The optimal dietary DL-methionine on growth performance, body composition and amino acids profile of Pangasius catfish (*Pangasius bocourti*)

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Abstract. This study evaluated the effects of methionine on growth performance, proximate and amino acids composition of Pangasius catfish, *Pangasius bocourti*. Two hundred individual fish similar size (161.74±9.59 g per fish) were divided into 5 treatments. The methionine concentration in each diet analyzed by HPLC were 0.46 (Diet 1 - control), 0.55 (Diet 2), 0.61 (Diet 3), 0.71 (Diet 4) and 0.81 g $100g^{-1}$ DL-methionine. The experiment was carried for 10 weeks and at the end of the feeding trial weight gain (WG), final weight (FW), specific growth rate (SGR), feed conversion ratio (FCR), nitrogen retention, protein efficiency ratio (PER), whole body proximate and amino acids composition were measured. A completely randomized design with four replications was applied. The results indicated that fish fed Diet 4 (0.71 g $100g^{-1}$ DL-methionine) showed significantly improved WG, PER and FCR compared with the control after 10 weeks of the feeding trial. However, fish fed DL-methionine diets did not affect the whole body proximate and amino acid composition of the fish ($p > 0.05$) and the total essential and no-essential amino acids ratio was closed to 1.0 of maximization amino acid utilization. In addition, dietary administration of 0.81 g $100g^{-1}$ DL-methionine showed the reduction on growth compared with Diet 4 (0.71 g $100g^{-1}$ L-methionine). No significant differences on SGR, nitrogen retention and survival rate were detected between DL-methionine supplemented groups and the control. Quadratic nonlinear regression analysis of SGR against intake methionine levels indicated that the estimated optimal dietary methionine for maximum growth *P. bocourti* was 0.63 g $100g^{-1}$ of dry diet.

Key Words: Pangasius catfish, methionine, maximum growth, feed utilization.

Introduction. Plant protein sources may be not totally suitable for aquatic animal feeds compared with fish meal because of the absence of several essential amino acids (Tacon 1994; Van den Ingh et al 1996). Li et al (2009) have reported that dietary administration of amino acids or derivatives may be alternatives to develop amino acid balanced feeds that can offset environmental impacts on aquaculture animals, improve growth performance, and profitability of the aquaculture industry. Consequently, the addition of amino acid to fish diet become a common practice as plant protein sources continue to replace fish meal and other animal protein feedstuffs in formulated feed (Yuan et al 2011). Methionine is an essential amino acid for normal growth of fish (Ahmed et al 2003; Schwarz et al 1998). It takes part in protein synthesis and other important physiological functions (Zhou et al 2006). Fish fed methionine diet has been reported to improved growth performance, fish utilization and immune response (Boonyoung et al 2013; Kuang et al 2012; Ma et al 2013; Rolland et al 2015; Tang et al 2009; Yuan et al 2011). On the other hand, studies have indicated that methionine deficiency led to depressed survival rate, reduced growth performance in Jian carp, *Cyprinus carpio* (Tang et al 2009); rainbow trout, *Oncorhynchus mykiss* (Poston 1986); juvenile red drum, *Sciaenops ocellatus* (Goff & Gatlin III 2004) and the development of lenticular cataracts in seabream, *Pagrus major* (Takagi et al 2001).

Pangasius catfish (*Pangasius bocourti*) is an economically important species in Mekong Delta basin, especially in Vietnam and Thailand (Jiwyam 2010). Fish production was an approximately 1 million ton with an exported value of USD 1.85 billion (Trifković 2014). The Pangasius catfish is an omnivorous species with a protein requirement of an approximately 60% and feed formulations for this species have typically incorporated high levels of fish meal (Hung et al 2003). With the increasing of fish meal cost, there has been a trend towards decreasing fish meal levels in fish diet and replacing it with plant ingredients (Ma et al 2013). Soybean meal with high protein content has been proved to become alternative protein source for fish culturing (Rumsey 1993; Siddiqui et al 2014). However, one of the factors limiting its use at higher inclusion levels appeared to be a deficiency of methionine (Luo et al 2005; Mai et al 2006; Espe et al 2008). Therefore the objective of this work was to evaluate the effects of methionine on growth performance, body proximate and amino acids composition of Pangasius catfish.

