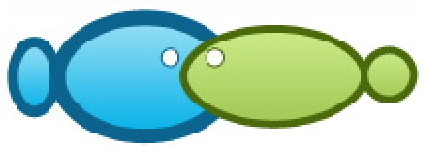

Dietary methionine optimization for the growth performance, nitrogen utilization and proteolytic enzymes activities of snakehead (*Channa striata*) fingerlings

[1]Narissara Suratip, [2]Rungkan Klahan, [1]Siripavee Charoenwattanasak, [1]Bundit Yuangsoi

[1] Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand; [2] Faculty of Agricultural Technology, Phetchaburi Rajabhat University, Phetchaburi, Thailand. Corresponding author: B. Yuangsoi, bundyu@kku.ac.th

Abstract. The objective of the study was to find the optimum methionine level for the snakehead fish (*Channa striata*) fingerlings (initial weight = 5.20±0.06 g fish^{-1}). The basal diet (without methionine supplementation) contained 0.79% of methionine (M1), and four experimental diets contained graded levels of methionine: 0.95% (M2), 1.10% (M3), 1.27% (M4), and 1.43% (M5). All five diets were isonitrogenous, isolipidic and contained a fixed cystine level of 0.62%. All fish were hand fed to satiation three times daily for eight weeks in four replicates. There were no significant differences in the survival rate and feed intake among the five groups ($p > 0.05$). However, weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio were significantly higher in M4 and M5 groups than in other three groups ($p < 0.05$). The highest protein utilization, N retention, and protein productive values were observed on the M4 group. Polynomial regression analysis of the specific growth rate indicated that the optimal dietary methionine requirement is 1.25% (2.98% of dietary protein). Whole body protein and methionine composition were significantly higher in M3-M5 groups than in M1 and M2 groups ($p < 0.05$). Specific trypsin activity was highest in groups M4 and M5 ($p < 0.05$). There was no evidence of histological alteration in the intestines of any of the five dietary groups. Our results indicate that dietary methionine levels between 1.10% (M4) and 1.30% (M5) promoted the growth rate, feed conversion ratio, nitrogen utilization, N retention, protein productive value, whole body protein content and amino acid composition, and increased the specific trypsin activity in snakehead fingerlings.
Key Words: optimum, methionine, growth performance, protein productive.

Introduction. Aquaculture has expanded rapidly due to the growing global population. In 2016, aquaculture accounted for 47 percent of the total global fish production. Snakehead (*Channa striata*), a carnivorous freshwater fish, comprises a minor proportion of the total global aquaculture, but at a yearly production of about 16,214 tons (FAO 2018) it has considerable economic value, especially in Southern and Southeastern Asia (Wattanakul et al 2016). In Thailand, the traditional snakehead fish culture feed was primarily based on so-called 'trash fish', comprising low value fish or small-size fish, often mixed with rice bran (Thongrod 2007). This type of feeding often causes water quality problems due to excess of leftover feed. In addition, this type of feed is often kept under unsuitable storage conditions, which can have strong negative effects on the feed quality and nutrient content, resulting in increased disease prevalence and high feed conversion rate. Consequently, a number of farmers switched to the high-protein formulated pellet feed produced for reared snakehead fingerling (Rachmansyah et al 2009). This feed, which contains > 40% of crude protein, proved to be cost-effective and high-quality feed, as well as reduce other problems such as water quality, nutrient leaching, and improve the digestibility of the feed.

A feed that meets protein and amino acid requirement of target species is necessary for obtaining high-quality fish meat (Santiago & Lovell 1988; Lall & Dumas 2015). Regarding the snakehead diet, guidelines suggest that the protein content should

range 40-55% and crude lipid 8-19% diet (Mohanty & Samantaray 1996; Samantaray & Mohanty 1997; Munir et al 2016; Hua et al 2019), but specific amino acid requirements remain poorly understood.

Methionine (Met) plays a vital role in protein synthesis; it is a precursor of S-adenosylmethionine (SAM) and a methyl group donor for all other sulfur-containing amino acids (cysteine, homocysteine, and taurine) (Brosnan & Brosnan 2006). As such it is the first limiting essential amino acid (EAA) in fish feed. There is little information on dietary methionine requirements of snakehead fingerlings. Hien et al (2018) reported that adding L-Met to 11.9 g Met kg^{-1} diet or 28.4 g kg^{-1} protein could provide the optimal weight gain, growth rate and protein efficiency ratio in snakehead fingerlings. For comparison, Met requirements in other carnivorous species are 1.19% of dry diet for juvenile cobia (*Rachycentron canadum*) (Zhou et al 2006), 8.0-9.1 g kg^{-1} for European sea bass (*Dicentrarchus labrax*) (Tulli et al 2010), and 1.82% for juvenile turbot (*Scophthalmus maximus*) (Gao et al 2019). Met deficiency is known to cause poor growth and reduced feed efficiency in a number of fish species: Atlantic salmon (*Salmo salar*) (Opstvedt et al 2003), juvenile European sea bass (*D. labrax*) (Tulli et al 2010), juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*) (Li et al 2009a), and immature rainbow trout (*Oncorhynchus mykiss*) (Séité et al 2018).

Sufficient Met levels can also optimize the activity of digestive enzymes, responsible for digestion and absorption potential in fish (Wu et al 2017). However, there are limited studies on the impact of dietary Met on the enzyme activities and intestinal histology of fingerling snakehead. Furthermore, Met deficiency significantly lowers the activities of trypsin. Gao et al (2019) found that the height of intestinal villi and microvilli in juvenile turbot (*S. maximus*) decreased after feeding it Met-deficient diet for 8 weeks.

Therefore, the aim of this study was to investigate the impact of different dietary methionine levels on the following parameters in snakehead fingerlings: 1) growth performance and nitrogen utilization, 2) chemical and amino acid composition, 3) proteolytic enzymes activities, and 4) intestinal histology.

