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Original Research Article

Optimizing protein and lipid levels in practical diet for juvenile northern snakehead fish (*Channa argus*)

Gladstone Sagada [a], Jianming Chen [b, *], Binqian Shen [b], Aixia Huang [b], Lihui Sun [b], Jianhu Jiang [b], Chunhua Jin [a, *]

[a] School of Marine Sciences, Ningbo University, Ningbo 315211, China
[b] Agriculture Ministry Key Laboratory of Healthy Freshwater Aquaculture, Zhejiang Institute of Freshwater Fisheries, Huzhou 313001, China

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ABSTRACT

A 3 × 3 factorial feeding trial was conducted to evaluate the production response of juvenile northern snakehead fish (*Channa argus*). Nine diets containing 3 protein levels (45%, 48% and 51%) and 3 lipid levels (9%, 12% and 15%) were formulated and fed to triplicate groups of juvenile northern snakehead (15.78 ± 0.09 g/fish) for 8 weeks. The formulated diets were named as P45L9, P45L12, P45L15, P48L9, P48L12, P48L15, P51L9, P51L12 and P51L15 (P-Protein, L-Lipid), respectively. Fish fed diets with the lowest protein and lipid combination (P45L9) had the lowest growth performance. Weight gains (WG) of fish fed the 4 diets P48L12, P48L15, P51L9, and P51L12 were not significantly different ($P > 0.05$), but significantly higher ($P < 0.05$) than those of fish fed the other diets. Fish fed diets P48L12 and P48L15 had significantly lower ($P < 0.05$) feed conversion ratios (FCR) than the rest of the treatments. Protein retentions (PR) among fish fed the diets P45L12, P45L15, P48L12, P48L15, P51L9, and P51L12 were similar and significantly higher ($P < 0.05$) than those of fish fed the remaining diets. Protein sparing effect was observed in the treatments when fish was fed diets containing 45% or 48% dietary protein levels with dietary lipid increased from 9% to 12%. Fish fed diets with 9% lipid tended to have lower viscerosomatic index (VSI), hepatosomatic index (HSI), and whole-body lipid. Increasing dietary protein level significantly increased ($P < 0.05$) liver moisture and lipid while dietary lipid level increased liver lipid. Intestinal lipase activity increased significantly ($P < 0.05$) with increasing dietary lipid and protein levels while intestinal α-amylase and protease activities were not significantly influenced ($P > 0.05$) by dietary treatments. Based on these results, the diet containing 48% protein with either 12% or 15% lipid is the optimal for supporting growth and feed utilization of juvenile northern snakehead under the current testing conditions.

1. Introduction

Fish farmers spend huge percentages of their total production budgets on feed, which makes it generally regarded as the most expensive item in aquaculture. Protein, being the most expensive ingredient in fish feed is also deemed the most important because its regular intake is required for the fish to utilize amino acids either to build new proteins during growth and reproduction or to replace existing ones during maintenance (NRC, 2011). High level of good quality protein feed yields good growth especially in carnivorous fish (Lee et al., 2002). Inadequate protein in feed results in growth reduction but when it is overloaded in a diet, the excess protein is converted to energy through direct oxidation of amino acids. This will lead to increased production cost and extra nitrogenous waste (Webb and Gatlin, 2003; Wu and Gatlin, 2014). Release of nitrogen into an aquatic system poses environmental concerns and might also impair feeding (Kaushik and Medale, 1994). The high cost can be avoided by offsetting diet's energy with lipid and carbohydrate. Copious reports intimated that efficient utilization of protein can be improved by inclusion of lipids and carbohydrates in fish diets (Cho and Kaushik, 1990; Kaushik and Medale, 1994). Between lipid and

* Corresponding authors.
E-mail addresses: aqua_labjm@163.com (J. Chen), jinchunhua@nbu.edu.cn (C. Jin).