Material and Method. This study was conducted in Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Thailand. The feeding trial was carried out 10 weeks (May-July, 2014).

Experimental diets. The ingredient composition of the experimental diets is presented in Table 1. Five isonitrogenous and isoenergetic diets were used fish meal, soybean meal and corn meal as intact protein sources. Soya and fish oil were supplemented as the lipid sources. The basal diet contained about 32% crude protein and 3% crude fat, which have been proved optimal for growth of Pangasius catfish. The experimental diets were formulated to simulate the methionine found in the fillets of Pangasius catfish (Men et al 2005) with varied levels of methionine ranged from 0 to 0.4 g 100g^{-1} dried matter. Then, the methionine concentration in each diet analyzed by HPLC were 0.46 (Diet 1 - Control), 0.55 (Diet 2), 0.61 (Diet 3), 0.71 (Diet 4) and 0.81 (Diet 5) g 100g^{-1} of diet, respectively (shown in Table 2). The ingredients were milled into powder to pass through 320 mm mesh and were thoroughly mixed with soya oil, fish oil, and then water was added to produce stiff dough. The dough was then passed through the mincer to form pellets. The pellets were finally dried in an oven at 50^{o}C to 10% and kept at -4^{o}C until use.

Table 1

Composition and proximate analysis of the experimental diets (g 100 g^{-1} dry matter)

Ingredient (g 100g^{-1})	*Diets (methionine level, g 100g^{-1})*				
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
Fish meal	4.76	4.76	4.76	4.76	4.76
Soybean meal	48.00	48.00	48.00	48.00	48.00
Corn meal	23.65	23.65	23.65	23.65	23.65
Rice bran	4.59	4.69	4.79	5.00	5.00
Soya oil	4	4	4	4	4
Fish oil	4	4	4	4	4
Alpha-starch	5	5	5	5	5
Di-calcium phosphate	1	1	1	1	1
Premix[1]	4	4	4	4	4
Methionine	0	0.10	0.20	0.30	0.40
Methylcellulose	1.00	0.80	0.60	0.29	0.19
Total	100	100	100	100	100
Total Met. (g 100g^{-1})	0.42	0.52	0.62	0.72	0.82
Proximate composition of the experimental diets (g kg^{-1} dry matter basis)					
Protein	34.18	34.34	34.87	34.65	34.97
Fat	2.45	2.50	2.33	2.74	2.47
Ash	8.62	8.83	8.74	8.88	8.96
Fiber	2.09	2.23	2.01	2.10	1.93
Dry matter	92.91	92.65	91.98	91.62	92.65
Gross energy (KJ g^{-1})	19.55	19.12	19.47	19.17	19.10

Note: [1](Vitamin and Mineral) mix kg^{-1}: Vit. A 36,000 I.U., Vit. D3 9,000 I.U., Vit. E 187 mg, Vit. K3 19 mg, Vit. B1 52 mg, Vit. B2 97 mg, Vit. B6 46 mg, Vit. C (coated) 68,800 mg activity, Vit. B12 60 mg, Panthothenic acid 93 mg, Niacin 130 mg., Folic acid 10 mg, Inositol 225 mg, Biotin 450 mg, manganese (Mn) 105 mg, copper (Cu) 9 mg, iron (Fe) 90 mg, Zinc (Zn) 90 mg, iodine (KI) 1.8 mg, cobalt (Co) 450 mg, magnesium (Mg) 1,900 mg, selenium (se) 150 mg, sodium (Na) 117 mg, potassium (K) 150 mg, calcium (Ca) 219 mg.

Table 2

Essential amino acid composition of the experimental diets (g $100g^{-1}$)