Material and Method

Experimental diets and procedures. Four experimental diets were formulated by adding crystalline DL-methionine in the in a stepwise manner with an increment of 20%. The basal diet (without methionine supplementation) contained 0.79% of methionine (M1), M2 contained 0.95%, M3 contained 1.10%, M4 contained 1.27%, and M5 contained 1.43%. All five experimental diets were formulated to be isonitrogenous (42% crude protein (CP)), isolipidic (10% crude fat (CF)), and all contained a fixed cystine level of 0.62%. Fishmeal, poultry meal and soybean meal were used as the protein source, while fish oil and soybean oil were used as the dietary lipid source (Table 1). All ingredients were finely ground in a hammer mill, weighted, and mixed. The liquid ingredients (fish oil, soybean oil and water) were then added to dry ingredients and extruded as pellet through a 2.5 mm of the hole diameter. The pellets were dried at 60ºC for 12 h to below 10% of moisture content. The experimental diets were formulated and made at the Sakon Nakhon Rajaphat University, Thailand. The experiment was conducted from March to September 2018.

Feeding trial. Snakehead fingerlings were obtained from a fish farm in the Ayutthaya Province (central Thailand), and acclimated in a hatchery in the Khon Kaen province for a week. After the acclimation, the fingerlings were stocked into a round 500 L plastic tank at facilities of the Department of Fisheries, Khon Kaen University. They were acclimated to experimental conditions for additional two weeks, during which time they were fed daily with commercial floating pellet. Before the trial, 600 healthy fingerlings (initial body weight: 5.20±0.47 g.) were randomly distributed into 20 half-filled plastic, round tanks (500 L total volume, filled up to 250 L) at a density of 30 fish per tank. Tanks were continuously aerated to maintain the dissolved oxygen levels, and water exchange level was 30% over five days. Tanks were randomly assigned to five diet groups, ensuring four replications per group (5x4=20). Fingerlings were fed by hand to apparent satiation three times a day (7:00, 12:00 and 17:00) for eight weeks. The feed intake and bulk fish

weight were recorded every two weeks. During the trial, the water temperature ranged between 25.4 and 29.1ºC, the dissolved oxygen (DO) concentration ranged between 6.08 and 8.22 mg L^{-1}, and the ammonia nitrogen was < 0.2 mg L^{-1}.

Table 1

Dietary formulation and chemical proximate analysis of experimental diets

Ingredients (%)	*Experimental diets*				
	M1	*M 2*	*M 3*	*M 4*	*M 5*
Fish meal[a]	20	20	20	20	20
Soybean meal[b]	30	30	30	30	30
Poultry meal[c]	22	22	22	22	22
Corn meal	7	7	7	7	7
Wheat flour	11.5	11.34	11.18	11.025	10.85
Soybean oil	4	4	4	4	4
Fish oil	2	2	2	2	2
Dicalciumphosphate	1.5	1.5	1.5	1.5	1.5
Vitamin premix[d]	1	1	1	1	1
Mineral premix[e]	1	1	1	1	1
DL-methionine[f]	0	0.16	0.32	0.485	0.65
Chemical composition by analysis (%)					
Crude protein	41.57	41.07	41.52	41.47	41.30
Crude lipid	9.47	9.52	9.59	9.45	9.58

Note: [a] Fish meal was obtained from the Thai Union Feed mill Co., Ltd. (crude protein 62.87%); [b] Soybean meal was obtained from the Thai Union Feed mill Co., Ltd. (crude protein 46.93%); [c] Poultry was obtained from the Thai Union Feed mill Co., Ltd. (crude protein 65.84%); [d] Vitamin premix (mg kg^{-1} diet): vitamin A, 36000 IU; vitamin D3, 9000 IU; vitamin E, 187; vitamin K, 19; vitamin B1, 52; vitamin B2, 97; vitamin B6, 46; vitamin B12, 0.06; vitamin C, 69800; pantothenic acid, 93; inositol, 225; Niacin, 130; folic acid, 10; D-biotin, 0.45; [e] Mineral premix (mg kg^{-1} diet): $MnSO_4 \cdot H_2O$, 105; $CuSO_4 \cdot 5H_2O$, 9; $FeSO_4 \cdot 7H_2O$, 90; $ZnSO_4 \cdot H_2O$, 90; KI, 1.8; $CoCl_2$, 0.45; $MgSO_4 \cdot 7H_2O$, 19000; NaCl, 117; Na_2SeO_3, 0.15; KCl, 3600; calcium $(IO_3)2$, 219; [f] Methionine: DL-methionine, purity 99.8%. Imported from Evonik Industries AG.

Performance calculation. At the end of experiment, fish were counted and weighted in groups in order to determine the growth performance and feed utilization according to the equations described below:

Survival (%) = ((100/fish quantity stocking) * fish quantity at harvest) (1)

Weight gain, WG (%) = $((W_f - W_i)/W_i) \times 100$ (where W_f is final body weight (g) and W_i is initial body weight (g)) (2)

Daily feed intake, DFI (% day^{-1}) = 100 × (Total feed intake (g) / ((W_f (g $fish^{-1}$) + W_i (g $fish^{-1}$))/2 × feeding day)) (3)

Average daily gain, ADG (g day^{-1}) = body weight gain/feeding day (4)

Specific growth rate, SGR (% day^{-1}) = ((Ln W_f - Ln W_i)/days) × 100 (5)

Feed conversion ratio, FCR = feed intake (g)/weight gain (g) (6)

Protein efficiency ratio, PER = wet weight gain (g)/ protein ingested (g) (7)

Nitrogen intake, NI (g *N* kg^{-1} MBW day^{-1}) = (feeding intake (g)× N content of the diet)/ (MBW × feeding day) (8)
(where MBW = $0.5 \times (W_f^{0.75} + W_i^{0.75})$

Nitrogen gain, NG (g *N* kg^{-1} MBW day^{-1}) = (W_f (g)× final body *N* (g)- W_i (g)× initial body *N* (g)/(MBW × feeding day) (9)

Nitrogen retention, NR (% *N* intake) = 100 × (*N* gain (g)/*N* intake (g)) (10)

Protein productive value, PPV (%) = 100 × (W_f × final body protein (g) - W_i × initial body protein)/ protein intake (g)) (11)

Hepatosomatic index, HSI (%) = 100 × (hepatic weight (g $fish^{-1}$)/body weight (g $fish^{-1}$)) (12)

Condition factor, CF (g/cm^{-3}) = (100 × body weight (g $fish^{-1}$))/ (body length$(cm)^3$) (13)

Sample collection. At the end of the trial, the snakehead fingerlings were allowed to fast for 16 hours, and 10 fish from each tank were individually weighted. After anesthetization with clove oil (7.4 mL L^{-1}, clove oil: ethanol = 1:4) (Rezende et al 2017),

the entire intestines were collected from five fish per each tank and immediately frozen at -20ºC for the proteolytic enzyme activity analysis. Additionally, posterior intestine samples from three fish per each tank were selected to be fixed in 10% formalin solution for the histological analysis. Twenty fingerlings were collected from each tank to measure the whole-body nutrient composition and amino acid accumulation.

