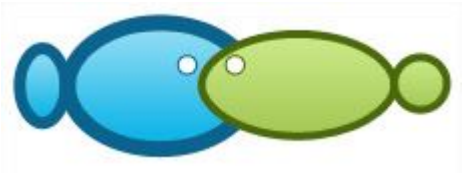

Dietary level of methionine:cysteine ratio for growth performance and feed utilisation in juvenile snakehead fish (*Channa striata*)

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Abstract. Total sulphur amino acid (TSAA); methionine and cysteine are generally known to play an important role in protein synthesis and other important physiological functions. This study evaluated the effects of dietary methionine:cysteine ratios (Met:Cyst) on growth performance and feed utilisation in juvenile snakehead fish (*Channa striata*). The five experimental diets were formulated with Met:Cyst at; 100:0 (control), 90:10 (MC1), 80:20 (MC2), 70:30 (MC3) and 60:40 (MC4). There were three replications for each diet formula. The similar sizes of healthy fish with average initial weight at 3.65±0.05 g fish-1 were distributed in plastic cabinet (40 cm × 54 cm × 34 cm with a volume of 60 litres) and they were fed twice a day for 60 days. The results showed no significant differences ($p > 0.05$) in growth and feed utilisation among experimental groups. The chemical composition in the whole body indicated that fish fed with the MC2 and MC4 diets had higher protein content than the control group ($p < 0.05$). Taurine accumulation in the whole body had the highest level in fish fed with the MC3 diet. Therefore, the present study indicated that cysteine could partially substitute methionine up to 40%. Based on above analysis, it is recommended the inclusion of methionine 0.68% and cysteine 0.97%, which had no negative effects on growth and feed utilisation in juvenile snakehead fish.
Key Words: snakehead fish, methionine, cysteine, growth, feed utilisation.

Introduction. The freshwater snakehead, *Channa striata* (Bloch, 1793) is one of the most popular and valuable fish in most southern and south-eastern Asian countries largely because its fast growth, disease resistance and tolerance in wide ranges of water quality (Webster & Lim 2002; Hossain et al 2008). Previously, the fish was mostly caught from the wild, some of the by-catch is used as raw material for fishmeal production. The fishmeal production is continuously decreasing every year and is also expected to be further declining in long run due to the reduction in the capture of fishery resources. (FAO 2020). Between 2009 and 2017 the amount of wild fish caught in nature was reduced from 25,500 tonnes to 14,500 tonnes (Department of Fisheries [DOF] 2019). Therefore, culturing this fish is basically required in order to maintain the market demand.

Fishmeal is one of main protein ingredients in aquafeed, nowadays the lack of it has forced the commercial business to find other alternative protein sources to replace it and fulfil market requirement. Plant-based protein sources are acknowledged as the best replacement source (Shukla et al 2019). However, the total sulphur amino acid (TSAA); methionine and cysteine are generally known to be present in low amounts when fishmeal is partly replaced by plant-based protein sources into commercial aquafeeds, which consequently influences an unsuitable portion of dietary methionine and cysteine levels in fish diets (Goff & Gatlin 2004). Methionine is classified as the first limit sulphur amino acid in fish which means it has effects on growth performance and physical development, whereas cysteine is considered to be a non-essential amino acid because it can be synthesised from methionine (Farthat & Khan 2014). Based on methionine biosynthesis pathway, it can be converted directly to cystine via trans-sulphuration; this

makes it to be considered as a supplier of cysteine in order to suit an appropriate replacement (Pillai et al 2006). Wilson (2002) referred to many studies which reported ranges of methionine requirements in common cultured fish between 1.8 and 3.2% of protein. However, a particular study of fingerling snakehead fish recently reported methionine requirements at 1.19% of diet (Hien et al 2018), plus many comparable studies in carnivorous fish also reported similar levels at 1.0% of diet for juvenile sea bass (*Dicentrarchus labrax*) (Thebault et al 1985), at 1.06% of diet for juvenile red drum (*Sciaenops ocellatus*) (Moon & Gatlin 1991), at 1.0% for juvenile yellow perch (*Perca flavescens*) (Twibell et al 2000) and at 1.19% of diet for juvenile cobia (*Rachycentron canadum*) along with cysteine existence at 0.67% of diet for maximum growth and feed utilisation (Zhou et al 2006). Nonetheless, methionine plays an important role in protein synthesis and other important physiological functions (Zhou et al 2006), so the lack of methionine in dietary feed affects many functions; it decreases weight gain which also affects growth, reduces feed efficiency and protein content which leads to fat accumulation in the body (Moran 1994; Abidi & Khan 2011; Khan & Abidi 2011). In contrast, snakehead fed with higher methionine levels than the suggested optimal level tended to have less growth due to excessive levels of methionine (Hien et al 2018).