Amino acid	*Diets (methionine level, g $100g^{-1}$)*				
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
MET	0.46	0.55	0.61	0.71	0.81
CYS	0.46	0.44	0.42	0.42	0.42
M+C	0.92	0.98	1.03	1.14	1.23
LYS	1.77	1.81	1.74	1.75	1.82
THR	1.15	1.22	1.12	1.18	1.16
TRP	0.37	0.38	0.37	0.37	0.38
ARG	2.10	2.14	2.05	2.06	2.12
ILE	1.34	1.35	1.32	1.32	1.34
LEU	2.32	2.35	2.28	2.28	2.34
VAL	1.46	1.50	1.47	1.46	1.49
HIS	0.84	0.87	0.81	0.84	0.87
PHE	1.54	1.56	1.48	1.51	1.56
TYR	2.11	1.12	1.08	1.10	1.04
GLY	1.60	1.67	1.63	1.62	1.67
SER	1.36	1.48	1.32	1.45	1.40
PRO	1.68	1.86	1.85	1.80	1.84
ALA	3.17	3.29	3.13	3.20	3.24
ASP	5.41	5.48	5.32	5.39	5.47

Note: MET: Methionine; CYS: Cysteine; M+C: Methionine plus Cystein; LYS: Lysine; THR: Threonine; TRP: Tryptophan; ARG: Arginine; ILE: Isoleucine; LEU: Leucine; VAL: Valine; HIS: Histidine; PHE: Phenylalanine; TYR: Tyrosine; GLY: Glycine; SER: Serine; PRO: Proline; ALA: Alanine; ASP: Aspartate.

Experiment design. Pangasius catfish fingerlings were obtained from Phayao Inland Fisheries Research and development Center, Thailand. Fish were placed in 1000-L tanks. Upon arrival the fish were fed with the commercial diet for 2 months. Prior to the experiments, fish were fed a control diet for 2 weeks. Two hundred individual fish of a similar size (161.74±9.59 g $fish^{-1}$) were distributed into 20 cement tank (capacity 1 m^3) 10 fish $tank^{-1}$. The experiment was laid in a Completely Randomized Design with four replications. During the experiment, the diets were hand-fed to the fish *ad libitum* twice a day at 9:00 a.m. and 5:00 p.m., water temperature was maintained at 25-29^{o}C, and pH in a range of 7.5-8.2. The dissolved oxygen was maintained no less than 5 mg L^{-1}. Twenty percent of the water was exchanged every three days.

Data collection and calculation. Fish from each tank was bulk weighed for every two weeks. Growth performance of Pangasius catfish were calculated using the following equations:

Weight gain (WG%) = 100 (final body weight - initial body weight)/Initial body weight;

Feed Conversion ratio = total feed intake (g)/(final body weight - initial body weight) (g);

Specific growth rate (SGR) = 100 (ln final weight - ln initial weight)/total duration of experiment);

Protein efficiency ratio (PER) = (final body weight - initial body weight) (g)/total protein intake (g).

Every two weeks, three randomly selected fish from each replication were sealed in plastic bags and stored frozen (-20°C) for body composition and amino acid profile analysis.

Analytical methods. Proximate composition analysis of feed ingredients and experimental diets was performed by the standard methods of Association of Official Analytical Chemists (AOAC 1984). Samples of diets and fish were dried to a constant weight at 105°C for the determination of dry matter. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl system method after an acid digestion using an auto Kjeldahl System; lipid by ether extraction using Soxhlet; ash by combustion at 550°C. Amino acid concentrations were analyzed in duplicate by using High Performance Liquid Chromatography (HPLC: LC 6A, Shimadzu Co., Tokyo, Japan) according to the method described by Teshima et al (1986).

Statistical analysis. Mean value and standard deviation (S.D.) were calculated from the results. One way analysis of variance (ANOVA) was applied for comparison of the mean values; $p < 0.05$ was established as significant. The nonlinear regression analysis was used to estimating the dietary methionine optimum of Pangasius catfish at a confidence interval of 95%.

Results and Discussion

Growth performance. Fish fed 0.71 g 100g^{-1} methionine (Diet 4) had significant higher final weight (FW) and weight gain (WG) than those of the control and other supplemented groups (Table 3). However, no significant differences on FW and WG were found between other methionine supplemented groups and the control.