Nutrient composition and amino acid analysis. For the nutrient composition, whole bodies of 10 fish from each tank were pooled, dried at 105°C and ground until they became homogenous. Whole body samples and diets were analyzed for dry matter (AOAC 2005). The total protein was determined following the method of Kjeldahl. The total lipid was measured by ether extraction using the Soxhlet method (Buchi B810, Switzerland). Gross energy content was determined using an Automatic Bomb Calorimeter (AC-500, LECO, United States). The total ash content was determined by burning samples at 550ºC in a muffle furnace. The amino acid hydrolysis was performed according to GB/T 18246-2000, the solution was then filtered through a 0.22 µm PTFE filter for amino acid analysis by reversed-phase high-performance liquid chromatography (RP-HPLC). Amino acid composition of the experimental diets is presented in Table 2.

Table 2

Amino acid composition of the experimental diets (%)

Amino acids	*Experimental diets*				
	M1	*M2*	*M3*	*M4*	*M5*
EAA[1]					
Methionine	0.78	0.86	0.96	1.10	1.30
Arginine	2.7	2.7	2.6	2.7	2.7
Lysine	2.3	2.3	2.3	2.3	2.3
Threonine	1.7	1.7	1.6	1.7	1.7
Histidine	0.9	0.9	0.9	0.9	0.9
Isoleucine	1.8	1.9	1.8	1.8	1.9
Leucine	3.0	3.0	3.0	3.0	3.1
Phenylalanine	1.9	1.9	1.8	1.9	1.9
Valine	2.0	2.0	2.0	2.0	2.0
NEAA[2]					
Alanine	2.4	2.4	2.3	2.4	2.4
Aspartic acid	4.0	4.0	3.9	4.0	4.2
Glutamine	5.9	5.8	5.8	5.9	5.9
Glycine	2.8	2.8	2.8	2.8	2.9
Serine	2.0	2.0	2.0	2.0	2.1
Tyrosine	1.2	1.1	1.1	1.1	1.2
Cystine	0.62	0.61	0.62	0.62	0.62
Proline	2.2	2.3	2.2	2.2	2.2
SUM AA[3]	37.42	37.41	36.72	37.32	38.02

Note: [1] EAA, essential amino acids; [2] NEAA, non-essential amino acids; [3] SUM AA, total amino acids.

Proteolytic enzymes activities analysis. Whole digestive tracts were homogenized on ice in phosphate buffer 8.2 using a homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 min at 4ºC. The crude enzyme extract was collected from the supernatant for enzymatic assays using a UV/VIS spectrophotometer (Biochrome Libra S80, Biochrome Ltd., Cambridge, UK). The concentration of protein was analyzed by the Lowry method (Lowry et al 1951) and expressed in g dL^{-1}. The total protease determination was conducted using azocasein as a substrate, trypsin determination using N_{α}-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA), and chymotrypsin determination using N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide (Rungruangsak-Torrissen et al 2006). The total protease was expressed in g dL^{-1}; trypsin and chymotrypsin were expressed as µmol p-nitroaniline produced per hour per mg protein.

Intestinal histology. The posterior intestinal samples fixed in 10% formalin solution were dehydrated in ethanol, embedded in paraffin, cut with a microtome at 4-µm sections, and stained with hematoxylin and eosin (H&E) according to the standard histology procedures. Tissue slides were digitally photographed with a light microscope (Nikon Eclipse Ci) equipped with a CCD camera and NIS-elements D software. Intestine diameter was measured from side-to-side of serosa (SE), villus height was measured from the lowest point between two longitudinal villi to its tip (Peng et al 2013). Width and density of villi were also measured.

Statistical analyses. Statistical analyses were performed using SPSS version 26.0 software. All data were analyzed using ANOVA and Duncan multiple comparison tests. The significance was set at $p < 0.05$. Data were presented as means±standard error. Second-degree polynomial regression analysis ($Y = a + bX + cX^2$) was used to estimate the optimum dietary methionine requirement of the fingerling snakehead fish based on SGR.

Results

Growth performance and nitrogen utilization. The growth performance of snakehead fingerlings fed with five different diets for eight weeks is shown in Table 3. Survival rate (SR) and daily feed intake (DFI) did not vary significantly different between diets ($p > 0.05$). Final body weight (FBW) and weight gain (WG) of M4 and M5 groups were significantly higher than those of M1 and M2 groups ($p < 0.05$). Likewise, average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) of the M4 and M5 groups were significantly ($p < 0.05$) better than in other groups (M1, 2, and 3). Polynomial regression analysis of SGR estimated that methionine optimum for snakehead fingerlings was 1.25% of diet, or 2.98% of dietary protein (Figure 1).

Table 3

Growth performance and nitrogen utilization of snakehead fingerlings fed with different methionine levels for 8 weeks