Therefore, the cysteine replacement for dietary methionine requirement in the diets can minimise feed costs (He et al 2016), since cysteine is cheaper than methionine. Consequently, the cysteine replacement for methionine is also important as it can decrease methionine levels in the diet without reducing growth (He et al 2016). Many previous studies mentioned the ability of the cysteine replacement for methionine that a portion of the methionine requirement can be spared by cysteine approximately at 40-60% in some fish species (Moon & Gatlin 1991; Goff & Gatlin 2004) such as; 40% in rohu fingerling (*Labeo rohita*) (Abidi & Khan 2011), 42% in rainbow trout (*Oncorhyncus mykiss*) (Kim et al 1992), 50% in yellow perch (*P. flavescens*) (Twibell et al 2000), 40-50% in red drum (*S. ocellatus*) (Moon & Gatlin 1991; Goff & Gatlin 2004) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*) (Griffin et al 1994), 60% in juvenile sea bass (*D. labrax*) (Hidalgo et al 1987) and channel catfish fingerling (*Ictalurus punctatus*) (Harding et al 1977).

Nonetheless, to our knowledge, the information on a nutrient requirement for this species are limited, especially the study of the cysteine replacement for methionine in commercial feed diets. For this reason, the objective of this study was to examine the optimum of dietary cysteine replacement for methionine of juvenile snakehead fish to balance the TSAA and to prepare cost-effective feeds for the future intensive aquafarming in this species.

Material and Method

Experimental diets. The experimental diets and analysed amino acid compositions are presented in Table 1 and Table 2. The experimental diets constantly consisted of the same basal ingredients except the dietary methionine:cysteine ratios, thereby the five isonitrogenous and isocaloric diets contained 35% crude protein and 415-434 kcal 100 g^{-1} gross energy (GE) and methionine requirement at 1.19% of diet which was determined by Hien et al (2018). Noticeably, the methionine levels in trial diets were obtained following 1.15% of diet in the control group then replacing with cysteine at 10-40% dividing into five dietary methionine: cysteine ratios (Met:Cyst) at; 1) 100:0 (control), 2) 90:10 (MC1), 3) 80:20 (MC2), 4) 70:30 (MC3), and 5) 60:40 (MC4), respectively. The ingredients were ground then thoroughly mixed with soya oil and fish oil. Water was later added to produce stiff dough. Afterwards, the mixture was extruded by a floating pellet extruder through a 2-3 mm diameter of die then the pelleted diets were later airing dried at 40-50ºC for two hours to reduce the moisture content which must not exceed than 10%. Then they were left to cool down at an ambient temperature, packed in bags and kept properly at room temperature without light accessing.

Table 1

Dietary formulations and chemical compositions of experimental diets

Ingredients	*Differential methionine:cysteine ratios*				
	Control (100:0)	*MC1 (90:10)*	*MC2 (80:20)*	*MC3 (70:30)*	*MC4 (60:40)*
Fish meal (64.02% CP)	16	16	16	16	16
Soybean meal (46.70% CP)	30	30	30	30	30
Poultry meal (65.43% CP)	19	19	19	19	19
Corn meal	10.12	10.12	10.12	10.12	10.12
Cassava starch	15	15	15	15	15
Soya oil	4	4	4	4	4
Fish oil	2	2	2	2	2
Dicalciumphosphate	1.14	1.14	1.14	1.14	1.14
Vitamin mix*	1	1	1	1	1
Mineral mix**	1	1	1	1	1
DL-Methionine	0.46	0.35	0.23	0.12	0
L-Cystine	0	0.11	0.23	0.34	0.46
L-Lysine	0.28	0.28	0.28	0.28	0.28
Total	100	100	100	100	100
Chemical composition (by analysis)					
Protein (%)	34.74	34.56	34.58	34.86	34.24
Fat (%)	8.81	6.96	6.01	5.39	5.83
Fibre (%)	1.77	1.46	1.79	1.79	1.82
Nitrogen free extract (%)	37.65	40.06	39.89	40.36	40.35
Moisture (%)	6.69	6.83	7.12	7.10	7.09
Ash (%)	10.34	10.13	10.61	10.50	10.67
Gross energy (GE) (kcal $100g^{-1}$)	434.88	426.30	416.75	414.42	415.01

*Vitamin mixtures provided the following per kg diet: vitamin A 1,130,000 IU, vitamin D3 1,043,170 IU, vitamin E 30,000 IU, vitamin K3 3.25 g, vitamin B1 12 g, vitamin B2 5 g, vitamin B6 30 g, vitamin B12 12 g, vitamin C 30 g, cholinechloride 5 g, niacin 10 g, pantothenic acid 27 g; **Mineral mixtures provided the following per kg diet: Na 3.278 g, Mg 25.25 g, K 76.612 g, Ca 49.096 g, Fe 4.821 g, Zn 0.667 g, Mn 0.433 g, Cu 0.069 g and I 0.015 g.

Table 2

Analysed amino acid compositions of different level methionine:cysteine ratios in test diets

Amino acids	*Amino acid composition (g 100 g^{-1} of dried diet)*				
	Control (100:0)	*MC1 (90:10)*	*MC2 (80:20)*	*MC3 (70:30)*	*MC4 (60:40)*
Phenylalanine	1.55	1.59	1.71	1.48	1.51
Valine	1.64	1.68	1.73	1.58	1.62
Tryptophan	0.37	0.39	0.42	0.34	0.34
Threonine	1.37	1.35	1.52	1.33	1.36
Isoleucine	1.53	1.55	1.61	1.51	1.52
Methionine	1.17	1.05	0.93	0.79	0.68
Histidine	0.81	0.8	0.85	0.78	0.81
Arginine	2.47	2.49	2.77	2.44	2.46
Leucine	2.53	2.58	2.73	2.55	2.53
Lysine	2.22	2.43	2.41	2.18	2.26
Cysteine	0.52	0.63	0.70	0.84	0.97

Experimental procedures. The 500 snakehead fish were obtained from a commercial farm in Phichit province, Thailand. Fish were stocked into two of 1000 L tanks at the Faculty of Agriculture, department of Fisheries, Khon Kaen University. They were acclimated to experimental conditions for one week with a control diet. There were three replications for each diet formula (5 treatments × 3 replications = 15 plastic cabinets).