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carbohydrate, lipids are preferred as energy sources in carnivorous species since carbohydrate is less available in their natural diets. Furthermore, dietary lipids serve as an important source of essential fatty acids, as well as carrier of fat-soluble vitamins (Watanabe, 1982; Mai et al., 1995; Lee et al., 2002). So it is important to optimize the level of combination of dietary protein and lipid in developing cost-effective feed to attain good growth in a culture species.

The snakeheads are air-breathing fresh water carnivorous fish having considerable value as food fish widely cultured in China with a total annual production of over 510,000 metric tons in 2014 (FAMAC, 2015). They are also prevalent in most southern and south-eastern Asian countries largely due to their good taste and fast growth as well as resistance to diseases, handling and tolerance to inferior water quality (Webster and Lim, 2002; Hossain et al., 2008). Snakehead fish culture highly depended on feeding with trash fish and diets made from animal raw materials such as poultry liver due to their strict carnivorous nature. In recent years, with the high demand and increasing price of trash fish as well as environmental and ecological concerns, it is important to develop formulated diets to support the sustainable production of this fish.

Striped snakehead (*Channa striatus*) is the most commonly cultured species in southern and south-eastern Asian countries. In China, the main farmed snakehead species include blotched snakehead (*Channa maculata*), northern snakehead (*Channa argus*) and hybrid snakehead (*Channa maculates* ♀ × *C. argus*♂). Blotched and hybrid snakeheads are mainly raised in southern provinces such as Guangdong and its adjacent provinces, where the climate is relatively warm with long feeding periods for the fish. Northern snakehead is mostly farmed around the Yangtze River catchment area and northeastern regions of China, due to its ability to survive during the colder winter weather. Commercial feeds for blotched and hybrid snakeheads have been developed based on nutritional requirement data available for striped and hybrid snakeheads (Samantaray and Mohanty, 1997; Aliyu-Paiko et al., 2010a, 2010b; Liu et al., 2011). However, similar formulated feed for blotched and hybrid snakeheads failed to support satisfactory growth in northern snakehead, suggesting that the feed developed for other snakeheads may not supply sufficient nutrients to meet requirement of the northern snakehead. Unfortunately, there are no commercially available feed formulated for this species and only little empirical information on its nutritional requirements has been reported so far (Shan et al., 2016). Therefore, the current study was conducted to evaluate the effect of dietary protein and lipid levels in practical diets on the growth performance, body composition and digestive enzyme activities of juvenile *C. argus*.

2. Materials and methods

2.1. Experimental design and diets

This study involved a 3 × 3 factorial design with 3 levels of dietary crude protein (CP) (45%, 48% and 51%) and 3 levels of dietary crude lipid (CL) (9%, 12% and 15%). The formulated diets were named as P45L9, P45L12, P45L15, P48L9, P48L12, P48L15, P51L9, P51L12 and P51L15 (P-Protein, L-Lipid), respectively (Table 1). The levels of protein and lipid in the formulation were based on earlier studies on a similar species, *C. striatus* (Samantaray and Mohanty, 1997; Aliyu-Paiko et al., 2010a, 2010b). All experimental diets were processed at 130 °C with a twin-screw extruder to 5 mm floating pellets and oven-dried at 70 °C in a local feed mill, DeQing Hong Li Feed Company Limited (Huzhou, Zhejiang, China) and then stored at 4 °C until subsequently crushed and sieved to desirable size for use.

2.2. Fish and experimental procedure

Snakehead juveniles from one nursery pond were obtained from a commercial hatchery (De Qing County Goayi farm, Huzhou, Zhejiang, China). The feeding trial was preceded by a 7-day acclimation period during which experimental fish were fed commercial diet containing 50% CP and 9% CL. A total of 2,700 pieces (initial average weight 15.78 ± 0.09 g/fish, $n = 100$) were randomly selected and distributed into 9 treatment groups with 3 replicates of 100 pieces each and stocked in in-door concrete flow-through tanks (200 cm × 150 cm × 60 cm) with 1,200 L of fresh water and flow rate 0.42 L/m at the Zhejiang Freshwater Breeding Center (Huzhou, Zhejiang, China). Temperature (23 to 26.5 °C), dissolved oxygen (4.8 to 9.3 mg/L) and pH (7.0 to 7.7) values were measured twice a day while total ammonia (0.11 to 0.26 mg/L) and nitrates (0.02 to 0.08 mg/L) were also monitored weekly. Dead fish were immediately removed from the tanks as soon as noticed and there was regular cleaning to get rid of feces and uneaten feed. Feeding lasted for 8 weeks with each tank fed 3 times daily (08:00, 12:00 and 16:00) to apparent satiation.