Parameters	*Experimental diets*					
	M1	*M2*	*M3*	*M4*	*M5*	*p-value*
IBW[1]	5.20±0.06	5.20±0.06	5.20±0.06	5.20±0.06	5.20±0.06	1.000
FBW[2]	13.66±1.02[a]	14.50±0.53[a]	15.43±0.88[ab]	17.53±1.11[b]	17.24±0.46[b]	0.021
SR[3]	90.0±4.3	88.4±1.0	85.8±3.4	85.0±5.0	84.2±4.4	0.817
WG[4]	162.6±19.6[a]	178.8±10.2[a]	196.6±16.9[ab]	237.1±21.4[b]	231.6±8.9[b]	0.021
DFI[5]	2.51±0.10	2.52±0.13	2.54±0.06	2.49±0.02	2.47±0.08	0.966
ADG[6]	0.015±0.02[a]	0.017±0.01[a]	0.018±0.02[ab]	0.022±0.02[b]	0.022±0.01[b]	0.020
SGR[7]	1.71±0.13[a]	1.83±0.07[a]	1.93±0.10[ab]	2.16±0.11[b]	2.14±0.05[b]	0.020
FCR[8]	1.61±0.11[a]	1.50±0.07[ab]	1.45±0.04[ab]	1.30±0.04[b]	1.29±0.05[b]	0.023
PER[9]	1.51±0.10[a]	1.60±0.07[a]	1.65±0.05[ab]	1.84±0.06[b]	1.86±0.07[b]	0.015
NI[10]	0.32±0.01	0.33±0.02	0.34±0.01	0.34±0.01	0.34±0.01	0.836
NG[11]	0.06±0.01	0.04±0.01	0.05±0.01	0.10±0.01	0.10±0.01	0.056
NR[12]	18.7±2.7	21.9±1.8	24.5±2.2	30.4±3.3	30.0±2.0	0.029
PPV[13]	37.3±4.2	38.2±4.9	37.8±0.8	37.5±2.2	39.0±3.2	0.996
HSI[14]	1.70±0.22	1.74±0.12	1.72±0.12	1.68±0.13	1.66±0.11	0.996
CF[15]	0.87±0.01[a]	0.94±0.02[ab]	0.94±0.03[ab]	0.96±0.03[b]	0.99±0.03[b]	0.038

Note: [1] IBW = initial body weight (g $fish^{-1}$); [2] FBW = final body weight (g $fish^{-1}$); [3] SR = survival rate (%); [4] WG = weight gain (%); [5] DFI = daily feed intake (%); [6] ADG = average daily gain (g day^{-1}); [7] SGR = specific growth rate (% day^{-1}); [8] FCR = feed conversion ratio; [9] PER = protein efficiency ratio (%); [10] NI = nitrogen intake (g N kg^{-1} MBW day^{-1}); [11] NG = nitrogen gain (g N kg^{-1} MBW day^{-1}); [12] NR = nitrogen retention (% N intake); [13] PPV = protein productive value (%); [14] HIS = hepatosomatic index (%); [15] CF = condition factor (g cm^{-3}).

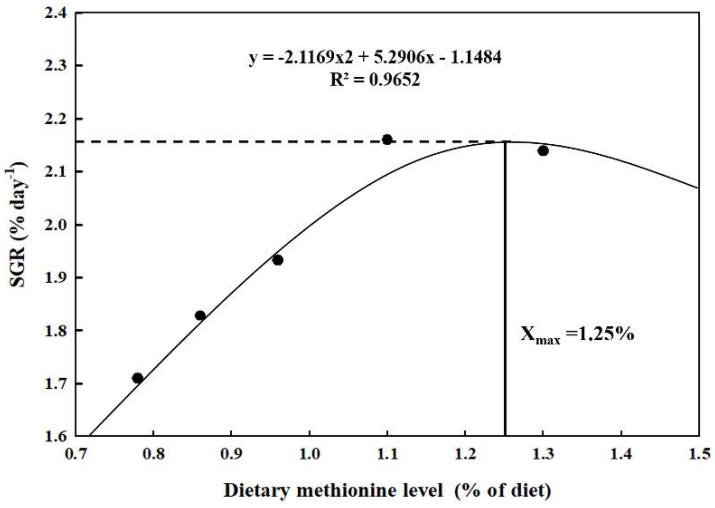

Figure 1. Second-order polynomial regression analysis of dietary methionine levels and specific growth rate (% day^{-1}) of snakehead fingerlings.

Nitrogen intake was unaffected by the dietary treatment. Nitrogen gain and nitrogen retention of the M4 and M5 groups was somewhat higher than in other groups (M 1, 2, and 3), but differences were not significant ($p > 0.05$) among the five groups.

In addition, protein productive value (PPV) was not significantly different ($p > 0.05$) between the five groups, but the highest value was observed in the M3 group, whilst the lowest value was observed in the M1 group. Fish in the M1 group had the highest HSI values (Hepatosomatic Index), but differences were not significant. Condition factor (CF) of the M4 and M5 groups was significantly improved in comparison with the group M2 ($p < 0.05$).

Chemical and amino acid composition. The impact of experimental diets on the proximate and amino acid composition of the whole body of snakehead fingerlings is presented in Table 4. Crude protein content in the whole body increased with increasing methionine levels, and M3-M5 groups had significantly higher values than the M1 group ($p < 0.05$). However, dietary methionine level did not significantly affect the crude lipid content ($p > 0.05$). As regards the essential amino acids (EAA), methionine concentration significantly increased in the M5 group, while arginine significantly increased in the M1 and M2 groups. As regards the non-essential amino acids (NEAA), the M3 group exhibited lower alanine levels than other groups ($p < 0.05$).

Proteolytic enzyme activities. The proteolytic enzyme activities are shown in Table 5. There were no differences in the activities of protease, chymotrypsin and the T/C ratio among the five diet groups. The M4 and M5 groups exhibited the highest activity of trypsin, while the M1 group exhibited the lowest value.

Intestinal histology. Histological sections of the posterior intestine of snakehead fingerlings from the five groups are shown in the Figure 2. The M1 group exhibited a non-significantly decreased height of villi in comparison to other groups. On the other hand, the fish fed M5 diet exhibited thinner villi, but differences were also insignificant among the groups. Villi density was improved with increasing dietary methionine concentration ($p > 0.05$) (Figure 3).

Table 4

Chemical composition (% dry matter basis; mean±SE; n = 3) and amino acid content (mean±SE; n = 2) in the whole body of snakehead fingerlings fed different levels of methionine for 8 weeks

