±2.26^{ab}	11.88±2.59^{ab}
SGR[4]	2.57±0.29^{a}	2.76±0.32^{c}	2.68±0.27^{bc}	2.62±0.26^{ab}	2.61±0.29^{ab}
FI[5]	11.58±0.41^{a}	13.66±0.76^{c}	12.99±0.40^{bc}	12.18±0.43^{ab}	12.18±0.51^{ab}
SR[6]	93.62±4.53	94.44±3.70	93.52±1.85	94.67±1.96	89.81±8.21

Abbreviations used: [1]In wt: initial weight (g), [2]Fn wt: final weight (g), [3]WG: percent weight gain (%), [4]SGR: specific growth rate (%day^{-1}), [5]FI: feed intake (g dry diet fish^{-1} for 60 days), SR[6]: Survival rate (%). *Values are means of quadruplicate groups±SD. Within a row, means with different superscripts indicates significant different (P<0.05). The absence of letters indicates no significant difference between treatments.

Table 5. Growth, feed intake and survival parameters in juvenile bagrid catfish fed with *C. caudatus* extract at different levels for 90 days

Parameters	Diet groups (Crude extract)				
	0.00% CE	0.01% CE	0.05% CE	0.10% CE	0.20% CE
In wt[1]	4.45±0.71	4.45±0.71	4.45±0.71	4.45±0.71	4.45±0.71
Fn wt[2]	16.66±2.15^{a}	18.93±3.98^{b}	22.96±4.21^{c}	22.83±4.28^{c}	19.87±3.89^{b}
WG[3]	12.25±2.15^{a}	14.50±3.98^{b}	18.51±4.21^{c}	18.38±4.28^{c}	15.42±3.89^{b}
SGR[4]	2.81±0.22^{a}	3.00±0.34^{b}	3.33±0.31^{c}	3.32±0.32^{c}	3.08±0.33^{b}
FI[5]	9.53±0.98	10.47±1.00	10.80±0.80	10.38±0.95	9.61±0.71
SR[6]	74.00±5.16	71.00±5.03	77.00±6.83	76.00±11.78	77.00±6.83

Abbreviations used: [1]In wt: initial weight (g), [2]Fn wt: final weight (g), [3]WG: percent weight gain (%), [4]SGR: specific growth rate (%day^{-1}), [5]FI: feed intake (g dry diet fish^{-1} for 60 days), SR[6]: Survival rate (%). *Values are means of quadruplicate groups±SD. Within a row, means with different superscripts indicates significant different (P<0.05). The absence of letters indicates no significant difference between treatments.

These results are aligned with other studies using herb inclusion as a supplement in fish feed either in extract or crude herb form (Harikrishnan *et al.*, 2011; Putra, Santoso, Lee, & Nan, 2013; Shalaby, Khattab, & Abdel Rahman, 2006; Talpur & Ikhwanuddin, 2013). The patterns of growth performance and survival in this study are similar to those reported by Gabriel *et al.* (2015) who supplemented *Aloe vera* in tilapia (GIFT). Furthermore, the survival range of *M. nemurus* in the current study is higher (90-95% for crude leaves and 71-77% for leaves extract) than the survival recorded by Islam *et al.* (2014) for Thai pangas using a commercial growth promoter (65-90%).

Since *C. caudatus* contains active components or phytobiotics such as phenolic compounds, vitamins and minerals (Citarasu, 2010; Dian-Nashiela, Noriham, Noorain, & Azizah, 2015; Moshawih *et al.*, 2017), it could enhance the growth performance of *M. nemurus* in this study. Even though the mechanisms of phytobiotic in *C. caudatus* are not yet clear in affecting growth performance of this fish, the final body weight and weight gained was better compared to the control groups. Therefore, supplementation of *C. caudatus* may have supported the growth of *M. nemurus* in this study.

Interesting findings of two optimum levels of *C. caudatus* were revealed during this study. By using crude leaves, the highest weight gain of *M. nemurus* fingerlings recorded from 0.50% treatment groups. Meanwhile, for leaves extract, the highest weight gain was recorded by fish in 0.05% treatment groups. The body weight of fish was reduced beyond these levels. So that, we suggest the optimum level of crude leaves and leaves extract of *C. caudatus* in fish supplementation to be 0.50% and 0.05% respectively.

According to Amna, Nooraain, Noriham, Azizah, and Husna (2013), *C. caudatus* is listed in Class Five, the lowest toxicity class based on the Organisation for Economic Cooperation and Development (OECD) guidelines. Moreover, an acute toxicity test found that *C. caudatus* ethanolic extract at levels of 2g/kg and 5g/kg were safe in rats. However, we still need to determine the favorable level of this herb in feed because most medicinal herbs may be toxic for certain animals when high concentrations are applied and finally may affect their growth and survival for certain durations. Properly tested feed additives are vital, so a safe dosage can be applied in feed nutrition and the farmers can get maximum benefit from their investment (Islam *et al.*, 2014).

In aquaculture, herbs used for fish feed are varied and depend on the response of the fish (Reverter, Bontemps, Lecchini, Banaigs, & Sasal, 2014). For example, Punitha *et al.* (2008) suggested 400 ppm (0.04%) of herbal mixture extract was optimal to increase the survival, growth and immune responses of *Ephinephelus tauvine*. However, Harikrishnan *et al.* (2011) studied green tea extracts and found that 0.01% and 0.1% concentrations in 100 g of feed enhanced non-specific humoral, cellular immunity and disease resistance in *E. brunues*. Putra *et al.* (2013) suggested a 1% supplementation of *Sauropus androgynous* extract improved the appetite, growth and food utilization of *E. coioides*. The levels of 2.5% and 5% of this herb reduced the growth performance. Therefore, these different levels applied to groupers showed a specific dosage of herbs application in diet.