Table 3

Growth performance and feed utilization of *Pangasius bocourti* fed with difference concentration of L-methionine for 10 weeks

Parameters	*Diets (methionine level, g 100 g^{-1})*				
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
FW	327.18±5.56^{b}	332.15±1.62^{b}	334.21±9.24^{b}	392.92±5.96^{a}	343.67±3.33^{b}
WG	166.65±7.70^{b}	175.53±7.21^{b}	172.40±8.40^{b}	217.73±4.76^{a}	188.60±1.60^{b}
FCR	2.18±0.09^{a}	1.87±0.07^{b}	2.01±0.10^{ab}	1.59±0.05^{c}	1.98±0.04^{ab}
SGR (%)	1.01±0.05	1.07±0.02	1.04±0.04	1.15±0.03	1.14±0.01
PER	1.35±0.10^{c}	1.56±0.11^{b}	1.44±0.12^{bc}	1.82±0.11^{a}	1.44±0.05^{bc}
Nitrogen retention	1.92±0.83	1.79±0.44	1.85±0.02	2.20±0.43	2.24±0.28
Intake Met/ABW/day	0.11±0.003^{c}	0.11±0.003^{c}	0.13±0.003^{b}	0.13±0.003^{b}	0.19±0.005^{a}
Intake Met+Cys/ ABW/day	0.22±0.003^{bc}	0.21±0.006^{c}	0.23±0.003^{b}	0.22±0.003^{bc}	0.29±0.005^{a}
Survival rate	100	100	100	100	100

Mean with the different letters in same row are significantly different at $p < 0.05$.

For feed conversion ratio (FCR) and protein efficiency ratio (PER), dietary administration of methionine showed significantly improved FCR and PER compared with the control, except for diets Diet 3 and Diet 5 (Table 3). Fish fed 0.71 g 100g^{-1} methionine (Diet 4) had significant lower FCR and higher PER compared with the control and other methionine supplemented groups. In addition, dietary administration of Diet 4 showed significantly increased the intake methionine compared with the control.

Fish fed 0.81 g 100g^{-1} methionine (Diet 5) showed the reduction in growth compared with Diet 4. No significant difference on survival rate was detected between methionine supplemented groups and the control after 10 weeks of the feeding trial.

Body composition. There was no significant difference on body composition and amino acid profile between dietary methionine supplemented group and the control (Tables 4 and 5). The dietary was formulated with concentration of soy protein are always absent of methionine (Yuan et al 2011). Therefore, an extra amount methionine is necessary to be added to these diets in order to promote optimal growth and health of fish (Yuan et al 2011). The administration of methionine in fish diets to alternative protein sources has been reported to improve growth performance and feed utilization of several aquatic species such as rainbow trout (Boonyoung et al 2013; Rolland et al 2015); grouper, *Epinephelus coioides* (Luo et al 2005); turbot, *Psetta maxima* (Ma et al 2013); yellow croaker, *Pseudosciaena crocea* (Mai et al 2006); Jian carp (Tang et al 2009); Chinese sucker, *Myxocyprinus asiaticus* (Yuan et al 2011), and juvenile Cobia, *Rachycentron canadum* (Zhou et al 2006). Similarity, Pangasius catfish fed 0.71 g 100g^{-1} methionine

(Diet 4) had significant higher FW, WG and PER than the control. In addition, fish fed with Diet 4 showed significantly lower FCR than the control after 10 weeks of the feeding trial. From the quadratic nonlinear regression analysis of SGR the optimal dietary methionine administration levels for growth of Pangasius catfish were estimated to be 0.63 g $100g^{-1}$ (0.63%) as shown in Figure 1.

Table 4

Whole body composition of *Pangasius bocourti* fed with difference concentration of methionine for 10 weeks

Parameters	*Diets*				
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
Protein	42.80±3.71	42.40±1.96	43.18±0.08	44.53±1.91	45.04±1.25
Fat	45.26±0.33	47.54±4.49	47.41±0.86	43.31±1.21	43.44±4.62
Ash	9.35±0.25	8.85±0.18	8.79±0.23	9.04±0.66	98.95±0.08
Dry matter	99.10±0.28	97.60±1.41	99.28±0.04	98.24±0.16	9.09±1.52

Table 5

Whole body amino acid composition of *Pangasius bocourti* fed with difference concentrations of methionine for 10 weeks