Parameters	*Experimental diets*					
	M1	*M2*	*M3*	*M4*	*M5*	*p-value*
Chemical composition (% dry matter basis)						
Crude protein	74.7±0.31[a]	75.2±0.16[ab]	75.5±0.17[b]	75.6±0.09[b]	75.6±0.05[b]	0.022
Crude lipid	9.62±0.07	9.64±0.14	9.64±0.26	9.67±0.10	9.68±0.03	0.997
EAA						
Methionine	1.27±0.01[ab]	1.26±0.02[a]	1.27±0.01[ab]	1.30±0.01[bc]	1.31±0.01[c]	0.026
Arginine	3.30±0.02[c]	3.30±0.02[c]	3.13±0.01[a]	3.23±0.02[b]	3.26±0.02[bc]	0.004
Lysine	3.76±0.10	3.85±0.14	3.65±0.11	3.76±0.09	3.80±0.10	0.751
Threonine	2.25±0.06	2.26±0.02	2.17±0.06	2.22±0.06	2.24±0.04	0.778
Histidine	1.06±0.02	1.09±0.01	1.05±0.01	1.07±0.01	1.09±0.02	0.217
Isoleucine	2.12±0.05	2.13±0.09	2.04±0.08	2.05±0.06	2.15±0.03	0.647
Leucine	3.59±0.03	3.65±0.00	3.51±0.02	3.57±0.06	3.64±0.05	0.177
Phenylalanine	2.13±0.02	2.15±0.00	2.09±0.02	2.14±0.03	2.15±0.04	0.486
Valine	2.32±0.10	2.34±0.12	2.24±0.10	2.26±0.09	2.34±0.05	0.890
NEAA						
Alanine	3.52±0.03[b]	3.51±0.04[b]	3.33±0.00[a]	3.45±0.01[ab]	3.50±0.05[b]	0.046
Aspartic acid	4.92±0.04	4.98±0.02	4.78±0.04	4.85±0.06	4.93±0.06	0.167
Glutamine	6.88±0.47	7.02±0.43	6.64±0.35	6.87±0.29	6.96±0.38	0.960
Glycine	4.40±0.25	4.39±0.21	4.05±0.13	4.29±0.16	4.31±0.22	0.722
Serine	2.24±0.14	2.26±0.11	2.16±0.13	2.24±0.13	2.23±0.08	0.978
Tyrosine	1.40±0.06	1.39±0.13	1.38±0.08	1.43±0.04	1.45±0.06	0.948
Cystine	0.44±0.01	0.44±0.01	0.43±0.02	0.44±0.01	0.44±0.01	0.994
Proline	2.79±0.04	2.77±0.07	2.65±0.16	2.74±0.07	2.73±0.06	0.846

[*] Different superscripts indicate significant differences between values in one row.

Table 5

Intestinal enzyme activity of snakehead fingerlings fed different levels of methionine for 8 weeks (mean±SE; n = 3)

Parameters	*Experimental diets*					
	M1	*M2*	*M3*	*M4*	*M5*	*p-value*
Protease[1]	0.23±0.00	0.24±0.01	0.23±0.01	0.23±0.02	0.23±0.01	0.988
Trypsin[2]	57.09±2.3[a]	60.42±1.68[ab]	63.56±0.86[bc]	66.99±1.27[c]	67.01±1.51[c]	0.005
Chymotrypsin[3]	49.63±0.83	49.73±1.27	49.08±2.14	50.73±0.70	50.60±1.17	0.885
T/C ratio[4]	1.15±0.06	1.22±0.06	1.30±0.06	1.32±0.04	1.33±0.06	0.197

Note: [1] Protease activity; [2] Trypsin activity; [3] Chymotrypsin activity: (μmol *p*-nitroanilide/mg protein); [4]Trypsin/Chymotrypsin ratio.

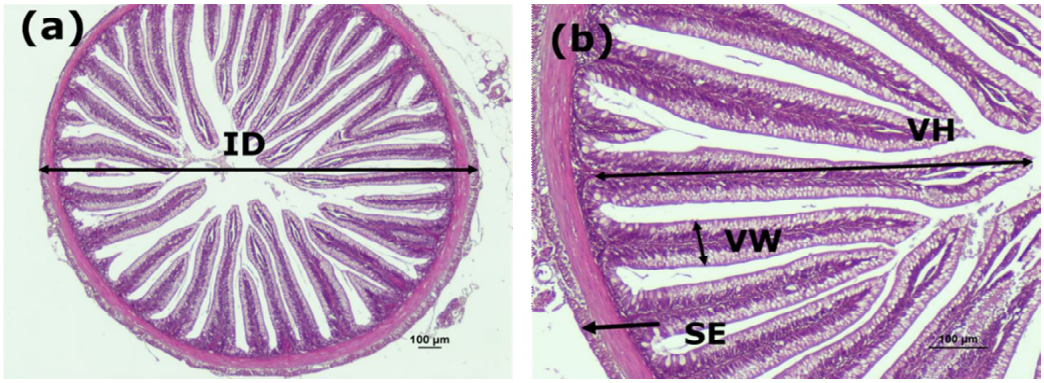

Figure 2. Light microscopic photographs of the posterior intestine cross-section of fingerling snakehead fed the control diet (M1 group). (a) The entire cross-section (4x), ID = Intestinal diameter, (b) Detail (10x), VH = villus height; VW = villus width; SE = serosa.