2.3. Sampling and chemical analysis

At the beginning of the trial, 10 fish were collected for initial body composition analyses. After the 8-week feeding period, fish were anaesthetized with eugenol (80 μL/L, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and recorded for total weights for each tank and then counted to determine average weight. Five fish per tank were used for whole body composition analysis. Twenty from each tank were individually measured, weighed and dissected to obtain muscle, liver and viscera for computing body condition indices such as condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI) as well as muscle and liver composition analysis. Five more samples from each tank were dissected and intestines immediately frozen in liquid nitrogen and then stored at −80 °C for subsequent digestive enzyme analysis. Proximate composition of the test diets and fish samples was analyzed following AOAC (1997) methods. Moisture was determined by drying in an oven with air circulation at 105 °C to constant weight. Crude protein (Nitrogen × 6.25) determination was by the Kjeldahl method after acid digestion using a BUCHI Digestion Unit K-435(BUCHI Labortechnik AG, Switzerland). Crude lipid determination was done by ethyl-ether extraction (BUCHI Labortechnik AG, Switzerland). Ash was incarcerated in a muffle furnace at 600 °C.

2.4. Digestive enzyme assay

Intestine samples were homogenized in normal saline (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) at a ratio of 1 g sample: 9 mL normal saline. The homogenates were centrifuged at 10,000 × *g* for 20 min and the supernatant re-centrifuged at 10,000 × *g* for 30 min to obtain a clearer supernatant (at 4 °C temperature) which was analyzed for enzyme activities. Proteolytic activity was determined according to the Folin phenol reagent method of Kunitz (1947) at 37 °C temperature and 7.5 pH. Lipase activity was determined at 37 °C according to procedures of the Nanjing Jiancheng Bioengineering Institute, test kit A054. Alpha-amylase activity was assayed according to the procedures of Bernfeld (1955) at 25 °C temperature and 6.9 pH. Proteolytic activity unit (U) was expressed as 1 μg tyrosine released per minute; lipase activity unit (U) as 1 μmol substrate consumed per minute; and amylase activity unit (U) as 1 μmol of maltose released per minute. Protein concentration in supernatant was determined with the Coomassie brilliant blue method of Bradford (1976).

Table 1
Formulation and proximate composition of the experimental diets (g/kg as fed).

Item	Diets[1]								
	P45L9	P45L12	P45L15	P48L9	P48L12	P48L15	P51L9	P51L12	P51L15
Ingredient									
Fish meal[2]	351	351	351	387	387	387	405	405	405
Soybean meal[3]	100	82	82	74	40	40	40	40	0
Poultry meal[4]	117	117	117	129	129	129	135	135	135
Gluten[3]	7	31	31	34	50	50	58	60	78
Spraying blood meal[5]	30	31	31	34	50	50	58	60	78
Peanut meal[3]	70	50	50	50	30	30	30	24	0
Flour[3]	215	198	198	184	175	175	178	170	169
Fish oil[2]	33	63	93	28	59	89	26	56	85
Squid offal[2]	10	10	10	10	10	10	10	10	10
Monocalcium phosphate[6]	12	12	12	15	15	15	15	15	15
Vitamin premix[6]	15	15	15	15	15	15	15	15	15
Mineral mixture[7]	10	10	10	10	10	10	10	10	10
Zeolite[8]	30	30	0	30	30	0	20	0	0
Proximate composition (as fed)									
Dry matter	91.3	91.1	92.3	92.2	91.8	92.2	91.9	91.6	90.5
Crude protein	46.7	46.3	46.2	48.2	48.1	49.0	51.6	50.8	51.2
Crude lipid	9.4	11.8	14.5	9.3	11.3	14.4	9.2	11.9	14.9
Ash	12.2	12.1	13.4	12.6	12.3	11.5	12.3	11.5	10.7
Carbohydrate[9]	23.0	21.0	18.2	22.0	20.1	17.3	18.9	17.4	13.8
Gross energy, kJ/g[10]	18.7	19.2	19.8	18.8	19.3	20.2	19.1	19.7	20.3
Protein:Energy ratio, mg/kJ	25.0	24.1	23.4	25.6	26.0	24.2	27.1	25.8	25.2