To start the experiment, each plastic cabinet (40 cm × 54 cm × 34 cm containing 60 litres) contained 20 similar sizes of healthy fish with an initial weight at 3.65 ± 0.05 g $fish^{-1}$, hereby the fish density was one fish per three litres. These rearing trials took 60 days (the experiment was started in February, 2019 and was finished in May, 2019) and fish were daily fed with 5.0-6.0% of their body weights at 8.00 am and 5.00 pm. Water was daily replaced between 20 to 50% in each cabinet.

Sampling and analytical methods. The fish were weighed every 10 days and feed intake in each cabinet was recorded daily. Growth performance and feed utilisation parameters were measured using the following equations:

Weight gain, WG (g) = final weight (g) – initial weight (g)

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

Specific growth rate, SGR (% day^{-1}) =((Ln final weight - Ln initial weight) / days) × 100

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

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

Survival (%) = 100 × (final number of fish / initial number of fish)

At the 60th day of the study, fish were fasted for 12 hours to empty their guts before sampling. Ten fish from each replicate were anaesthetised by using 100 mg L^{-1} concentration of clove oil for surgical dissection. Afterward, the livers were collected and weighed then liver weight data were later used to calculate the hepatosomatic index (HSI). Whole fish and liver tissues were sealed in plastic bags separately and stored frozen at -20ºC for amino acid composition and taurine accumulation analysis.

Proximate composition of experimental diets and fish whole body composition were estimated according to AOAC (1995). Crude protein (N×6.25) was determined by the Kjeldahl method after an acid digestion followed by using the auto Kjeldahl system (1030 Auto-analyzer, Tecator, Sweden), crude lipid was regulated by the ether-extraction method using the Soxtec System HT (Soxtec System HT6, Tecator, Sweden), moisture was determined by using an oven dryer at 105ºC for 24 hours and ash was determined by using a muffle furnace at 550ºC for 24 hours.

The amino acid and taurine accumulation analysis were performed at the end of the experiment (60 days), hereby proximation composition analysis of the experimental diets and fish carcasses was determined using the Automated Amino Acid Analyser. Firstly, the amino acid accumulation analysis was performed after digesting acid hydrolysis of 6 M HCI for 22 hours (Blankenship et al 1989). For methionine determination, liver and carcass samples were extracted using 0.1 M HCl; derivatisation was performed using O-phthalaldehyde (Aristoy & Toldrá 1991). Secondly, taurine accumulation in the whole body and liver tissue were performed using 0.1 M HCl and then sample concentrations were later operated using dansyl chloride (McCarthy et al 2000). The sample precolumn derivatisations were then quantified using o-phthalaldehyde before injecting HPLC and fluorescent detector (Agilent 1090 system, Palo Alto, CA) according to Fleming et al (1992).

Histological analysis was adeptly obtained following the method mentioned in Prisingkorn et al (2017). At the end of the experiment, livers and posterior intestines of three fish in each replicate were dissected and fixed in 10% phosphate-buffered formalin with pH 7.2 and stored at room temperature for dehydration. Afterward, the samples were embedded in paraffin then cut into 5 µm sections. The tissue sections were later stained with haematoxylin and eosin (H&E). The samples were observed under a light microscope (Nikon Eclipse Ci), photographed by using a digital camera (Nikon DS-Fi2) and visualised with NIS-Elements Microscope Imaging Software (Nikon Corporation, Tokyo, Japan).

Statistical analysis. All data from each parameter were statistically analysed using the SPSS version 11.5 (SPSS, Chicago, IL, USA), then subjecting to one-way analysis of variances (ANOVA). Significant differences among the group means were compared using Duncan's multiple range tests at $p < 0.05$ significance threshold. Results were expressed as mean±SD.

Results

Growth performance. The results of growth parameters and feed utilisation are shown in Table 3. After 60 days of experimental trials, the results in final weight (FW), weight gain (WG), average daily gain (ADG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish fed with the MC1, MC2, MC3 and MC4 diets showed slight increase trends from the result of the control group, but no significant differences ($p > 0.05$) were found among all groups. However, the maximum and minimum results of FW were found in the MC4 at 15.00±1.65 g and the control group at 13.63±1.11 g, respectively. There were slightly differences on ADG among groups between 0.17-0.19 g day^{-1}. The survival rate of all groups was over 90% and the MC3 group had the highest level at 96.67±5.77%. Therefore, FCR values showed a slight decrease trend respectively at 2.01±0.28 for the control group to 1.69±0.30 for the MC4 group. In contrast, PER values showed a slight increase trend respectively from the control group to the MC4 groups from 1.33±0.17 to 1.59±0.29.

