Dietary methionine and lysine requirement of snakehead (*Channa striata*) fingerlings

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Abstract-This study was aimed determine the dietary methionine (Met) and lysine (Lys) requirement for snakehead fingerlings (2-4 g fish^{-1}). Basal diets in two experiments contained approximately isonitrogenous 42 % and isoenergetic 20.3 KJ g^{-1}. In the first experiment on Met requirement, L-Met was added to the basal diets including six treatments containing from 7.3 to 14.8 g Met kg^{-1} diet (17.5 to 35.3 g Met kg^{-1} protein) interval increasing of 1.5 g kg^{-1} diet. In the second experiment determining Lys requirement, L-Lys HCL was added to basal diets including seven treatments containing from 12.6 to 36.6 g Lys kg^{-1} diet (30.1 to 87.2 g Lys kg^{-1} protein), interval increasing of about 4 g kg^{-1} diet. The experiements were randomly designed with four replications for each treatment. The first experiment indicated that optimal weight gain, special growth rate, protein efficiency ratio was found in the diet containing 28.2 g Met kg^{-1} protein and there were significant differences in those parameters between diet treatment containing 24.8 g Met kg^{-1} protein and other diets containing lower Met levels. The hepatosomatic index and protein content in whole-body fish were significantly affected by dietary Met levels. Feed conversion ratio (FCR) was significantly improved with the increase of dietary Met level in diet to 28.2 g kg^{-1} protein (P<0.05). Results of the second experiment showed that optimal growth rate and protein efficiency ratio were found in diet containing 73.1 g. Lys kg^{-1} protein and there were significant differences in those parameters between diet treatment containing 73.1 g Lys kg^{-1} protein and other diets containing lower Lys levels. The hepatosomatic index, protein and fat content in whole-body fish were significantly affected by dietary Lys levels. The FCR was significantly improved by increasing dietary Lys concentration to approximately 77.9 g Lys kg^{-1} protein. Fish survival rate were not significant differences among treatments in both experiments. Broken-line analysis on the basis of optimal growth rate showed that the dietary Met requirement was 11.9 g Met kg^{-1}diet (28.4 g kg^{-1} protein) and the dietary Lys requirement of snakehead was 30.7 g Lys kg^{-1}diet (73.1 g kg^{-1} protein).

Keywords: methionine requirements, lysine, snakehead, *Channa striata*

I. INTRODUCTION

Protein, especially when derived from fish meal, is the most expensive nutrient in the dietary formulation for aquatic organisms. Therefore, it is important to incorporate inexpensive protein ingredients in the formulation of fish feed by taking care of essential amino acids (EAA) balances (Sardar *et al*., 2009). By doing this, lysine (Lys) and methionine (Met) are known as the most limiting amino acids in feed ingredients used in diets for fish (Small and Soares, 2000), especial, when plant protein sources are used to replace fish meal (Abimorad *et al*., 2009; Sardar *et al*., 2009, Mai *et al*., 2006).

Of all essential amino acids, Lys is one of special concern because it helps to absorb the calcium, maintain healthy blood vessels, and produce antibodies, enzymes, and collagen and repair tissues. Dietary Lys supplementation is related to advantages on weight gain feed conversion, nitrogen retention and reduction in body lipid contents (Santiago and Lovell, 1988) estimated dietary requirement of 14.3 g Lys kg^{-1} diet (51 g kg^{-1} protein) for Nile tilapia fingerlings. Wang *et al*. (2005) evaluated the digestible Lys requirement of grass carp fry and estimated a 20.7 g kg^{-1} diet level for maximum growth, corresponding to 55.5 g kg^{-1} of dietary protein. Furuya *et al*. (2006) determined the digestible Lys requirement of Nile tilapia juveniles to be 14.4 g kg^{-1} diet for highest weight gain, which corresponded to 17.2 g kg^{-1}diet crude Lys in the dry diet and 52.3 g kg^{-1} in the protein fraction. For striped catfish *(Pangasianodon hypophthalmus)*, the demand for Lys and Met was 20.7 g kg^{-1} diet (53.5 g kg^{-1} protein) and 10.1 g kg^{-1} diet (26.7 g kg^{-1} protein) respectively, with protein content of 38% (Hien *et al*., 2009a; 2009b).

Besides Lys, Met is also limiting amino acid in some plant protein sources when the fish meal is replaced (Mai *et al*., 2006; Sardar *et al*., 2009). Met deficiency results in slow growth and reduced feed efficiency in Atlantic salmon (*Salmo salar L.*) (Opstvedt *et al*., 2003), juvenile cobia (*Rachycentron canadum*) (Zhou *et al*., 2006), juvenile rockfish (*Sebastes schlegeli*) (Yan *et al*., 2007). The dietary Met supplementation has been applied mainly in soybean-protein based diets, which have Met as the first limiting amino acid for carnivorous fish. Met requirements have been reported varying from 2% to 3.75% of dietary protein in different fish species (Ahemd *et al*., 2003; Mai *et al*., 2006; Jia *et al*., 2013).