[1] Diets: P45L9 = 45% crude protein, 9% crude lipid; P45L12 = 45% crude protein, 12% crude lipid; P45L15 = 45% crude protein, 15% crude lipid, etc.
[2] Rongchen Haida Fish Meal Company Ltd., Weihai, China; fishmeal: domestic brown fishmeal.
[3] Cargill Green and Oil (Nantong) Company Ltd., Nantong, China.
[4] Pet grade poultry: American Proteins Inc, Cumming, GA, USA.
[5] Zhejiang Sonac Biology Company Ltd., Anji, China.
[6] Hangzhou Minshen Bio-Tech Co., Ltd. Hangzhou, China; Vitamin premix (g/kg mixture): retinol acetate, 0.80; cholecalciferol, 0.06; α-tocopherol acetate, 4.00; menadione, 8.00; thiamin, 2.00; riboflavin, 2.00; pantothenic acid, 6.00; pyridoxine, 2.00; folic acid, 0.50; niacin, 15; cyanocobalamin, 0.02; ascorbic acid, 20.00.
[7] Mineral mixture (g/kg mixture): $FeSO_4$, 40; $CuSO_4 \cdot H_2O$, 25; $MnSO_4 \cdot H_2O$, 10; $ZnSO_4 \cdot H_2O$, 100; $MgSO_4 \cdot 7H_2O$, 200; $CaCO_3$, 0.35; KI, 0.05; Na_2SeO_3, 0.30 and zeolite, 624.3. Hangzhou Minshen Bio-Tech Co., Ltd. Hangzhou, China.
[8] Ningbo Jiahe New Material Technology Company Ltd., Ningbo, China.
[9] Carbohydrate (%) = 100 − (%crude protein + %crude lipid + %moisture + %ash).
[10] Calculated based on 17.2 kJ/g carbohydrate; 23.6 kJ/g protein and 39.5 kJ/g lipid.

2.5. Calculations and statistical analyses

$$\text{Feed conversion ratio(FCR)} = \text{Feed weight as dry(g)} / \text{Wet weight gain(g)};$$

$$\text{Weight gain(WG\%)} = 100 \times [\text{Final body weight(g)} - \text{Initial body weight(g)}] / \text{Initial body weight(g)};$$

$$\text{Specific growth rate(SGR, \%)} = 100 \times [\text{Ln Final body weight(g)} - \text{Ln Initial body weight(g)}] / \text{Feeding period(days)};$$

$$\text{HSI(\%)} = 100 \times \text{Liver weight (g)} / \text{Body weight (g)};$$

$$\text{VSI (\%)} = 100 \times \text{Viscera weight(g)} / \text{Body weight(g)};$$

$$\text{CF} = \text{Body weight(g)} / \text{Total length}^3\text{(m)};$$

$$\text{Protein retention(PR, \%)} = 100 \times [\text{Final fish body protein(g)} - \text{Initial fish body protein(g)}] / \times \text{Total protein fed(g)}.$$

All data were subjected to one-way ANOVA to determine the significance due to effects of dietary treatments, and two-way ANOVA to determine the significance due to levels of protein, lipid or their interaction. Post Hoc was analyzed by Tukey's HSD test with statistical significance determined at $P < 0.05$. All statistical analyses were carried out using software SPSS (version 20, SPSS Inc. Chicago IL, USA).