Table 3

Growth parameters and feed utilisation in juvenile snakehead fish fed with different methionine:cysteine ratios for 60 days

Parameters	*Differential methionine:cysteine ratios*					*p-value*
	Control (100:0)	*MC1 (90:10)*	*MC2 (80:20)*	*MC3 (70:30)*	*MC4 (60:40)*	
IW (g $fish^{-1}$)	3.67±0.03	3.65±0.05	3.64±0.05	3.64±0.05	3.65±0.04	0.966
FW (g $fish^{-1}$)	13.63±1.11	13.83±0.07	14.07±0.76	14.35±0.60	15.00±1.65	0.514
WG (g $fish^{-1}$)	9.96±1.09	10.19±0.05	10.43±0.70	10.71±0.55	11.35±1.63	0.479
ADG (g $fish^{-1}$ day^{-1})	0.17±0.02	0.17±0.00	0.17±0.01	0.18±0.00	0.19±0.03	0.479
SGR (% day^{-1})	2.18±0.13	2.22±0.02	2.25±0.07	2.29±0.05	2.35±0.18	0.409
SR (%)	93.33±5.77	95.00±5.00	95.00±5.00	96.67±5.77	93.33±2.89	0.913
FCR	2.01±0.28	1.90±0.10	1.83±0.08	1.83±0.12	1.69±0.30	0.436
PER	1.33±0.17	1.39±0.07	1.44±0.06	1.45±0.10	1.59±0.29	0.371

Note: initial weight (IW), final weight (FW), weight gain (WG), average daily gain (ADG), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR) and protein efficiency ratio (PER). Values are presented as means±SD of three replicates. Values within the same row with different superscripts are significantly different ($p < 0.05$).

Body composition. The composition of the whole body has been analyzed (Table 4). Moisture had no significant differences ($p > 0.05$) among all groups. Fish fed with the MC2 and MC4 diets had significantly higher protein accumulation ($p < 0.05$) (59.54± 1.06% and 58.55±0.35% respectively) than fish fed with the control, MC1 and MC3 diets, which were recorded at 56.78±0.11, 55.86±0.01 and 56.33±0.09%, respectively. The lowest value of the fat content was observed in fish fed with the MC2 diet at 15.47±0.19% of dried matter which was significantly lower ($p < 0.05$) than the highest level in control group at 18.09±0.71% of dried matter, as well as the MC3 group also showed the lower significant level ($p < 0.05$) at 16.06±0.12% of dried matter comparing to the control group. However, in the MC1 and MC4 groups similar levels were observed and no significant differences ($p > 0.05$) found with the control group. Fish fed with the MC1 diet had the highest level of ash composition at 15.86±0.00% of dried matter which was also significantly higher ($p < 0.05$) than other groups and followed by the MC2 group. Contrarily, the MC4 group had the lowest level at 13.84±0.17% of dried matter which was significantly lower ($p < 0.05$) among the other experimental groups. However, the MC2 and MC3 groups recorded parallel results correspondingly at 15.55±0.26 and 15.25±0.24% of dried matter and were also significantly higher ($p < 0.05$) than the control group which resulted at 14.67±0.14% of dried matter.

Table 4

Body composition in whole body (% of DM) of juvenile snakehead fish fed with different methionine:cysteine ratios for 60 days

Parameters	Differential methionine:cysteine ratios					p-value
	Control (100:0)	MC1 (90:10)	MC2 (80:20)	MC3 (70:30)	MC4 (60:40)	
Protein	56.78±0.11^{b}	55.86±0.01^{b}	59.54±1.06^{a}	56.33±0.09^{b}	58.55±0.35^{a}	0.003
Fat	18.09±0.71^{a}	17.98±0.07^{a}	15.47±0.19^{b}	16.06±0.12^{b}	17.79±0.15^{a}	0.002
Ash	14.67±0.14^{c}	15.86±0.00^{a}	15.55±0.26^{ab}	15.25±0.24^{b}	13.84±0.17^{d}	0.000
Moisture	1.90±0.22	1.61±0.73	1.85±0.33	1.73±0.32	1.52±0.44	0.893

Note: Values are presented as means±SD of three replications. Values within the same row with different superscripts are significantly different ($p < 0.05$).

Hepatosomatic index. HSI values are presented in Table 5. There were no significant differences among groups ($p > 0.05$). Therefore, fish fed with the MC4 diet had the highest value at 2.24±0.28 followed by the control diet at 2.23±0.21, whereas the MC1 group had the lowest value at 2.13±0.20. Moreover, the MC2 and MC3 groups were recorded similar values at 2.17±0.25 and 2.20±0.2, respectively.

Table 5

Body composition in whole body (% of DM) of juvenile snakehead fish fed with different methionine:cysteine ratios for 60 days

Parameter	Differential methionine:cysteine ratios					p-value
	Control (100:0)	MC1 (90:10)	MC2 (80:20)	MC3 (70:30)	MC4 (60:40)	
HSI	2.23±0.21	2.13±0.20	2.17±0.25	2.20±0.25	2.24±0.28	0.733

Note: Hepatosomatic Index (HSI). Values are presented as means±SD of three replicates. Values within the same row with different superscripts are significantly different ($p < 0.05$).