As a carnivorous species, snakehead, a valuable commercial fish species culture in Asian countries, requires a high protein level in the diet (Samantary and Mohanty, 1997). As a result, feed cost covered more than 80% of the total snakehead production cost (Hien *et al.*, 2018). Reducing cost, fish meal protein could be replacement by defatted soybean meal 40% with adding Met, Lys and phytase enzyme (Hien *et al*., 2015). However, there is no published information on dietary Met and Lys requirements for snakehead to formulate the Lys and Met-balanced practical feeds. This study was aimed at determining optimum dietary Lys and Met requirements for optimizing growth, feed conversion, and body composition of snakehead (*Channa striata*) fingerlings.

II. MATERIALS AND METHODS

2.1 Experimental diets

Experiment 1: Dietary Met requirement for snakehead

The experiment included 6 diets with the iso-protein level of 42% and iso-energy of 20.3 $KJ.g^{-1}$. The control treatment without Met supplement contaimed a Met level available in feed of 7.3g. kg^{-1} diet (corresponding to 17.5 $g.kg^{-1}$protein). Met was added to the experimental diets from 0 to 7.5 g $.kg^{-1}$ diet (increase of 1.5g. kg^{-1} diet)., at a constant dietary level cysteine of 2.66 g kg^{-1} diet were formulated. The fish meal, wheat gluten and gelatin were used as as main protein sources, and all diets were supplemented with crystalline amino acids mixture (Table 1) without Met to meet the requirements of other EAA according to the whole body amino acids profiles of snakehead.

Experiment 2: Dietary Lys requirement

The experiment was conducted with 7 diets with the same protein level of 42% and energy of 20.3 $KJ.g^{-1}$. The control treatment was without Lys supplementation, where Lys content of 12.6 $g.kg^{-1}$ diet (corresponding to 30.1 $g.kg^{-1}$ protein) is available in feed. Lys was added to the experimental diets from 0 to 24 g kg^{-1} diet (an increase of 4 g kg^{-1} diet). The fish meal, wheat gluten and gelatin were used as as main protein sources, and all diets were supplemented with crystalline amino acids mixture (Table 2) without Lys to meet the requirements of other EAA according to the whole body amino acids profiles of snakehead. except Met level was added 28.4 g kg^{-1} protein. All ingredients were mixed mechanically with water for 30 minutes and the dough was then passed through an extruder to obtain pellets of 2-mm diameter. Diets were dried in the oven at 60^{o}C for 6 h, then cooled at room temperature for 30 minutes, and finally stored in airtight plastic bags in the freezer -20^{o}C until use throughout the experiment (i.e., all diets were made at one time only). Proximate composition of diets was analyzed using methods of AOAC (2000).

2.2 Fish and experimental condition

Snakehead fingerlings used in the experiments were bought from a hatchery in An Giang province. Before starting each experiment, fish were acclimated in a 2,000-L circular tank and fed on a commercial diet for 15 days. The average initial weight per fish was 2-3 g. At the start of the experiments, fingerlings were randomly distributed into 18 composite tanks for Met experiment and 21 tanks for Lys experiment (120-L capacity, filled with 100 L of water) at a stocking density of 30 $fish.tank^{-1}$. Each tank was provided with continuous aeration and flow-through water supply with 30% water exchange d^{-1}. Fish were fed twice a day (0900 and 1500) to satiation. The amount of consumed feed and uneaten feed in each tank was recorded daily (uneaten feed was siphoned out 30 min after feeding began, dried and weighed). Any fish mortality was recorded daily and dead fish were removed and weighed immediately. Each experiment lasted for 8 weeks. Water parameters were recorded. Temperature ranged from 26.7-28.8 ºC, dissolved oxygen from 5.4 to 5.7 mg L-1, pH from 7.4 to 7.7, NO2 < 0.1 mg L-1 and NH3<0.1 mg L-1, so the water quality parameters in all treatments were in a suitable range for the normal growth and development of this species. At the end of the experiments, the survival rate and fish weight was determined by counting and weighing all fish in each tank. Each specimen was kept in the freezer at -20°C for the chemical compositions analysis of the fish according to the method of AOAC (2000).

2.3. Data collection and calculations

Growth rate was calculated and expressed as weight gain (WG), daily weight gain (DWG), specific growth rate (SGR) according to the following equations: WG(g) = Wf – Wi, DWG (g/day) = WG/t, SGR (%/day) = ((ln (Wf) - ln (Wi))/t) x 100. The survival rate of the fish in each tank was measured using the following fomula: survival rate (%): SR = (the number of the fish after 8 weeks per the number fish at commencement) x 100. Feed conversion ratio (FCR) and protein efficiency rate (PER) were calculated by the following equations: FCR = consumed feed (dry weight (g))/weight gain of fish (g), PER = (Wf - Wi)/protein intake. NPU = (the amount of fish body protein in the final fish – the amount of fish body protein in the initial fish)/the amount of protein intake. Where Wi is the initial weight of fish (g), Wf is the final weight of fish (g), t is the experimental period (day).

2.4 Statistical analysis

Mean differences in growth parameters and feed efficiency among treatments were tested by using ANOVA followed by DUNCAN tests at the significant level of 0.05. Statistical analyses were performed by using SPSS 16.0 software. Met and Lys requirements were calculated according to a model of broken line Zeitoun *et al*. (1976).