3. Results

3.1. Growth performance and feed utilization

Growth performance, feed utilization and survival rate of northern snakehead juveniles fed the different diets after 8 weeks of feeding are shown in Table 2. According to one-way ANOVA, diets P48L12, P48L15, P51L9 and P51L12 produced significantly higher ($P < 0.05$) WG values than the rest. Specific growth rate of fish fed diets P48L12 and P51L9 were significantly higher ($P < 0.05$) than those of P45L9, P45L12, P45L15, P48L9 and P51L15, but not significantly different ($P > 0.05$) from those of P48L15 and P51L12; while diets P48L12 and P48L15 produced lower FCR values than the rest of the treatments. In terms of protein retention (PR), diets P45L12, P45L15, P48L12, P48L15, P51L9 and P51L12 had significantly higher values ($P < 0.05$) than P45L9, P48L9, and P51L15. Based on two-way ANOVA, dietary CP and CL levels significantly affected ($P < 0.05$) WG, SGR, PR and FCR of fish. Significant improvements in WG, SGR and FCR were observed by increment of dietary CP levels from 45% to 48% ($P < 0.05$). However, a further increase of CP level to 51% resulted in a significant increase in FCR and reduction in PR ($P < 0.05$), while WG and SGR were not affected significantly. Weight gain and SGR were significantly enhanced ($P < 0.05$) by increment

Table 2
Growth performance and feed utilization of northern snakehead juveniles fed diets of varying protein and lipid levels for 8 weeks.

Item	WG, %	SGR, %	FCR	PR, %	Survival, %
Individual treatment means[1]					
P45L9	286.0^{x}	2.4^{z}	1.6^{v}	29.2^{w}	96.3
P45L12	335.2^{w}	2.6^{wxy}	1.2^{y}	36.5^{v}	98.7
P45L15	334.9^{w}	2.6^{xy}	1.3^{x}	34.9^{v}	97.0
P48L9	323.4^{w}	2.6^{y}	1.6^{v}	30.1^{w}	95.7
P48L12	364.4^{v}	2.7^{v}	1.0^{z}	38.7^{v}	99.0
P48L15	360.6^{v}	2.7^{vw}	1.0^{z}	38.5^{v}	98.0
P51L9	365.7^{v}	2.8^{v}	1.2^{y}	36.1^{v}	98.7
P51L12	360.3^{v}	2.7^{vwx}	1.1^{y}	37.5^{v}	98.0
P51L15	312.9^{w}	2.5^{y}	1.5^{w}	29.6^{w}	96.0
Pooled SEM[2]	5.26	0.02	0.04	0.75	0.31
Means of main effects[3]					
Protein					
45	318.7^{b}	2.6^{b}	1.4^{a}	33.5^{b}	97.3
48	349.5^{a}	2.7^{a}	1.3^{c}	35.7^{a}	97.6
51	346.3^{a}	2.7^{a}	1.3^{b}	34.4^{ab}	97.6
Lipid					
9	325.1^{C}	2.6^{C}	1.5^{A}	31.8^{C}	96.9^{B}
12	353.3^{A}	2.7^{A}	1.1^{C}	37.5^{A}	98.6^{A}
15	336.1^{B}	2.6^{B}	1.3^{B}	34.3^{B}	97.0^{B}
Two-way ANOVA (*P*-value)					
Protein	<0.001	<0.001	<0.001	0.015	0.912
Lipid	<0.001	<0.001	<0.001	<0.001	0.02
Protein × Lipid	<0.001	<0.001	<0.001	<0.001	0.028

WG = percentage weight gain; SGR = specific growth rate; FCR = feed conversion ratio; PR = protein retention; P45L9 = 45% crude protein, 9% crude lipid; P45L12 = 45% crude protein, 12% crude lipid; P45L15 = 45% crude protein, 15% crude lipid, etc.

[1] Treatment means represent the average values of 3 tanks per treatment. Within the same column, means with the same superscript letter (v, w, x, y, z) are not significantly different ($P > 0.05$).

[2] Standard error of the mean (pooled).