Chemical analysis

Amino acid accumulation. The whole body carcases were analysed for amino acid accumulation and the results are shown in Table 6. Most of amino acid accumulation levels among trial groups were slightly different except the methionine and cysteine levels, which were relevant to amino acid levels in diets. Based on the replacement levels of cysteine for methionine in diets, the results of methionine accumulations in fish showed a decrease trend respectively from 1.83 g 100 g^{-1} of dried matter in the control group to 1.53 g 100 g^{-1} of dried matter in the MC4 group, whereas cysteine accumulations were correspondingly increased following its substitute proportions in the diets at 0.74 g 100 g^{-1} of dried matter in the control group and 1.36 g 100 g^{-1} of dried matter in the MC4 group.

Table 6

Amino acid accumulation (g 100 g^{-1} of DM) in whole body of juvenile snakehead fish fed with different methionine:cysteine ratios for 60 days

Amino acids	Differential methionine:cysteine ratios				
	Control (100:0)	MC1 (90:10)	MC2 (80:20)	MC3 (70:30)	MC4 (60:40)
Phenylalanine	2.37	2.41	2.46	2.47	2.45
Valine	2.61	2.73	2.56	2.64	2.58
Tryptophan	ND	ND	ND	ND	ND
Threonine	2.53	2.64	2.67	2.76	2.63
Isoleucine	2.48	2.51	2.56	2.53	2.47
Methionine	1.83	1.78	1.66	1.69	1.53
Histidine	1.43	1.32	1.36	1.24	1.27
Arginine	3.74	3.68	3.54	3.81	3.73
Leucine	4.45	4.57	4.48	4.28	4.43
Lysine	4.61	4.69	4.51	4.28	4.48
Cysteine	0.74	1.12	1.34	1.37	1.36

Note: Not detected (ND).

Taurine accumulation. The taurine accumulation levels in the whole body and liver tissue are displayed in Table 7. In the liver, the highest level was observed in fish fed with the MC4 diet at 57.32 µmol g^{-1} and respectively showed a decrease trend from 46.19 µmol g^{-1} in the MC3 group, 39.92 µmol g^{-1} in the MC2 group, 36.81 µmol g^{-1} in the MC1 group and the lowest level in the control group at 32.42 µmol g^{-1}. However, the accumulation levels in the whole body carcasses of fish fed with the MC2 diet surprisingly resulted in the highest level at 55.09 µmol g^{-1} reasonably followed by the MC4 group at 43.13 µmol g^{-1}. The lowest level was observed in the control group at 26.27 µmol g^{-1} which did not much differ from the MC1 group at 30.92 µmol g^{-1}, hereby the MC3 group had a similar result to the MC4 group at 41.91 µmol g^{-1}.

Table 7

Taurine accumulation (µmol g^{-1}) in juvenile snakehead fish fed with different methionine: cysteine ratios for 60 days

Targets	*Different methionine:cysteine ratios*				
	Control (100:0)	*MC1 (90:10)*	*MC2 (80:20)*	*MC3 (70:30)*	*MC4 (60:40)*
Liver	32.42	36.81	39.92	46.19	57.32
Whole body	26.27	30.92	55.09	41.91	43.13

Histological analysis. Liver and posterior intestine histology are shown in Figures 1 and 2. The histological analysis results in both liver and posterior intestine areas indicated no significant difference observed among the experimental groups. In the liver, the lipid vacuole diffusions, hepatocyte hypertrophies, nucleus absences and abnormalities were correspondingly found in fish fed with all trial diets. The posterior intestine results showed similar effects on intestinal villi and lamina propria and muscular layers among all groups. It was noticeable that the muscular layers of the MC3 and MC4 groups were slightly thicker than others.