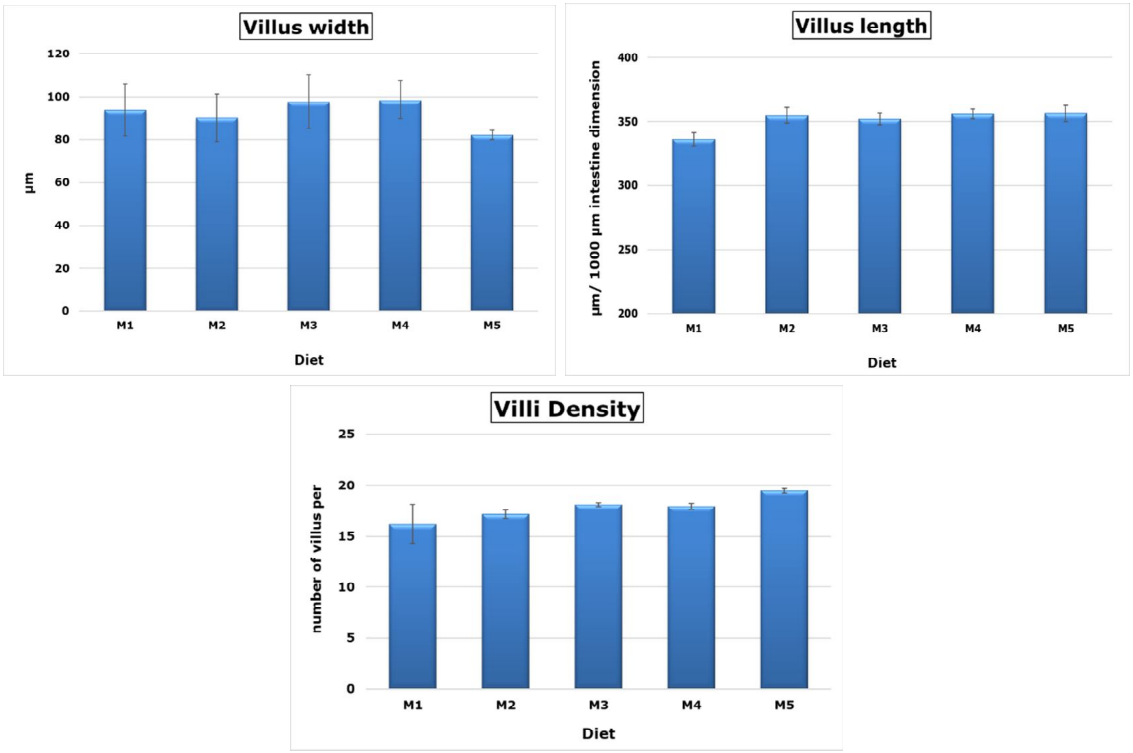

Figure 3. The length, width, and density of villi of snakehead juveniles fed graded levels of methionine for 8 weeks ($p > 0.05$).

Discussion. The results of this study demonstrated that the dietary concentration of methionine between 1.10% (M4) and 1.30% (M4) (corresponding to 2.62-3.10% methionine of dietary protein) significantly improved growth, nutrient utilization, whole-body composition and trypsin activity of snakehead fingerlings. This result was congruent with some previous studies in this species. Hien et al (2018) reported that the optimum dietary methionine requirement of snakehead fingerlings was 1.19% (corresponding to 2.48% Met of dietary protein); this level promoted growth performance, diet utilization, protein content in fish body, and hepatosomatic index. These results are also similar to the methionine requirements in other fish species, such as juvenile cobia (*Rachycentron canadum*) (2.33% Met of dietary protein, Zhou et al (2006)), juvenile turbot (*Scophthalmus maximus*) (3.31% Met of dietary protein, Ma et al (2013)), yellow croaker (*Pseudosciaena crocea*) (3.34% Met of dietary protein, Mai et al (2006)), and juvenile yellowtail kingfish (*Seriola lalandi*) (2.18% Met of dietary protein, Candebat et al (2020)). The highest protein utilization, N retention, and protein productive value were observed

in snakehead fingerlings fed a dietary methionine level of 1.10% (M4). Recent evidence shows that adequate amounts of all essential amino acids are crucial for growth, development, and health of aquatic animals (Li et al 2009b). These results highlight the necessity of a balanced diet in terms of the content of individual essential amino acids. Van Nguyen et al (2019) found that a disbalanced diet in terms of essential amino acids can lead to increased oxidation of amino acids, and consequently generate adverse effects on the growth performance. Excessive concentrations of methionine in fish diet would also be likely to result in decreased growth and feed conversion rates, but herein we did not observe any negative impacts of the highest studied methionine supplementation level (1.30%) on the snakehead fingerlings. Methionine is metabolized in the liver, so dietary methionine imbalance could affect the transcriptional regulation of genes involved in lipid metabolism in the liver, which in turn can be reflected on the liver size (HSI) and cause decreased protein utilization (Mato et al 2002; Rønnestad et al 2007; Skiba-Cassy et al 2016; Wang et al 2016). However, Coloso et al (1999) reported that HSI was not affected by the dietary methionine in juvenile Asian sea bass. The condition factor (body length-weight relationship) is often used to assess the growth stage and fatness of fish (Thomas et al 2003). In the present study, the condition factor of snakehead fish fingerlings fed with dietary methionine range between 0.86 and 1.30% significantly higher than that of fish fed was dietary methionine 0.78%. Similarly, the condition factor of juvenile Ussuri catfish (*Pseudobagrus ussuriensis*) (Wang et al 2016) and juvenile cobia (*Rachycentron canadum*) (Van Nguyen et al 2019) was positively responsive to dietary methionine levels. Our findings are in agreement with the above two studies.

We also found that increased dietary methionine levels significantly influenced the protein and methionine level in the whole body. This supports previous findings in other fish species. For example, Zhou et al (2011) reported that dietary methionine supplementation had a positive correlation with the protein content and methionine level in the whole body of juvenile black sea bream. In contrast, supplementation of lysine and methionine had no effect on the whole-body composition in juvenile black sea bream (Lu et al 2014). As for other essential amino acids, arginine and alanine were also influenced by the dietary methionine in our study. Chamruspollert et al (2002) indicated that the interrelationships of arginine and methionine observed that an availability of those mechanisms interaction had an involvement with the creatine synthesis pathway for muscle creatine of broiler chicks. Methionine is a main source of the methyl group needed for the synthesis of glycocyamine, a biological precursor of creatine biosynthesis (Arg and Gly are required for creatine synthesis) (Walker 1979). The interaction of dietary methionine and arginine also affects the growth performance of turkeys (Jankowski et al 2020). The interaction between methionine and alanine needs to be further studied.

Methionine and cysteine are important for the maintenance of intestinal function, including the digestion, absorption, and nutrient metabolism (Fang et al 2010). In the present study, trypsin activity was another parameter that was positively associated with the dietary methionine. Enhanced trypsin activity of snakehead fingerlings fed diets with supplemented methionine could explain their improved growth performance and feed utilization. Numerous studies have found that methionine intake affects the enzyme activity in fish species. Lee (2013) has reported that dietary methionine deficiency resulted in a significant decrease of pepsin, trypsin, chymotrypsin, and brush border enzymes, including aminopeptidase and alkaline phosphatase, in the Atlantic salmon. Nevertheless, effects of dietary methionine on the activities of brush border enzymes need to be further studied in snakehead. The activities of trypsin and non-specific lipase were decreased in *Sparidentex hasta* fed a diet deficient on essential amino acids (Mozanzadeh et al 2018). Herein, we found that the activities of trypsin and chymotrypsin were higher when methionine increased. Similarly, the ratio of trypsin and chymotrypsin (T/C ratio) was higher during a rapid growth phase, and lower during a slow growth phase in the Atlantic salmon (Rungruangsak-Torrissen et al 2006).