[3] Main effect means followed by the same superscript letter (dietary protein = lowercase; dietary lipid = upper case) in the same column are not significantly different ($P > 0.05$).

Table 3
Body condition indices of northern snakehead juveniles fed diets of varying protein and lipid levels for 8 weeks.

Item	CF, g/cm	VSI, %	HSI, %
Individual treatment means[1]			
P45L9	1.3	7.9^{wx}	2.7
P45L12	1.2	8.4^{vw}	2.7
P45L15	1.3	8.9^{v}	2.9
P48L9	1.3	7.4^{x}	2.4
P48L12	1.3	9.1^{v}	2.8
P48L15	1.3	8.5^{vw}	2.8
P51L9	1.3	7.6^{x}	2.4
P51L12	1.3	8.5^{vw}	2.8
P51L15	1.3	8.7^{v}	2.8
Pooled SEM[2]	0.01	0.11	0.05
Means of main effects[3]			
Protein			
45	1.3	8.4	2.8
48	1.3	8.3	2.7
51	1.3	8.3	2.6
Lipid			
9	1.3	7.6^{B}	2.5^{B}
12	1.3	8.6^{A}	2.8^{A}
15	1.3	8.7^{A}	2.8^{A}
Two-way ANOVA (*P*-value)			
Protein	0.143	0.35	0.514
Lipid	0.669	<0.001	0.017
Protein × Lipid	0.174	0.011	0.487

CF = condition factor; VSI = viscerosomatic index; HSI = hepatosomatic index. P45L9 = 45% crude protein, 9% crude lipid; P45L12 = 45% crude protein, 12% crude lipid. P45L15 = 45% crude protein, 15% crude lipid, etc.

[1] Treatment means represent the average values of 3 tanks per treatment. Within the same column, means with the same superscript letter (v, w, x, y, z) are not significantly different ($P > 0.05$).

[2] Standard error of the mean (pooled).

[3] Main effect means followed by the same superscript letter (dietary lipid = upper case) in a same column are not significantly different ($P > 0.05$).

of dietary CL levels from 9% to 12% and decreased as CL further increased to 15%. The FCR of fish fed diets with 9% CL was significantly higher ($P < 0.05$) than those fed 12% and 15% CL, with fish fed 15% CL also significantly higher ($P < 0.05$) in FCR than those fed 12% CL. Fish fed 12% dietary CL had significantly higher PR ($P < 0.05$) than those fed 9% and 15% CL. At the end of the feeding trial, the lowest survival rate was above 95%. Survival rates were not significantly affected by dietary treatments. There were significant interactions ($P < 0.05$) in WG, SGR, FCR, PR and survival between dietary protein and lipid.

3.2. Body condition indices

Viscerosomatic index was significantly affected by dietary treatments (Table 3). Two-way ANOVA revealed that CF, HSI, and VSI of experimental fish were not significantly affected by different levels of dietary CP in this study. Dietary CL had no significant effect on CF. An increase of CL from 9% to 12% caused significant increases ($P < 0.05$) in HSI and VSI, while a further increase to 15% resulted in slight increases in both parameters (without significant differences). There was also a significant effect of interaction between dietary protein and lipid on VSI but not on HSI ($P < 0.05$).