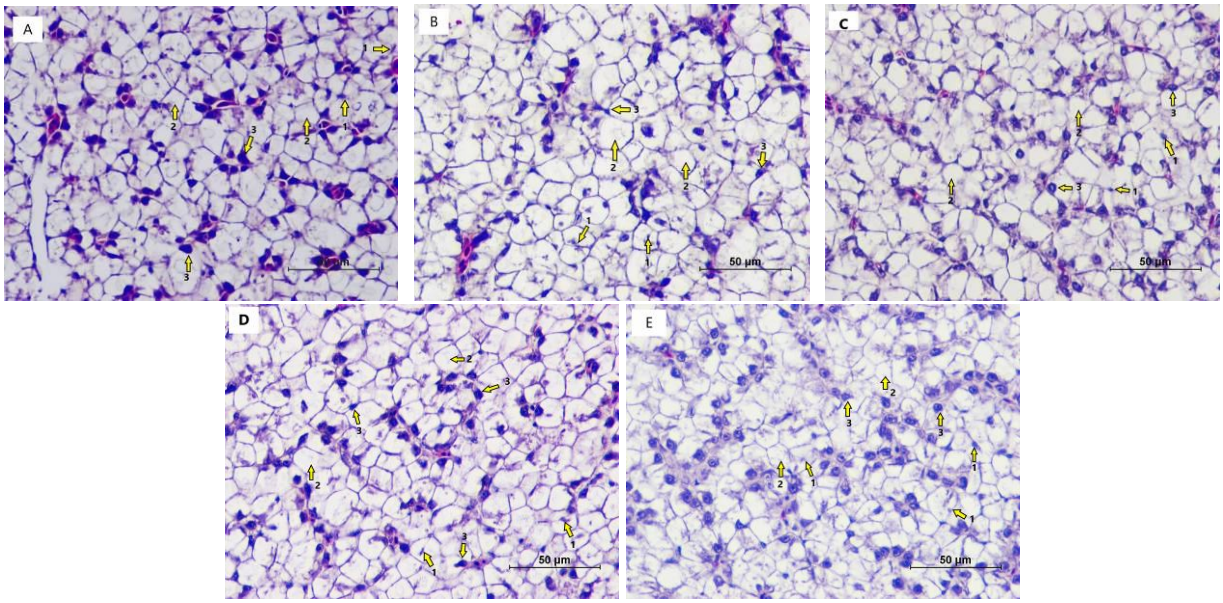

Figure 1. Liver histology of juvenile snakehead fish fed with different methionine:cysteine ratios for 60 days (Notes: Diets; A) control, B) MC1, C) MC2, D) MC3 and E) MC4. Labels; 1 = hepatocyte hypertrophy, 2 = absent nucleus, and 3 = abnormal nucleus).

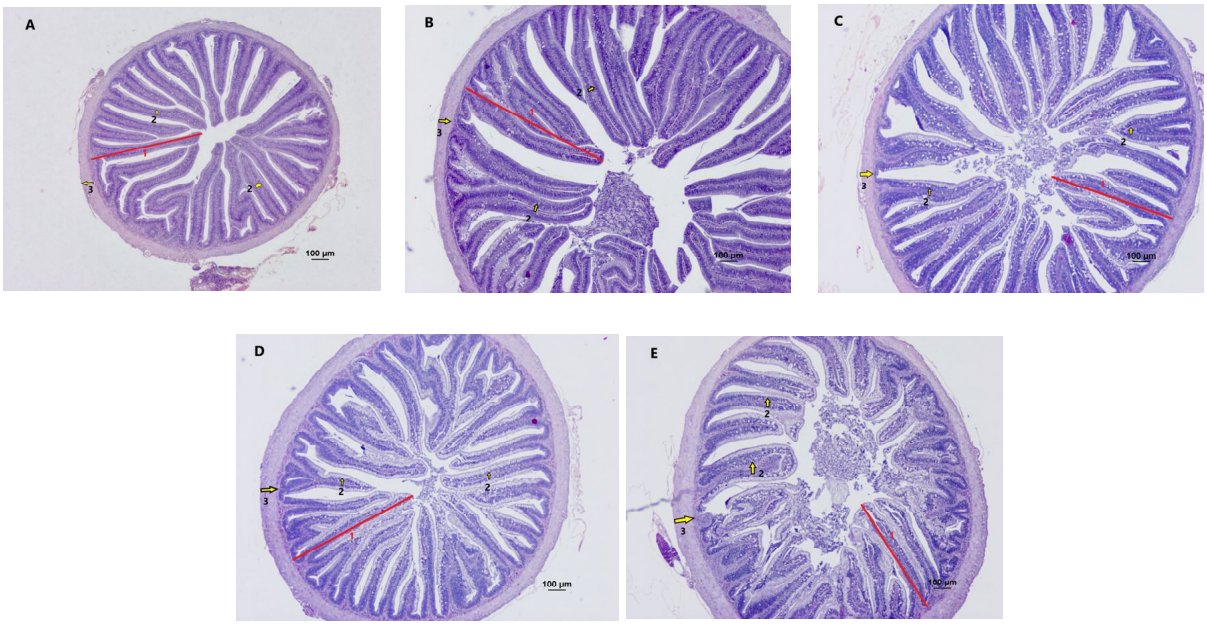

Figure 2. Posterior intestine histology in juvenile snakehead fish fed with different methionine: cysteine ratios for 60 days (Notes: Diets; A) control, B) MC1, C) MC2, D) MC3 and E) MC4. Labels; 1 = intestinal villous area, 2 = lamina propria, and 3 = muscular layer).

Discussion. To our knowledge, methionine is officially classified as the first limited sulphur amino acid in fish and fish cannot synthesise methionine by themselves (Parker 2011), which means they only get it through diet, especially when diets were based on plant protein source (Michelato et al 2013). Therefore, the methionine supplementation is required in order to meet fish requirements for growth and physical developments. Although, cysteine can be synthesised from methionine via a trans-sulphuration pathway, it was considered as a nonessential amino acid, moreover it also undergoes conversion into taurine via cysteinesulphinate decarboxylate (CSD). However, cysteine replacement is one of alternative options to spare the methionine level in the diet from converting to cysteine. The existence of cysteine in the diet demonstrates its sparing effect for reducing the need for required methionine, so if cysteine presents in the diet then methionine may be spared for maximum growth (Luo et al 2005; Mai et al 2006). Nevertheless, the optimal ratio of dietary cysteine replacement for methionine in juvenile snakehead fish was not yet clarified, more research is necessary into this specific species.