Amino acid	*Diets*				
	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
Essential amino acid (EAA)					
MET	1.93±0.11	1.96±0.08	1.96±0.07	2.02±0.05	1.98±0.11
CYS	0.75±0.05	0.74±0.03	0.73±0.01	0.78±0.01	0.76±0.06
M+C	2.68±0.16	2.70±0.09	2.70±0.08	2.80±0.06	2.74±0.16
LYS	6.32±0.20	6.32±0.29	6.31±0.20	6.41±0.16	6.35±0.25
THR	3.31±0.14	3.27±0.06	3.29±0.04	3.30±0.04	3.28±0.11
TRP	0.74±0.03	0.75±0.02	0.75±0.02	0.76±0.03	0.71±0.04
ARG	4.64±0.26	4.70±0.09	4.80±0.10	4.57±0.25	4.64±0.15
ILE	3.24±0.10	3.29±0.13	3.29±0.10	3.26±0.07	3.30±0.08
LEU	5.51±0.21	5.51±0.17	5.56±0.08	5.46±0.11	5.52±0.17
VAL	3.60±0.08	3.64±0.13	3.67±0.08	3.65±0.04	3.68±0.09
HIS	1.67±0.07	1.70±0.06	1.73±0.01	1.72±0.06	1.69±0.05
PHE	2.99±0.11	3.02±0.08	3.02±0.05	3.01±0.08	3.04±0.10
Non-essential amino acid (NEAA)					
TYR	0	0	0	0	0
GLY	6.43±0.12	6.35±0.04	6.40±0.09	6.34±0.14	6.50±0.05
SER	3.11±0.19	3.08±0.04	3.14±0.10	3.13±0.04	3.10±0.08
PRO	3.97±0.17	3.99±0.13	4.05±0.05	3.88±0.17	4.19±0.07
LAN	0	0	0	0	0
ALA	4.99±0.11	5.00±0.10	5.03±0.04	5.01±0.06	5.08±0.10
ASP	7.24±0.30	7.25±0.20	7.30±0.08	7.30±0.08	7.20±0.22
GLU	10.81±0.42	10.74±0.25	10.89±0.12	10.91±0.22	10.77±0.27
∑EAA	33.97±1.29	34.16±1.07	34.39±0.51	34.16±0.65	34.18±0.11
∑NEAA	37.29±0.91	37.15±0.51	37.54±0.12	37.36±0.36	37.59±0.69
∑EAA/∑NEAA	0.91±0.01	0.92±0.02	0.91±0.02	0.91±0.11	0.91±0.02

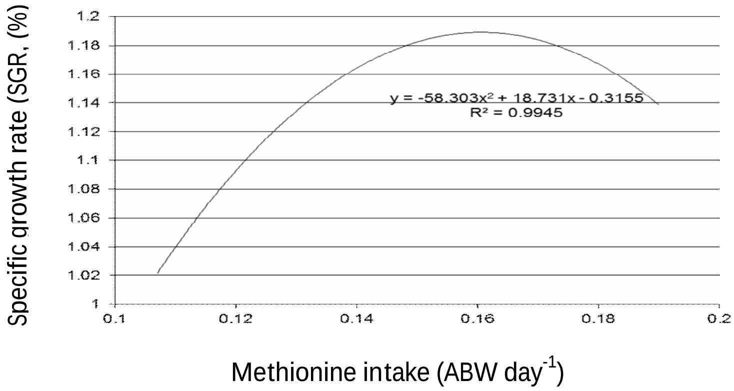

Figure 1. Second-degree polynomial relationship of specific growth rate (SGR) to methionine intake of borcurti's catfish at 10 weeks.

This was similar to previous studies on rainbow trout - 0.52% (Kim et al 1992a); hybrid striped bass - 0.87% (Keembiyehetty & Gatlin 1997). In contrast, the result in this experiment was lower than results from previous studies on Japanese flounder, *Paralichthys olivaceus* (1.49%) (Alam et al 2001) and large yellow croaker, *Pseudosciaena crocea* (1.42%) (Mai et al 2006) and turbot, *Psetta maxima* (Ma et al 2013). The differences in methionine requirement may be due to fish species and size, feed ingredients and dietary cystine content, feeding regime, environmental conditions, and method of data analysis (Goff & Gatlin III 2004; Kim et al 1992b; Shearer 2000). In addition, it was known that dietary methionine can be converted to cystine when necessary in the body. The presence of cystine in the fish diet may reduce the amount of methionine required for maximum growth (Ahmed et al 2003). Therefore, the requirement for total sulfur amino acids can be satisfied by either methionine alone or the proper combination of methionine and cystine (Ahmed et al 2003). The cystine could replace methionine has been demonstrated in several fish species such as about 42% in rainbow trout (Kim et al 1992b), 50% in yellow perch, *Perca flavescens* (Twibell et al 2000); 40–50% in red drum, *Sciaenops ocellatus* (Moon & Gatlin III 1991; Goff & Gatlin III 2004) and in hybrid striped bass (Griffin et al 1994). However, the action mechanism of the conversion efficiency of methionine to cystine and cystine sparing effect for methionine in fish is not clarified. More studies are necessary to clarify this phenomenon.