3.3. Body compositions and digestive enzyme assay

Based on one-way ANOVA, whole body moisture, lipid and protein were significantly affected by dietary treatments (Table 4). Two-way ANOVA showed that dietary protein levels had no significant influence on whole-body lipid of experimental fish. Meanwhile, treatments fed 51% dietary CP had significantly higher ($P < 0.05$) whole-body protein and significantly lower ($P < 0.05$) moisture content than those of 45% and 48% CP diets. Whole-body protein was not significantly affected by dietary CL levels. Lipid content in whole-body of fish increased and moisture content decreased with increasing dietary lipid. Significant interactions ($P < 0.05$) between dietary CP and CL were portrayed in whole-body moisture, lipid and protein compositions. Whole-body ash content and muscle proximate composition (Table 5) were not significantly affected by dietary CP and CL levels. For hepatic composition analyses (Table 5), lipid content was significantly affected ($P < 0.05$) by individual dietary treatments. Protein content was not significantly affected by dietary CP levels. Liver lipid content of fish fed 48% CP was significantly higher ($P < 0.05$) than that of fish fed 45% CP, but did not significantly differ from those fed 51% CP. Treatments fed 51% CP diet had higher liver moisture content than those of 45% and 48% with significant difference only between the 48% and 51% CP treatments. Fish fed 9% CL diet had significantly higher ($P < 0.05$) liver protein than that of fish fed 12% and slightly higher (without significant difference) than that of 15% CL fish. Fish fed 12% CL diet had significantly higher ($P < 0.05$) hepatic lipid content than those fed 9% CL, but not significantly different from those fed 15% CL. Dietary CL levels had no significant influence on liver moisture content. Significant interactions ($P < 0.05$) between dietary CP and CL in liver compositions were noticeable. Intestinal protease and α-amylase activities were not significantly affected by dietary levels of protein or lipid, while lipase activity increased with increasing dietary lipid and protein levels. According to one-way ANOVA, diet P48L15 had the highest lipase activity than the rest of the treatments (Table 6).

Table 4
Whole-body composition (fresh-weight basis) of northern snakehead juveniles fed diets of varying protein and lipid levels for 8 weeks.

Item	Moisture, %	Ash, %	Lipid, %	Protein, %
Individual treatment means[1]				
P45L9	76.5^{vwx}	3.7	4.8^{x}	14.1^{xy}
P45L12	77.4^{v}	3.7	4.8^{x}	14.0^{y}
P45L15	74.7^{y}	3.7	5.9^{vw}	15.3^{vwx}
P48L9	77.2^{v}	3.7	4.7^{x}	15.0^{vwxy}
P48L12	75.5^{wxy}	3.7	6.3^{v}	14.2^{xy}
P48L15	76.6^{vw}	3.7	4.8^{x}	14.4^{wxy}
P51L9	75.4^{wxy}	3.7	4.7^{x}	15.8^{v}
P51L12	75.3^{wxy}	3.7	5.4^{wx}	15.4^{vw}
P51L15	75.0^{xy}	3.7	6.1^{vw}	14.9^{vwxy}
Pooled SEM[2]	0.20	0.02	0.13	0.13
Means of main effects[3]				
Protein				
45	76.2^{a}	3.7	5.17	14.4^{b}
48	76.4^{a}	3.7	5.27	14.5^{b}
51	75.2^{b}	3.7	5.37	15.4^{a}
Lipid				
9	76.4^{A}	3.7	4.70^{B}	14.9
12	76.1^{AB}	3.4	5.50^{A}	14.5
15	75.4^{B}	3.7	5.60^{A}	14.8
Two-way ANOVA (*P*-value)				
Protein	<0.001	0.958	0.374	<0.001
Lipid	0.006	0.893	<0.001	0.075
Protein × Lipid	<0.001	0.861	<0.001	0.003

P45L9 = 45% crude protein, 9% crude lipid; P45L12 = 45% crude protein, 12% crude lipid. P45L15 = 45% crude protein, 15% crude lipid, etc.
[1] Treatment means represent the average values of 3 tanks per treatment. Within the same column, means with the same superscript letter (v, w, x, y, z) are not significantly different ($P > 0.05$).
[2] Standard error of the mean (pooled).
[3] Main effect means followed by the same superscript letter (dietary protein = lower case; dietary lipid = upper case) in the same column are not significantly different ($P > 0.05$).