The present study was aimed to examine the optimum level of cysteine replacement for methionine requirement in snakehead fish diet. Nonetheless, for a safe starting point of new culture species 30-40% of cysteine replacement was assumed as the maximum contribution for the TSAA (Twibell et al 2000). Therefore, the methionine requirement for juvenile snakehead fish with average initial weight at 3.65±0.05 g fish^{-1} in this study was determined at 1.15% of diet as the control group then decreasing by cysteine replacement at 10-40% in other test diets. The results indicated that the MC4 diet, which had the highest level of cysteine replacement at 40%, resulted in no significant differences ($p > 0.05$) among all groups on growth and feed utilisation parameters; FW, WG, ADG, SGR, SR, FCR and PER (Table 3), which could be assumed to confirm an ability of cysteine replacement for dietary methionine. However, the balanceable and suitable cysteine replacement could spare the methionine from cysteine synthesis, plus either methionine itself or the combination with cysteine can reach the TSAA requirement in fish (Ahmed et al 2003). Moreover, there were many conditions involved to properly estimate a suitable portion of cysteine replacement for methionine such as the diet ingredients and compositions, fish sizes, different fish genetically and species and culture conditions (Mai et al 2006). The similar result was recently reported in juvenile tilapia fed with various ratios of cystine replacements significantly showed no effects on growth parameters and confirmed that cysteine could spare at 47% of

methionine in diet (He et al 2016). Moreover, Farthat & Khan (2014) especially indicated cysteine replacement approximately at 39.6-40.2% for methionine requirement gave no adverse effects on growth in fingerling stinging catfish (*Heteropneustes fossilis*). However, other previous studies reported the cysteine replacements in various levels at 40-50% of dietary methionine requirement in some fish species such as; 42% in rainbow trout (*O. mykiss*) (Kim et al 1992), 50% in yellow perch (*P. flavescens*) (Twibell et al 2000), 40-50% in red drum (*S. ocellatus*) (Moon & Gatlin 1991; Goff & Gatlin 2004) and hybrid striped bass (*M. chrysops* × *M. saxatilis*) (Griffin et al 1994). Conversly, the higher cysteine replacement levels might have reverse effects in fingerling stinging catfish. According to the earlier study, it was found that fish growth was significantly depressed when being fed with 50-60% of cysteine replacement levels (Farthat & Khan 2014). Moreover, in mammals, the excess dietary cysteine was reported as the cause of the reduction on growth in rat (Harper et al 1970), considered it was a toxic in mice when exists in over requirement (Stipanuk et al 2006).

The body composition results were analysed and interestingly indicated that fish fed with the MC4 diet gave no significant differences ($p > 0.05$) on the protein content in comparison with the highest protein composition in fish fed with the MC2 diet, plus both of their levels were significantly higher than the control group (Table 4), whereas the MC1 and MC3 diets had no significant differences ($p > 0.05$) compared with the control diet. Fat composition level in the fish fed with the MC4 diet also gave no significant differences ($p > 0.05$) in comparison with the control and MC1 diets. Therefore, this could be suggested that cysteine replacement for methionine up to 40% had no negative effects on the body composition of juvenile snakehead fish under the present trial conditions, which relatively agreed by some previous studies reported no significant effects ($p > 0.05$) on body composition in fingerling *Catla catla* when methionine was partly replaced by cysteine up to 40% (Zehra & Khan 2014) and in fingerling stinging catfish (*H. fossillis*) (Farthat & Khan 2014). On the other hand, the results of the beyond 40% replacement levels in both previous studies fell in reverse protein composition levels and high fat levels.

In addition, methionine is also generally known as one of factors that is relevant to the HSI level in teleost fishes, plus there were some previous studies of methionine requirement in some fish species which reported the effect of dietary methionine on liver. In one particular study of fingerling snakehead fish there were found significant increases in liver weight when fish was fed with supplementary methionine in trial diets (Hien et al 2018). Other reports also observed similar significant results of methionine effects on the liver such as; in juvenile rockfish (*Sebastes schlegeli*) (Yan et al 2007), in Atlantic salmon (*Salmo salar*) (Espe et al 2008), in juvenile jian carp (*Cyprinus carpio*) (Tang et al 2009), in juvenile yellow catfish (*Pelteobagrus fulvidraco*) (Chen et al 2014) and in pre-adult gibel carp (*Carassius auratus gibeilo*) (Wang et al 2016). On the other hand, HSI value of juvenile meagre (*Argyrosomus regius*) fed with dietary methionine supplementation was recorded no relevant effects (De Moura 2018). Nevertheless, most of these studies were focused only on the methionine requirement, unlike the present study which focused on the balancing of the TSAA requirement through various dietary methionine and cysteine ratios. A similar study into juvenile Asian sea bass (*Lates calcarifer*) fed with different sulphur amino acid ratios were found no significant differences related to HSI value (Coloso et al 1999). The present study, the HSI results were not significantly different among all experiment groups ($p > 0.05$), hereby fish fed with the MC4 diet was detected as the closest level to the control group. Additionally, the explanation behind these results might be that methionine and cysteine are both sulphur amino acids and as mentioned before, methionine is a precursor of cysteine, so by replacing cysteine into the diet could probably maintain the balancing of amino acids requirement in juvenile snakehead fish. Consequently, this could be indicated that the cysteine replacement for methionine requirement up to 40% of diet in the present study had no relative effects on HSI values in this species. Contrarily, a study of rainbow trout fed with different methionine and cysteine ratios concluded its effects on HSI values by observing the decreasing trend of HIS results when fish were fed with methionine and cysteine supplementation ratios (Walton et al 1982). Nonetheless, there were no negative results

yet found in this study, hence in order to illuminate a true effect of cysteine replacement in this species the beyond 40% of replacement levels is needed to carry out in future study.