III. RESULTS AND DISCUSSION

3.1 Dietary Met requirement

Growth out and survival rate of fish fed different dietary Met levels

The survival rate of snakehead fish was not significantly different among dietary Met treatments, ranging from 77.5% to 84.2%. Growth rate was increased with increasing dietary Met levels from 17.5 to 28.2 g kg^{-1} protein ($P < 0.05$), but decreased with dietary Met levels from 31.9 g kg^{-1} protein. At the highest Met level of 35.5 g kg^{-1} protein, the growth of fish was the lowest (6.81g $fish^{-1}$ and DWG 0.1 g $fish^{-1}$ day^{-1}). Weight gain

(11.57 g) and DWG (0.21 g fish^{-1} day^{-1}) of fish were the highest at the dietary Met level of 28.2 g kg^{-1} protein (Table 3).

The broken-line analysis of the relationship between the dietary Met levels and DWG (Figure 1) was given by two equations y = -0.0117x + 0.5388 (R^2 = 0.9381) and y = 0.0058x + 0.0424 (R^2 = 0.8905). The highest DWG at the broken - line was estimated at the optimal Met level of x = 28.4 g kg^{-1} protein (11.9 g Met kg^{-1} diet) with dietary protein level of 42%.

Feed utilization efficiency of fish fed different dietary Met levels

The fish had higher FI in diets containing Met levels from 24.8 to 28.2 g kg^{-1} protein compared to the fish in other treatments (Table 4). The FCR of the treatments tended to decrease when fish fed the dietary Met levels increased. The highest FCR (1.15) was found in the lowest dietary Met at 17.5 g.kg^{-1} protein and significantly different from the dietary Met treatment of 28.2 g kg^{-1} protein (P <0.05). When fish fed with higher dietary Met levels (from 31.9 to 35.5 g kg^{-1} protein, FCR of fish tended to increase but not significantly from the lowest one (P> 0.05). The efficiency of protein utilization (PER) and net protein utilization (NPU) tended to increase (from 2.08 to 2.17) and (0.29 to 0.32) when fish fed dietary Met level increased from 17.5 to 24.8 g kg^{-1} protein, but remained relatively constant from 28.2 to 35.5 g kg^{-1} protein.

Body composition of fish in the dietary Met experiment

Levels of dietary Met had effects on body compositions and HSI of snakehead (Table 5). Crude protein and ash were significant differences among the dietary treatments (P>0.05). HSI content significantly increased with higher dietary Met levels (P<0.05), and highest HIS was observed at 35.5 g.kg^{-1} protein.

3.2 Dietary Lys requirement

Growth out and survival rate of fish fed different dietary Lys levels

The survival rates of snakehead fish were not significantly different among dietary Lys treatments, ranging from 72.5% to 82.5% (P>0.05). The growth of fish (DWG) increased with increasing dietary Lys levels from 30.1 to 68.4 g.kg^{-1} protein (P < 0.05) but remained relatively constant from 68.4 to 87.2 g kg^{-1} protein (table 6).

The broken-line analysis of the relationship between the dietary Lys levels in the diet and the DWG (Figure 2) was based on two equations y = 0.0013x + 0.0697 (R^2 = 0.9373) and y = -0.00001x + 0.16583. The optimal Lys level estimated at the highest DWG was x = 73.1 g kg^{-1} protein (30.7g Lys kg^{-1} diet) with protein level diet of 42%.

Feed utilization efficiency of fish fed different dietary Lys levels

The feed conversion ratio (FCR) of the treatments tended to decrease from 1.19 to 1.04 when fish fed increasing dietary Lys levels from 30.1 to 87.2 g kg^{-1} protein (Table 7). The highest FCR was 1.19 at 30.1 g kg^{-1} protein treatment and significantly different from the remaining treatments (P<0.05). The efficiency of protein utilization (PER) and net protein utilization (NPU) tended to increase when the dietary Lys level increased from 30.1 to 64.4 g kg^{-1} protein, but remained relatively constant with higher dietary Lys levles. In this experiment, the NPU values increased from 0.28 to 0.36 when the fish fed Lys content of 30.1 to 68.4 g kg^{-1} protein. At high Lys levels from 58.7to 87.2 g.kg^{-1} protein, there were no significant differences in NPU among treatments.

Fish body composition in dietary Lys treatments

Protein in the fish body tended to increase when fish fed increasing dietary Lys levels from 30.1 to 87.2 g kg^{-1} protein (Table 8). However, the difference was not significant among treatments with Lys content of 30.1 to 68.4 g kg^{-1} protein (P> 0.05). Meanwhile, lipid levels in the fish body decreased when fish fed increasing dietary Lys levels from 30.1 to 68.4 g kg^{-1} protein. However, there were not significant difference in body lipid content of fish fed dietary Lys of 39.6 to 87.2 g kg^{-1} protein (P>0.05). The ash in fish body was not affected by the Lys levels in the feed. The HSI of snakehead fish increased (2.19 to 2.68) with the increase of dietary Lys levels. The lowest HSI of fish (2.19) was found at the lowest Lys content of 30.1 g kg^{-1} protein.