The diet is primary source of amino acids for the intestinal mucosa, which are vital for maintaining the mucosal mass and integrity (Windmueller 1982). Gao et al (2019) reported that methionine-restricted diet affected the villi and microvilli in juvenile turbot,

and that it might reduce the efficiency of nutrient absorption in the fish intestine. In the present study, we did not identify any signs of inflammatory or degenerative changes in the intestinal morphology in any of the experimental groups.

Conclusions. The present study aimed to investigate the optimum methionine level for the snakehead fingerlings. We focused on identifying the effects of dietary methionine on growth performance, nitrogen utilization, whole body composition, proteolytic enzyme activities, and intestinal histology. The results indicate that dietary methionine supplementation of 1.10% with a constant cystine level at 0.62% (corresponding to 2.62% methionine of the total dietary protein) is advantageous for the growth rate, feed conversion ratio, protein utilization, specific trypsin activity and whole-body protein content. The level of dietary methionine ranging from 0.78 up to 1.30% did not apparently affect the intestinal health of the experimental fish.

Acknowledgements. The authors would like to thank Evonik (SEA, Singapore) for providing DL-methionine and partial funding for this research, Thai Union Feedmill for providing some of the raw materials used for the diets, and Betagro for conducting the chemical composition analyses of raw materials.

References

AOAC, 2005 Official methods of analysis of AOAC International. 18th edition, AOAC International, Gaithersburg, MD, USA.

Brosnan J. T., Brosnan M. E., 2006 The sulfur-containing amino acids: an overview. Journal of Nutrition 136(6):1636-1640.

Candebat C. L., Booth M., Codabaccus M. B., Pirozzi I., 2020 Dietary methionine spares the requirement for taurine in juvenile yellowtail kingfish (*Seriola lalandi*). Aquaculture 522:735090.

Chamruspollert M., Pesti G. M., Bakalli R. I., 2002 Dietary interrelationships among arginine, methionine, and lysine in young broiler chicks. British Journal of Nutrition 88(6):655-660.

Coloso R. M., Murillo-Gurrea D. P., Borlongan I. G., Catacutan M. R., 1999 Sulphur amino acid requirement of juvenile Asian sea bass *Lates calcarifer*. Journal of Applied Ichthyology 15(2):54-58.

Fang Z., Yao K., Zhang X., Zhao S., Sun Z., Tian G., Yu B., Lin Y., Zhu B., Jia G., Zhang K., Chen D., Wu D., 2010 Nutrition and health relevant regulation of intestinal sulfur amino acid metabolism. Amino Acids 39(3):633-640.

FAO, 2018 The state of world fisheries and aquaculture 2018 - meeting the sustainable development goals. FAO, Rome, 227 pp.

Gao Z., Wang X., Tan C., Zhou H., Mai K., He G., 2019 Effect of dietary methionine levels on growth performance, amino acid metabolism and intestinal homeostasis in turbot (*Scophthalmus maximus* L.). Aquaculture 498:335-342.

Hien T. T. T., Duyen N. T. C., Van Nguyen K., Phu M. T., 2018 Dietary methionine and lysine requirement of snakehead (*Channa striata*) fingerlings. International Journal of Scientific and Research Publications 8(8):795-805.

Hua K., Koppe W., Fontanillas R., 2019 Effects of dietary protein and lipid levels on growth, body composition and nutrient utilization of *Channa striata*. Aquaculture 501:368-373.

Jankowski J., Mikulski D., Mikulska M., Ognik K., Całyniuk Z., Mróz E., Zduńczyk Z., 2020 The effect of different dietary ratios of arginine, methionine, and lysine on the performance, carcass traits, and immune status of turkeys. Poultry Science 99(2):1028-1037.

Lall S. P., Dumas A., 2015 Nutritional requirements of cultured fish: formulating nutritionally adequate feeds. In: Feed and feeding practices in aquaculture. Woodhead Publishing, pp. 53-109.

Lee B. J., 2013 Effects of dietary level of indispensable amino acids and feeding strategies on growth and biochemical responses in Atlantic salmon *Salmo salar* L. (Publication No. 10473898). Doctoral dissertation, The Ohio State University, ProQuest Dissertations Publishing, 141 pp.