The amino acid analysis showed levels of methionine and cysteine accumulations in the whole body correspondingly decreased or increased following their substitute proportions in diets, suggesting they relevantly responded to their own dietary levels in trial diets. These results could be implied that by sparing dietary methionine with cysteine affected their amino acid accumulation levels in the whole body of juvenile snakehead fish. The similar result was reported in pangasius catfish (*Pangasius bocourti*) where it has been observed a slight rise trend of DL-methionine in the whole body which corresponded with its levels in trial diets, but no significant difference was found in a comparison with the control diet (Yuangsoi et al 2016). Moreover, the study in juvenile Nile tilapia had previously reported amino acids accumulation in fish whole body was not affected by dietary methionine to cysteine ratios (He et al 2016). Though, increasing cysteine levels in the whole body may also affect its conversion to taurine and consequently cause an effect to taurine accumulation in fish.

Taurine accumulations of fish whole body and liver tissues were slightly increased in trends directly related to the variations of the cysteine replacement levels in experimental diets. However, while fish fed with the control diet gave the lowest level of taurine accumulation in the whole body and in liver tissue, fish fed with the MC4 diet gave the highest accumulation levels in both whole body and liver tissue. Likewise, the similar study in rainbow trout found the increase taurine levels in plasma and liver samples consequently related with the increasing of the dietary methionine and cysteine levels in aquafeeds, suggesting a considerable taurine synthesis from cysteine through either dietary level or biosynthetic pathway (Walton et al 1982). Based on a methionine biosynthesis pathway, methionine is metabolised to cysteine then taurine which made it to be classified as a precursor of both cysteine and taurine. However, this pathway has to occur through homocysteine which is a key intermediate in sulphur metabolism because it could be trans-sulphureted directly to cystathionine then cysteine or re-methylated or transmethylated back to methionine. Although, no analysis was made in this study for homocysteine which could be an ancillary for cystathionine formation. Therefore, a cysteine replacement is one of the alternative options to avoid its loss from trans-methylated back to methionine. Furthermore, cysteine is believed to be a precursor for taurine biosynthesis in teleost which could be assumed that snakehead fish may be able to synthesise dietary cystine for taurine biosynthesis. This could suggest that the improvement levels of taurine accumulations in juvenile snakehead fish were caused by the cysteine replacements in experimental diets, assumingly fish could possibly synthesise taurine from dietary cysteine. On the other hand, juvenile Japanese flounder (*Paralichthys olivaceus*) was reported unable to biosynthesise taurine from cystine (Park et al 2002). However, taurine supplementation is fundamental in the diets of carnivorous fish species when fishmeal is partially or totally replaced by plant protein sources, hence fish fed with a taurine deficient diet were reported to have interference in growth performance and to develop green liver syndrome (Takagi et al 2011). In the present study, taurine accumulations in either the whole body or liver tissue of all experimental groups gave only slight differences among all groups and no green liver symptoms were found in any of the groups, suggesting that by replacing cysteine for methionine up to 40% is probably an appropriate portion and could improve the taurine accumulations in juvenile snakehead fish. Although, taurine requirement, metabolism, physiological function and synthesis mechanism also are not entirely be cleared, hence more future studies in this species should be carried out.

The liver histology of fish fed with different cysteine replacement levels for methionine showed no significant differences among treatments. However, the higher levels of glycogen contents in liver induced a large number of lipid vacuoles diffusion and hepatocyte hypertrophies. The absent and abnormal nuclei in hepatocytes were also found in all experimental groups. The previous study of juvenile meagre reported macro-vesicular lipid accumulated diffusely in liver tissue when fish fed with different dietary protein levels with the methionine and lysine supplementations (Güroy et al 2017). It

could be assumed that these effects might be caused by the high dietary carbohydrate levels in diet formulations, as in the previous study of blunt snout bream (*Megalobrama amblycephala*) that reported the negative effects on liver health when fish were fed with high carbohydrate contents in diet (Prisingkorn et al 2017). Moreover, all cysteine replacement ratios for methionine in this study had no negative effects on nutrient absorption of the posterior intestine in all groups, relatively agreeing with growth performance and feed efficiency.

Conclusions. The results of the present study clearly indicated that the dietary of methionine:cysteine ratios (Met:Cyst) had no adverse effect on the growth performance, feed utilisation and body composition of juvenile snakehead fish (*Channa striata*). The cysteine could partially substitute methionine up to 40% and based on above analysis, it is recommended the inclusion of methionine 0.68% and cysteine 0.97%. Therefore, data generated during this study would be useful in formulating the total sulphur amino acid balance for this species.

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