3.2 Discussion

The demand for EAA should be met the needs of aquatic animal health and growth Results from this study showed that growth rate of snakehead increased with increasing dietary levels of Met (the optimal level of 28.8 g kg^{-1} protein) and Lys (the optimal level of 73.1 g kg^{-1} protein). Final body weight, WG and SGR increased with increasing dietary Met level from 17.5 to 28.2 g kg^{-1} protein while FCR showed the adverse tendency. The growth of experimental snakehead declined insignificantly at higher Met level diets from 31.9 g kg^{-1} protein when compared with those fish fed optimal Met level diet as excessive level of Met. It could be the exceeded Met level leading to accumulate and oxidate of Met to ketones and other toxic metabolites thereby it occurred the reduction in the growth rate (Murthy & Varghese, 1998) and superfluous level of Met in fish body would result in extra energy expenditure towards deamination and excretion (Sveier *et al*., 2001). Liou *et al.* (2014) reported that weight gain increased with increasing dietary Met level from 11.5 to 36.5 g kg^{-1} protein and then decreased insignificantly when dietary Met treached 45.3 g kg^{-1} protein, while FCR showed adverse tendency of *Megalobrama amblycephala*.

Comparing to other species, Met in feed for snakehead fish was similar to the Met requirement of *L. rohita*, 28.8 g kg^{-1} protein (Murthy and Varghese, 1998), Striped catfish *(P. hypophthalmus)* 26.7 g kg^{-1} protein (Hien *et al*., 2009b), Chanel catfish (*Ictalury punctatus*) 23.4 g kg^{-1} protein (Harding, 1977); carp (*Carassius auratus gibeilo*) 23 g kg^{-1} protein (Wang *et al*., 2016) and marine fish, i.e. *Sebastes schlegeli* 28.0 g kg^{-1} protein (Yan *et al*., 2007); Seed germs 25 g kg^{-1} protein (Liao *et al*., 2014). However, Met requirement for snakehead is lower than Chinese sucker (*Myxocyprinus asiaticus)* 32.0 g kg^{-1} protein (Chu *et al*., 2014); and marine fish, i.e. *Sparus macrocephalus* 45.3 g kg^{-1} protein (Zhou *et al*., 2011). Generally, the Met requirement for fish ranges from 20 to 40 g kg^{-1} protein and according to species (Wilson *et al*., 1989 ; Ahemd *et al*., 2003; Mai *et al*., 2006; Jia *et al*., 2013). Results of previous studies on Met requirements in snakehead are consistent with this study. The FCR of snakehead of dietary Met treatments tended to decrease when fish fed the dietary Met levels increased. Yan et al. (2007) study on Rockfish (*S. schlegeli*) showed that FCR decreased when fish fed increased dietary Met levels in feed increased higher than growth demand FCR of fish was significant. Similar results were also observed in other fishes, juvenile Japanese flounder (Alam *et al*., 2001), juvenile grouper *Epinephelus coioides* (Luo *et al*., 2005), and Atlantic salmon (Espe *et al*., 2008).

Relating to the fish body composition, many studies have shown that the Met levels in the diet affected the protein and lipid contents in fish body compositon. In the present study, protein contents in whole body of snake head tended to increase with dietary Met level up to the near requirement level, beyond which it remained nearly unchanged, which is in agreement with other reports (Kim *et al*., 1992 ; Ruchimat *et al*., 1997 ; Alam *et al*., 2000 ; Luo *et al*., 2005). The results on snakehead was similar with the results of Luo *et al*. (2005), Yan *et al*. (2007), Zhou *et al*. (2011), Niu *et al*. (2013), Liao *et al*. (2014). These authors reported that protein in the fish body increased significantly with the increase of Met level in the diet and at the highest dietary Met level, the protein in the fish body slightly reduced. The lipid content in snakehead body slight increased (from 3.54 to 3.68%) with the increase in Met level in the diet (from 17.5 to 35.5 g kg^{-1} protein). There are various reports showed similar results as that of current study, i.e. Wang *et al*. (2016), Niu *et al*. (2013), the lipid content in the fish body increasing with the increase levels of dietary Met but Met in the diet exceeding the requirement, slightly increased lipid content in the fish body found. This might be because of better utilization of protein with reduced deposition of lipid in the presence of Met resulting lean growth of fish (Sardar *et al*., 2009). This study results also were consistent with Kim *et al*. (1992) and Schwarz *et al*. (1998), but in contrast with the study in grouper (Luo *et al*., 2005). The liver is the organ that performs the highest Met hylation reaction as well as the sulfur metabolic reaction (Mato *et al*., 2002). The present study showed that liver weight was significantly increased with dietary Met supplementation. Similar results were observed in juvenile Jian carp, *Cyprinus carpio* (Tang *et al*., 2009), juvenile rockfish (Yan *et al*., 2007) and Atlantic salmon (Espe *et al*., 2008). Wang *et al*. (2016) showed that fish HSI increased with the increase in Met level in feed. An increased HSI was also observed when Atlantic salmon was fed too low levels of Lys (Espe *et al*., 2007) or Met (Espe, *et al*., 2008).

Apart from Met, the results from the second experiment showed that growth rates of snakehead increased and FCR decreased with increasing dietary Lys level from 30.1 to 68.4 g kg^{-1} protein. Higher levels of dietary Lys (77.9 g kg^{-1} protein) influenced negatively on growth and FCR of snakehead. Depressed growth and lower feed conversion were more commonly noted in *H. fossilis* receiving dietary Lys below and above 52.9 g kg^{-1} protein (20 g kg^{-1} diet). Studies by Dupree and Halver (1970) also demonstrated that Lys-deficient diets when fed to chinook salmon and channel catfish caused poor growth.