Whole body proximate analysis and biometric indices*:* The whole body proximate composition of fish from two experiments are shown in Table 6 and 7. It was found that

Table 6. Whole body analysis of bagrid catfish fed with different diets for 60 days

Parameter	Diet groups (crude leave)				
	0.00% CL	0.50% CL	1.00% CL	1.50% CL	2.00% CL
Moisture	71.94±0.58	71.70±0.19	72.00±0.30	71.81±0.45	72.00±0.50
Crude protein	15.70±0.79	16.47±1.56	16.74±1.90	15.90±2.74	15.83±1.77
Crude lipid	7.24±0.94	7.00±0.96	7.77±0.67	8.12±1.05	7.22±0.35
Crude ash	3.68±0.89	3.86±0.17	3.78±0.12	3.80±0.26	3.81±0.12
CF[1]	0.68±0.05[b]	0.64±0.05[a]	0.69±0.05[b]	0.69±0.05[b]	0.71±0.06[c]
HSI[2]	1.12±0.10	1.15±0.09	1.18±0.12	1.11±0.07	1.13±0.14

[1]CF: condition factor (%). [2]HSI: hepatosomatic index. *Values are means of quadruplicate groups±SD. Within a row, means with different superscripts indicates significant different (P<0.05). The absence of letters indicates no significant difference between treatments.

Table 7. Whole body analysis of bagrid catfish fed with different diets for 90 days

Parameter	Diet groups (crude extract)				
	0% CE	0.01% CE	0.05% CE	0.10% CE	0.20% CE
Moisture	72.85±1.22	72.94±1.66	71.96±1.95	70.74±1.80	72.52±1.80
Protein	15.18±0.65	15.12±0.67	15.57±0.48	15.67±0.55	14.96±0.19
Lipid	6.48±0.23[a]	7.00±0.29[ab]	7.80±1.47[b]	7.18±0.85[ab]	7.93±0.38[b]
Ash	4.09±0.17	4.08±0.06	4.03±0.10	4.03±0.33	3.79±0.30
CF[1]	0.65±0.08[b]	0.65±0.07[b]	0.59±0.07[a]	0.61±0.08[a]	0.64±0.08[b]
HSI[2]	1.08±0.11	0.98±0.21	0.93±0.16	1.26±0.20	0.96±0.12

[1]CF: condition factor (%). [2]HSI: hepatosomatic index. *Values are means of quadruplicate groups±SD. Within a row, means with different superscripts indicates significant different (P<0.05). The absence of letters indicates no significant difference between treatments.

fish fed with *C. caudatus* crude leaves (Experiment 1) had higher protein content than the control groups. Increasing and decreasing levels of each parameter was seen among the treatments. In Experiment 2 using *C. caudatus* leaves extract, the protein content was higher than the control for the treatment at 0.05% and 0.10%. However, there were no significant differences (P>0.05) among the treatments for whole body analyses in both experiments.

The results of the dietary treatments using crude leaves or leaves extract had no significant influence on the whole body proximate composition of *M. nemurus* fingerlings. Although the lipid content in Experiment 2 (leaves extract) was different among the treatment groups, the differences were not significant. In contrast, the condition factor (CF) data showed significant differences (P<0.05) among the treatment groups for both Experiments 1 and 2.

Proximate analyses of whole body samples showed no significant differences (P>0.05) among the treatments for fish treated with *C. caudatus* crude leaves. The same result was also obtained for fish treated with *C. caudatus* extract. The dietary treatments using crude leaves or leaves extract had no significant influence on the whole body proximate composition of *M. nemurus* fingerlings. Even though the amount of protein was reported to be 4.22±0.12g per fresh kg weight of *C. caudatus* (Andarwulan *et al.*, 2012), only a small amount of herb was used in the feed formulation thus the protein content in the fish was not affected. However, according to Ji *et al.* (2009), high protein retention was observed when a herbal mixture was used for red sea bream feed. Besides that, the lower plasma triglyceride and high plasma HDL-CHO (high density lipoprotein cholesterol) levels in herbal mixture diets improves fatty acid utilization (Ji *et al.*, 2007). A study by Turan, Ganpolat, and Aygen (2016) on African catfish feed supplementation with *Laurus noblis* (Bay Laurel) also resulted in a significantly higher protein concentration compared to the control.

From this study, the condition factor (CF) showed plateau data, but there were significant differences among the treatment groups in Experiments 1 and 2. This is probably due to the significantly higher weight and length observed in fish treated with *C. caudatus* either by crude leaves or extract. This is also may be due to the calcium and anti-oxidant content in this herb which affected the fish condition factor in this study. As mentioned by Cheng *et al.* (2015), their study on ovariectomised rats as a model for postmenopausal osteoporosis also produced a positive result for groups supplemented with 500mg/kg *C. caudatus* aqueous extract. They found that *C. caudatus* helped to restore the bone structural parameter to a normal level.

4. Conclusions

Supplementation of *C. caudatus* enhanced growth performance of *M. nemurus* in this study. The level of the herb leaves to be incorporated in feed formulation is dependent on its form. For crude leaves, 0.50% of herb

inclusion was found effective to stimulate bagrid catfish growth while for leaves extract, the level was 0.05%. The condition factors for both experiments were significantly different among their treatment groups. No significant differences were observed for whole body analyses of fish supplemented with crude leaves or crude extract and supplementation also did not affect the fish survival. From the result obtained, we can suggest *C. caudatus* as a growth promoter for *M. nemurus* fingerlings. However, further analysis and tests should be conducted for the physiological study of the fish.

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