Li P., Burr G. S., Wen Q., Goff J. B., Murthy H. S., Gatlin D. M., 2009a Dietary sufficiency of sulfur amino acid compounds influences plasma ascorbic acid concentrations and liver peroxidation of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*). Aquaculture 287(3-4):414-418.
Li P., Mai K., Trushenski J., Wu G., 2009b New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37:43-53.
Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., 1951 Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 193(1):265-275.
Lu J., Hua Y., Fu W., Zhou F., Yang B., Xiao J., Liu M., Shao Q., 2014 Effects of supplementation coated lysine and methionine in mixture protein diets on growth performance, digestibility and serum biochemical indices of juvenile black sea bream, *Acanthopagrus schlegelii*. Turkish Journal of Fisheries and Aquatic Sciences 14:633-642.
Ma R., Hou H., Mai K., Bharadwaj A. S., Cao H., Ji F., Zhang W., 2013 Comparative study on the effects of L-methionine or 2-hydroxy-4-(methylthio) butanoic acid as dietary methionine source on growth performance and anti-oxidative responses of turbot (*Psetta maxima*). Aquaculture 412-413:136-143.
Mai K., Wan J., Ai Q., Xu W., Liufu Z., Zhang L., Zhang C., Li H., 2006 Dietary methionine requirement of large yellow croaker, *Pseudosciaena crocea* R. Aquaculture 253(1-4):564-572.
Mato J. M., Corrales F. J., Lu S. C., Avila M. A., 2002 S-Adenosylmethionine: a control switch that regulates liver function. The FASEB Journal 16(1):15-26.
Mohanty S. S., Samantaray K., 1996 Effect of varying levels of dietary protein on the growth performance and feed conversion efficiency of snakehead *Channa striata* fry. Aquaculture Nutrition 2(2):89-94.
Mozanzadeh M. T., Yaghoubi M., Marammazi J. G., Safari O., Gisbert E., 2018 Effects of dietary protein and essential amino acid deficiencies on growth, body composition, and digestive enzyme activities of silvery-black porgy (*Sparidentex hasta*). International Aquatic Research 10(1):45-55.
Munir M. B., Hashim R., Manaf M. S. A., Nor S. A. M., 2016 Dietary prebiotics and probiotics influence the growth performance, feed utilization, and body indices of snakehead (*Channa striata*) fingerlings. Tropical Life Sciences Research 27(2):111-125.
Opstvedt J., Aksnes A., Hope B., Pike I. H., 2003 Efficiency of feed utilization in Atlantic salmon (*Salmo salar* L.) fed diets with increasing substitution of fish meal with vegetable proteins. Aquaculture 221(1-4):365-379.
Peng M., Xu W., Ai Q., Mai K., Liufu Z., Zhang K., 2013 Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.). Aquaculture 392-395:51-58.
Rachmansyah U., Palinggi N. N., Williams K., 2009 Formulated feed for tiger grouper grow-out. Aquaculture Asia Magazine 14:30-35.
Rezende F. P., Pascoal L. M., Vianna R. A., Lanna E. A. T., 2017 Sedation of Nile tilapia with essential oils: tea tree, clove, eucalyptus, and mint oils. Revista Caatinga 30(2):479-486.
Rønnestad I., Kamisaka Y., Conceição L. E. C., Morais S., Tonheim S. K., 2007 Digestive physiology of marine fish larvae: hormonal control and processing capacity for proteins, peptides and amino acids. Aquaculture 268(1-4):82-97.
Rungruangsak-Torrissen K., Moss R., Andresen L. H., Berg A., Waagbø R., 2006 Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (*Salmo salar* L.). Fish Physiology and Biochemistry 32(1):7-23.
Samantaray K., Mohanty S. S., 1997 Interactions of dietary levels of protein and energy on fingerling snakehead, *Channa striata*. Aquaculture 156(3-4):241-249.
Santiago C. B., Lovell R. T., 1988 Amino acid requirements for growth of Nile tilapia. Journal of Nutrition 118(12):1540-1546.
Séité S., Mourie A., Camougrand N., Sal B., et al, 2018 Dietary methionine deficiency affects oxidative status, mitochondrial integrity and mitophagy in the liver of rainbow trout (*Oncorhynchus mykiss*). Scientific Reports 8:10151.
Skiba-Cassy S., Geurden I., Panserat S., Seiliez I., 2016 Dietary methionine imbalance alters the transcriptional regulation of genes involved in glucose, lipid and amino acid metabolism in the liver of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 454:56-65.

Thomas J., Venu S., Kurup B. M., 2003 Length-weight relationship of some deep-sea fish inhabiting the continental slope beyond 250m depth along the west coast of India. Naga, WorldFish Center Quaterly 26(2):17-21.
Thongrod S., 2007 Analysis of feeds and fertilizers for sustainable aquaculture development in Thailand. In: Study and analysis of feeds and fertilizers for sustainable aquaculture development. Hasan M. R., Hecht T., De Silva S. S., Tacon A. G. J. (eds), FAO, Rome, Fisheries Technical Paper 497:309-330.
Tulli F., Messina M., Calligaris M., Tibaldi E., 2010 Response of European sea bass (*Dicentrarchus labrax*) to graded levels of methionine (total sulfur amino acids) in soya protein-based semi-purified diets. British Journal of Nutrition 104(5):664-673.
Van Nguyen M., Espe M., Conceição L. E. C., Le H. M., Yúfera M., Engrola S. A. D., Jordal A. E. O., Rønnestad I., 2019 The role of dietary methionine concentrations on growth, metabolism and N-retention in cobia (*Rachycentron canadum*) at elevated water temperatures. Aquaculture Nutrition 25(2):495-507.
Walker J. B., 1979 Creatine: biosynthesis, regulation, and function. Advances in Enzymology and Related Areas of Molecular Biology 50:177-242.
Wang Y. Y., Che J. F., Tang B. B., Yu S. L., Wang Y. Y., Yang Y. H., 2016 Dietary methionine requirement of juvenile *Pseudobagrus ussuriensis*. Aquaculture Nutrition 22(6):1293-1300.
Wang Z., Mai K., Xu W., Zhang Y., Liu Y., Ai Q., 2016 Dietary methionine level influences growth and lipid metabolism via GCN2 pathway in cobia (*Rachycentron canadum)*. Aquaculture 454:148-156.
Wattanakul W., Wattanakul U., Thongprajukaew K., Muenpo C., 2016 Fish condensate as effective replacer of fish meal protein in diet for striped snakehead, *Channa striata* (Bloch). Fish Physiology and Biochemistry 43:217-228.
Windmueller H. G., 1982 Glutamine utilization by the small intestine. Advances in Enzymology and Related Areas of Molecular Biology 53:201-237.
Wu P., Tang L., Jiang W., Hu K., Liu Y., Jiang J., Kuang S., Tang L., Tang W., Zhang Y., Zhou X., Feng L., 2017 The relationship between dietary methionine and growth, digestion, absorption, and antioxidant status in intestinal and hepatopancreatic tissues of sub-adult grass carp (*Ctenopharyngodon idella*). Journal of Animal Science and Biotechnology 8:63.
Zhou F., Xiao J. X., Hua Y., Ngandzali B. O., Shao Q. J., 2011 Dietary L-methionine requirement of juvenile black sea bream (*Sparus macrocephalus*) at a constant dietary cystine level. Aquaculture Nutrition 17(5):469-481.
Zhou Q. C., Wu Z. H., Tan B. P., Chi S. Y., Yang Q. H., 2006 Optimal dietary methionine requirement for juvenile cobia (*Rachycentron canadum*). Aquaculture 258(1-4):551-557.

Received: 21 August 2020. Accepted: 28 September 2020. Published online: 30 October 2020.
Authors:
Narissara Suratip, Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand 40002, e-mail: narissara.sur@gmail.com
Rungkan Klahan, Faculty of Agricultural Technology, Phetchaburi Rajabhat University, Phetchaburi, Thailand 76000, e-mail: supatcharin5@hotmail.com
Siripavee Charoenwattanasak, Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand 40002, e-mail: kobkaew@kku.ac.th
Bundit Yuangsoi, Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand 40002, e-mail: bundyu@kku.ac.th

How to cite this article:
Suratip N., Klahan R., Charoenwattanasak S., Yuangsoi B., 2020 Dietary methionine optimization for the growth performance, nitrogen utilization and proteolytic enzymes activities of snakehead (*Channa striata*) fingerlings. AACL Bioflux 13(5):3056-3067.