The Lys requirement for optimal growth of snakehead was greater than that of other freshwater fish species such as 41.3 g kg^{-1} protein for *S. quinqueradiata* (Ruchimat *et al*., 2001); 31.4 g kg^{-1} protein for freshwater mantle *M. nemurus* (Tantikitti and Chimsung, 2001); 59.6 g .kg^{-1} protein for grass carp *Bidyanus bidyanus* (Wang *et al*., 2005); 50.4 g kg^{-1} protein for *S. aurata* (Marcouli, 2006); 53.5 g kg^{-1} protein for stripted catfish (Hien *et al*., 2009a); 57 g kg^{-1} protein for common carp *Cyprinus carpio* (Nose, 1979). In contrast, the Lys demand for snakehead was lower than the Lys requirement of *Sparus macrocephalus* 86.4 g kg^{-1}protein (Zhou *et al*., 2010); or Goldfish (*Pelteobagrus fulvidraco*) 83.2 g kg^{-1} protein (Cao *et al*., 2012). The different results for determining the Lys requirement for fish species may be due to differences in methodology, protein sources in feed, protein content in diet, amino acid compositions in formula, culture conditions and species (Forster and Ogata, 1998 ; Kim et al., 1992). The feed conversion ratio (FCR) of the treatments tended to decrease when the fish were fed increasing dietary Lys levels while PER showed the adverse tendency. Lin et al. (2013) when studied on Chinese sucker (*Myxocyprinus asiaticus*), FCR decreased significantly with increasing dietary Lys from 28.3 to 54.9g.kg^{-1} protein and then showed no significant difference as the Lys level increases from 54.9 to 96.19g kg^{-1} protein, while the highest FCR was observed for fish fed with the diet containing 28.3 kg^{-1} protein. The studies of Cao *et al.* (2012) on the goldfish (*Pelteobagrus fulvidraco*), Lin *et al*. (2013) on chinese sucker (*Myxocyprinus asiaticus*) and Abimorad *et al*. (2010) on pacu juveniles showed that the PER increased with the increase of Lys level in the diet and the highest PER was reached when the diet contained the highest Lys content.

The protein content in the fish body tended to increase when the fish fed increasing dietary Lys levels from 30.1 to 87.2 g kg^{-1} protein. This result is similar to some study's results Zhou *et al*. (2010), Cao *et al*. (2012), Lin *et al*. (2013) and Wang *et al*. (2016). In this study, when snakehead fed high dietary Lys levels showed an increase in protein content in the body but lipid content decreased. This trend of increase in protein deposition and muscle gain, and reduction in carcass fat in response to required dietary Lys intake has also been reported for various other cultivable fish species (Luo *et al*., 2006). Highest whole-body lipid was noted in fish fed lowest level of Lys at 30.1g kg^{-1} protein of the diet which was found to decrease with increasing dietary Lys levels. This finding is in line with the trend in other studies reporting that Lys supplementation leads to increased protein content and decreased lipid content in fish (Zarate and Lovell, 1997; Mai *et al*., 2006; Zhang *et al*., 2008). Lys is active in promoting the transport of long-chain fatty acids across the inner mitochondrial membrane, resulting in extra energy from b-oxidation (Tanphaichitr, Horneand Broquist, 1971). However, dietary Lys deficiency suppresses the oxidation of these fatty acids, thereby increasing their availability or esterification to triacylglycerol and deposition in the various lipid storage tissues. Therefore, fish fed the lowest level of dietary Lys accumulated highest body fat. The HSI of snakehead fish increased (2.19 to 2.68) with the increase of dietary Lys levels. Wang *et al*. (2005) when studying the Lys demand of grass carp (*Ctenopharyngodon idella*) was reported: HSI increased from 2.2 to 2.6% when the fish fed Lys increased from 1.87 to 8,32 g kg^{-1} protein (P<0.05). the study of Lin *et al*. (2013) on Chinese sucker (*Myxocyprinus asiaticus*) also showed that HSI increased from 1.54 to 1.85% when fish fed Lys increased feed. The differences in HSI findings as well as chemical compositions in fish can be attributed to the ability of each species to absorb amino acids, amino acid changes in the body, amino acid sources in the diet and methods processing, but most probably due to species differences (Lou and Liu, 2006).

IV. CONCLUSION

In conclusion, the optimum requirements of dietary Met and Lys for snakehead were 11.9 g Met kg^{-1} diet (28.4 g kg^{-1} protein) and 30.7g Lys kg^{-1} diet (73.1 g kg^{-1} protein) based on the broken-line regression analysis of daily weight gain versus different dietary Met and Lys levels. At these dietary levels of Met and Lys, the experimental fish expressed the best growth, the lowest feed conversion ratio (FCR) and the highest protein utilization (PER). Moreover, the increase of dietary levels of both Met and Lys tended to increase the protein content in the fish body. Especially, the supplementation of Lys in the diet helped to reduce the lipid content in the fish body and the liver weight (hepasomatic index – HIS) of fish.