It was interested to note that fish fed 0.81 g $100g^{-1}$ L-methionine (Diet 5) had significant lower FW, WG and higher FCR than the Diet 4. Similar trend was also observed on several fish species (Ahmed et al 2003; Mai et al 2006; Murthy & Varghese 1998). The reduction in growth at high levels of dietary methionine could be attributable to amino acid toxicity and catabolism of excessive methionine. The accumulation of amino acid or its degradation products in body pools may stress enzymatic systems and lead to further accumulation and possible toxicity (Ahmed et al 2003). It has also been reported that excessive levels of amino acids may become toxic and may have adverse effect on growth, because the disproportionate intake affects the absorption and utilization of other amino acids or decrease the diet's palatability (Murthy & Varghese 1998). However, the exact mechanism of excessive methionine affects the growth of the fish is needed to prove this phenomenon.

Present study indicated that whole body proximate composition of Pangasius catfish was not significantly affected by dietary methionine. This was in agreement with previous studies on grass carp, *Ctenopharyngodon idella* (Wang et al 2005); sea bream, *Pagrus major* (Chatzifotis et al 1996); freshwater catfish, *Mystus nemurus* (Tantikitti & Chimsung 2001); turbot (Kroeckel et al 2013) and Chinese sucker, *Myxocyprinus asiaticus* (Chu et al 2014; Yuan et al 2011). The reason for this may be attributable to an increase in nitrogen retention with increasing dietary methionine (Kim et al 1992b; Ruchimat et al 1997). Furthermore, dietary restriction of one essential amino acid led to an increase in oxidation of other essential and non-essential amino acids present at normal levels in the diets (Rodehutscord et al 2000; Ozório et al 2002). The difference in these findings may be due to the difference of fish size, species, diet composition and experimental conditions. For whole body amino acid composition, no significant difference was found between dietary DL-methionine and the control. This was similar to the observations that have been reported in juvenile Chinese sucker (Chu et al 2014) and rainbow trout (Kim et al 1992b). Moreover, the essential amino acid (EAA) content increased with the increasing of the diet methionine supplementation in the diet. The reason for probably because of dietary restriction of one EAA led to an increase in oxidation of other essential and non-essential amino acids present at normal levels in the diets (Rodehutscord et al 2000). In addition, a relationship between tissue EAA levels and dietary requirements has been suggested (Cowey 1994). Increased level of dietary methionine did not affect to essential amino acids (EAA) and non-essential amino acids (NEAA) ratio. This ∑EAA/∑NEAA ratio was held close to 1 to maximize amino acid utilization. Few studies have also looked into the potential of some of the ratios between dietary ∑EAA/∑NEAA (Hughes 1985; Mambrini & Kaushik 1994). Maximum mean N retention and minimum mean N excretion gain was achieved by rainbow trout fed with diets containing ∑EAA/∑NEAA ratio at 57:43 (Green et al 2002). In the result of the present study the ∑EAA/∑NEAA ratio was similarly as in gilthead seabream, a dietary ∑EAA/∑NEAA ratio of 1.1 was found to be better than a ratio of 0.8 (Gómez-Requeni et al 2003).

Conclusions. The current results on feeding methionine to Pangasius catfish (initial average weight 161.74 g per fish) showed the improvement effects on growth performance and feed utilization. This study indicated that this information is important for practical feed formulation for Pangasius catfish diet. Analysis of dose response with nonlinear regression on the basis of SGR, the optimal dietary methionine requirements of *Pangasius bocourti* intimately was 0.63 g $100g^{-1}$ of dry diet for maximum growth and feed utilization.

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