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Table 1. Formulation and proximate composition of the Met experimental diets (dry matter basis)

	Treatment diets					
Ingredients (g kg^{-1})	**M 17.5**	**M 20.9**	**M 24.8**	**M 28.2**	**M 31.9**	**M 35.5**
Fish meal	120	120	120	120	120	120
Wheat gluten	150	150	150	150	150	150
Dextrin	275	275	275	275	275	275
Gelatin	100	100	100	100	100	100
Cassava meal	150	150	150	150	150	150
MCP	10	10	10	10	10	10
Guar gum	0.5	0.5	0.5	0.5	0.5	0.5
Essential amino acids (EAA)	48.8	48.8	48.8	48.8	48.8	48.8
Nonessentials AA mixture	63.7	62.2	60.7	59.2	57.7	56.2
L – Methionine	**0**	**1.5**	**3**	**4.5**	**6**	**7.5**
Fish Oil	52	52	52	52	52	52
Mineral and vitamin premix*	30	30	30	30	30	30
Total	1000	1000	1000	1000	1000	1000
Chemical composition (%)						
Crude Protein	41.8	42.3	41.7	41.9	41.8	41.7
Crude fat	6.44	6.48	6.43	6.42	6.48	6.47
Ash	6.66	6.65	6.66	6.65	6.66	6.65
Gross energy (MJ kg^{-1})	20.2	20.3	20.2	20.4	20.3	20.1
Methionine						
Met g kg^{-1} diet	7.3	8.8	10.3	11.8	13.3	14.8
Met g kg^{-1} protein	17.5	20.9	24.8	28.2	31.9	35.5

Premix mineral and vitamin (unit.kg^{-1}): Vitamin A 2,000,000 IU; Vitamin D 400,000 IU; Vitamin E 6g; Vitamin B_1 800mg; Vitamin B_2 800mg; Vitamin B_{12} 2mg; Calcium D Pantothenate 2g; Folic acid 160mg; Choline Chloride 100g; Iron (Fe^{2+}) 1g; Zinc (Zn^{2+}) 3g; Manganese (Mn^{2+}) 2g; Copper (Cu^{2+}) 100mg; Iodine (I^-) 20mg; Cobalt (Co^{2+}) 10mg.

Table 2. Formulation and proximate composition of the Lys experimental diets (dry matter basis)

	Treatment diets						
Ingredients (g kg^{-1})	**L30.1**	**L39.6**	**L49.1**	**L58.7**	**L68.4**	**L77.9**	**L87.2**
Fish meal	120	120	120	120	120	120	120
Wheat gluten	150	150	150	150	150	150	150
Dextrin	275	275	275	275	275	275	275
Gelatin	100	100	100	100	100	100	100
Cassava meal	150	150	150	150	150	150	150
MCP	10	10	10	10	10	10	10
Guar gum	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Essential amino acids (EAA)	33.6	33.6	33.6	33.6	33.6	33.6	33.6
Nonessentials AA mixture	78.9	74.9	70.9	66.9	62.9	58.9	54.9
L – Lys	0	4	8	12	16	20	24
Fish Oil	52	52	52	52	52	52	52
Mineral and vitamin premix	30	30	30	30	30	30	30
Total	1000	1000	1000	1000	1000	1000	1000
Chemical composition (%)							
Crude Protein	41.5	42.1	41.9	41.9	41.7	41.8	41.9
Crude fat	6.47	6.45	6.45	6.47	6.47	6.47	6.49
Ash	6.70	6.63	6.66	6.65	6.65	6.66	6.66
Gross energy (MJ kg^{-1})	20.3	20.3	20.2	20.2	20.0	20.3	20.3
Lys							
Lys g kg^{-1} feed	12.6	16.6	20.6	24.6	28.6	32.6	36.6
Lys g kg^{-1} protein	30.1	39.6	49.1	58.7	68.4	77.9	87.2

Premix mineral and vitamin (unit.kg^{-1}): Vitamin A 2,000,000 IU; Vitamin D 400,000 IU; Vitamin E 6g; Vitamin B_1 800mg; Vitamin B_2 800mg; Vitamin B_{12} 2mg; Calcium D Pantothenate 2g; Folic acid 160mg; Choline Chloride 100g; Iron (Fe^{2+}) 1g; Zinc (Zn^{2+}) 3g; Manganese (Mn^{2+}) 2g; Copper (Cu^{2+}) 100mg; Iodine (I^-) 20mg; Cobalt (Co^{2+}) 10mg.

Table 3. Growth out and survival rate of snakehead fed with six dietary Met levels for 8 weeks

Met (g kg^{-1} protein)	Wi (g fish^{-1})	Wf (g fish^{-1})	WG (g fish^{-1})	DWG (g day^{-1})	SGR (% day^{-1})	SR (%)
17.5	2.95±0.02^{a}	10.87±0.3^{d}	7.93±0.39^{d}	0.14±0.01^{d}	2.51±0.08^{d}	77.5±6.87
20.9	2.92±0.03^{a}	12.36±0.70^{c}	9.44±0.68^{c}	0.17±0.01^{c}	2.77±0.10^{c}	80.84±4.19
24.8	2.88±0.10^{a}	13.17±0.22^{b}	10.28±0.29^{b}	0.19±0.01^{b}	2.92±0.09^{b}	84.17±11.02
28.2	2.95±0.02^{a}	14.52±0.5^{a}	11.57±0.52^{a}	0.21±0.01^{a}	3.07±0.07^{a}	82.50±05
31.9	2.95±0.03^{a}	12.67±0.36^{bc}	9.72±0.35^{bc}	0.17±0.01^{bc}	2.80±0.05^{bc}	80.83±1.67
35.5	2.88±0.07^{a}	9.70±0.56^{e}	6.81±0.61^{e}	0.12±0.01^{e}	2.33±0.15^{e}	79.17±6.94

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05).

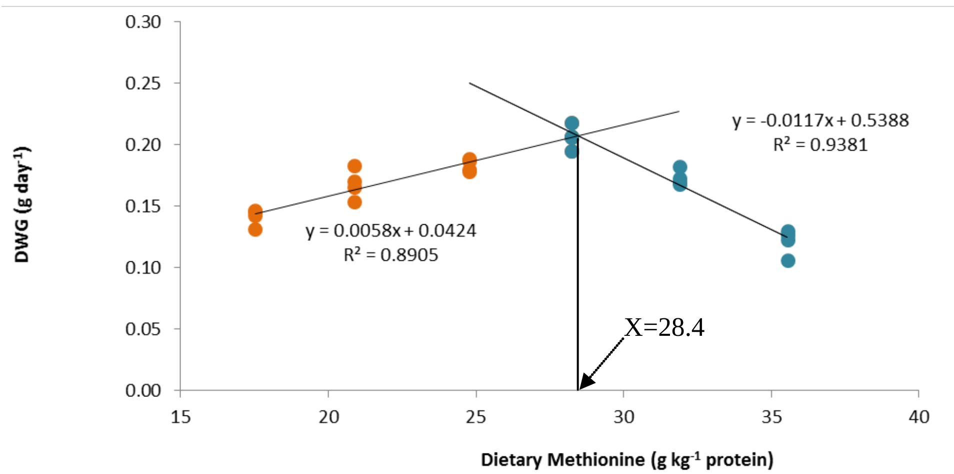

Figure 1. The relationship between Daily weight gain (DWG) and dietary Met levels (g kg^{-1} protein of snaked fed with the experimental diets for 8 weeks.

Table 4. Feed intake (FI), feed conversion ratio (FCR), protein efficiency (PER) and net protein utilization (NPU) of snakehead fed with six dietary Met levels for 8 weeks

Met (g kg^{-1} protein)	FI (% BW)	FCR	PER	NPU
17.5	2.50±0.20^{bc}	1.15±0.04^{a}	2.08±0.07^{b}	0.29±0.01^{b}
20.9	2.69±0.08^{ab}	1.13±0.01^{ab}	2.09±0.01^{b}	0.29±0.01^{b}
24.8	2.87±0.18^{a}	1.14±0.01^{ab}	2.11±0.02^{ab}	0.32±0.01^{a}
28.2	2.90±0.13^{a}	1.10±0.01^{b}	2.17±0.03^{a}	0.33±0.014^{a}
31.9	2.71±0.05^{ab}	1.11±0.02^{ab}	2.15±0.04^{ab}	0.32±0.01^{a}
35.5	2.39±0.17^{c}	1.13±0.03^{ab}	2.12±0.05^{ab}	0.32±0.01^{a}

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05).

Table 5. Effect of dietary Met levels on body composition (g kg^{-1}) of snakehead fed with the dietary Met levels for 8 week

Met (g kg^{-1}) protein	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	HSI
17.5	73.91±0.22^{a}	14.01±0.63^{b}	3.54±0.17^{a}	4.48±0.21^{a}	2.58±0.04^{b}
20.9	74.06±0.91^{a}	14.06±0.45^{b}	3.54±0.14^{a}	4.27±0.22^{a}	2.98±0.23^{ab}
24.8	73.34±0.74^{a}	15.05±0.37^{a}	3.63±0.12^{a}	4.43±0.14^{a}	2.86±0.04^{ab}
28.2	73.20±0.65^{a}	15.21±0.40^{a}	3.64±0.07^{a}	4.47±0.14^{a}	2.95±0.08^{ab}
31.9	73.08±0.38^{a}	15.20±0.25^{a}	3.67±0.06^{a}	4.41±0.6^{a}	2.89±0.08^{ab}
35.5	73.39±0.94^{a}	15.26±0.08^{a}	3.68±0.14^{a}	4.38±0.12^{a}	3.32±0.09^{a}

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05).

Table 6. Growth rate and survival rate of snakehead fed with seven dietary Lys levels for 8 weeks

Lys (g kg^{-1} protein)	Wi (g fish^{-1})	Wf (g fish^{-1})	WG (g fish^{-1})	DWG (g day^{-1})	SGR (% day^{-1})	SR (%)
30.1	2.36±0.05^{a}	8.56±0.06^{e}	6.20±0.09^{e}	0.11±0.001^{d}	2.48±0.05^{e}	72.50±8.77
39.6	2.36±0.05^{a}	9.30±0.16^{d}	6.94±0.12^{d}	0.13±0.006^{c}	2.64±0.03^{d}	75.84±6.87
49.2	2.36±0.04^{a}	9.67±0.05^{c}	7.31±0.04^{c}	0.13±0.001^{c}	2.71±0.03^{c}	78.33±3.34
58.7	2.35±0.05^{a}	10.23±0.10^{b}	7.88±0.14^{b}	0.14±0.001^{b}	2.83±0.06^{d}	71.67±8.39
68.4	2.38±0.04^{a}	11.63±0.12^{a}	9.25±0.14^{a}	0.17±0.005^{a}	3.04±0.04^{a}	70.84±11.35
77.9	2.37±0.05^{a}	11.58±0.10^{a}	9.21±0.13^{a}	0.17±0.006^{a}	3.05±0.02^{a}	79.17±11.01
87.2	2.34±0.05^{a}	11.57±0.09^{a}	9.23±0.05^{a}	0.16±0.005^{a}	3.07±0.02^{a}	82.50±5.0

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different (P>0.05).

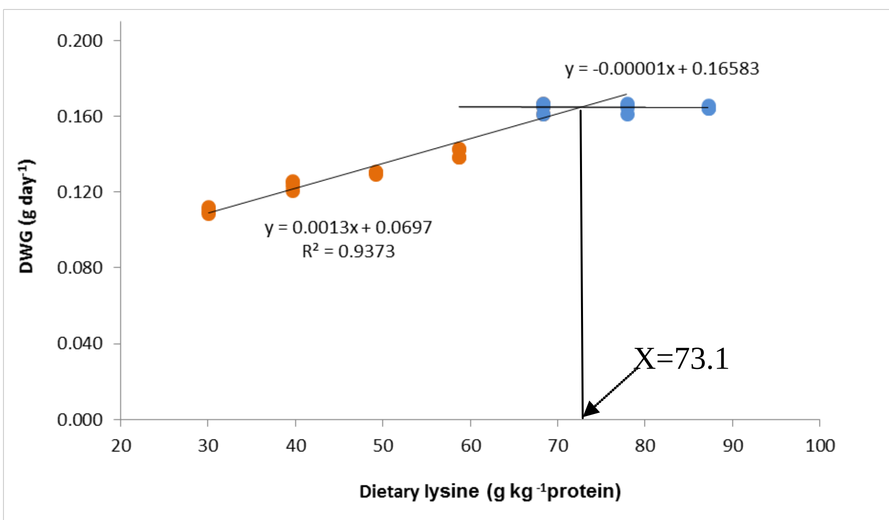

Figure 2. The relationship between DWG and dietary Lys levels (g kg^{-1} protein of snakead fed with the experimental diets for 8 weeks.

Table 7. Feed intake (FI), feed conversion ratio (FCR), protein efficiency (PER) and net protein utilization (NPU) of snakehead fed with seven dietary Lys levels for 8 weeks

Lys (g kg^{-1} protein)	FI (% BW)	FCR	PER	NPU
30.1	1.83±0.12^{a}	1.19±0.04^{a}	2.01±0.07^{c}	0.28±0.02^{c}
39.6	1.84±0.08^{a}	1.15±0.02^{ab}	2.08±0.02^{bc}	0.30±0.001^{bc}
49.2	1.90±0.16^{a}	1.11±0.01^{b}	2.16±0.02^{b}	0.31±0.03^{bc}
58.7	1.80±0.06^{a}	1.10±0.03^{b}	2.16±0.05^{b}	0.32±0.01^{ab}
68.4	1.78±0.22^{a}	1.05±0.04^{c}	2.29±0.09^{a}	0.36±0.05^{a}
77.9	1.96±0.21^{a}	1.02±0.03^{c}	2.35±0.08^{a}	0.34±0.03^{ab}
87.2	1.94±0.18^{a}	1.04±0.04^{c}	2.30±0.09^{a}	0.33±0.03^{ab}

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different(P>0.05).

Table 8. Body composition of snakehead fed with the dietary Lys levels for 8 weeks

NT Lys (g kg^{-1} protein)	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	HSI
30.1	72.43±0.29^{a}	14.67±0.15^{b}	4.69±0.16^{a}	6.14±0.23^{a}	2.19±0.07^{b}
39.6	72.17±0.18^{a}	14.73±0.19^{b}	4.54±0.21^{ab}	6.18±0.22^{a}	2.50±0.29^{ab}
49.2	72.14±0.59^{a}	15.03±0.31^{b}	4.50±0.08^{ab}	6.04±0.16^{a}	2.49±0.46^{ab}
58.7	72.75±0.14^{a}	15.01±0.01^{b}	4.42±0.05^{b}	6.00±0.04^{a}	2.55±0.19^{ab}
68.4	73.12±0.47^{a}	15.07±0.18^{ab}	4.37±0.15^{b}	5.90±0.26^{a}	2.53±0.05^{ab}
77.9	71.83±0.82^{a}	15.47±0.56^{a}	4.38±0.13^{b}	5.95±0.18^{a}	2.62±0.25^{ab}
87.2	72.84±0.41^{a}	14.99±0.16^{b}	4.42±0.05^{b}	6.10±0.47^{a}	2.69±0.10^{a}

Values (mean ± SD) in a column followed by the same superscript letter are not significantly different(P>0.05).