4. Discussion

In this study, 45% dietary protein level generally produced low performance in terms of WG, SGR and FCR. This may be caused mainly by deficiency in dietary amino acids as a result of inadequate dietary protein, which was also evinced in studies on other carnivorous fish including hybrid clarias catfish (*Clarias batrachus* × *Clarias gariepinus*) (Giri et al., 2003); hybrid grouper, (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*) (Jiang et al., 2015) and brown-marbled grouper, *E. fuscoguttatus* (Shapawi et al., 2014). In general, an increase of CP from 48% to 51% did not cause any significant improvement in fish growth and PR. This could be an indication that minimal amino acid requirement was met at 48% protein and excess protein wastefully converted to energy at 51%. Similar observations were documented in earlier reports (Yang et al., 2002; Tu et al., 2015). Fish fed diet P45L9 had the lowest growth performance, possibly indicating that the combination did not meet nutritional requirements of *C. argus* under the conditions of this study. Meanwhile, diets P48L12, P48L15, P51L9 and P51L12 might have met the requirements, hence producing significantly higher growth. In terms of FCR, fish fed diets P48L12 and P48L15 had better values than those fed P51L9 and P51L12, meaning the feed was best utilized in the former 2 diets. Consequently, 48% CP can be deemed as more conducive than 51% for formulating practical diets for juvenile northern snakehead. This result showed that juvenile northern snakehead fingerlings may have higher protein requirement than the other snakehead species. In earlier studies, the protein requirements for striped and hybrid (*C. maculates* ♀ × *C. argus*♂) snakeheads were reported to be 43% to 45% and 46% respectively (Boonyaratpalin, 1980, 1981; Samantaray and Mohanty, 1997; Aliyu-Paiko et al., 2010a, 2010b; Liu et al., 2011), while another species of the same genus, spotted snakehead

Table 5
Muscle and liver composition (fresh-weight basis) of northern snakehead juveniles fed diets of varying protein and lipid levels for 8 weeks.

Item	Muscle				Liver		
	Moisture, %	Ash, %	Lipid, %	Protein, %	Moisture, %	Lipid, %	Protein, %
Individual treatment means[1]							
P45L9	79.0	1.2	1.2	19.3	70.2	3.3^{y}	12.0
P45L12	79.1	1.2	1.1	19.3	70.7	5.0^{wx}	11.6
P45L15	79.0	1.1	1.1	19.2	71.9	6.1^{vw}	12.6
P48L9	79.3	1.2	1.1	19.2	71.0	5.2^{wx}	12.7
P48L12	79.0	1.1	1.1	19.3	70.7	6.9^{v}	12.2
P48L15	79.1	1.1	1.1	19.6	70.7	4.8^{x}	11.7
P51L9	79.5	1.1	1.2	19.2	72.0	5.1^{wx}	12.5
P51L12	79.0	1.1	1.1	19.2	71.9	4.9^{wx}	11.9
P51L15	79.1	1.2	1.2	19.2	71.0	5.4^{wx}	12.7
Pooled SEM[2]	0.07	0.01	0.01	0.05	0.16	0.19	0.10
Means of main effects[3]							
Protein							
45	79.1	1.2	1.1	19.2	70.9^{ab}	4.8^{b}	12.1
48	79.1	1.2	1.1	19.4	70.8^{b}	5.6^{a}	12.2
51	79.3	1.1	1.2	19.2	71.7^{a}	5.2^{ab}	12.4
Lipid							
9	79.3	1.2	1.1	19.3	71.1	4.6^{B}	12.4^{A}
12	79.1	1.2	1.1	19.3	71.1	5.6^{A}	11.9^{B}
15	79.2	1.1	1.1	19.3	71.2	5.4^{A}	12.3^{AB}
Two-way ANOVA (*P*-value)							
Protein	0.301	0.374	0.083	0.248	0.023	0.005	0.284
Lipid	0.409	0.730	0.947	0.941	0.935	<0.001	0.026
Protein × Lipid	0.569	0.354	0.600	0.520	0.021	<0.001	0.010

P45L9 = 45% crude protein, 9% crude lipid; P45L12 = 45% crude protein, 12% crude lipid; P45L15 = 45% crude protein, 15% crude lipid, etc.
[1] Treatment means represent the average values for 3 tanks per treatment. Within the same column, means with the same superscript letter (v, w, x, y, z) are not significantly different ($P > 0.05$).
[2] Standard error of the mean (pooled).
[3] Main effect means followed by the same superscript letter (dietary protein = lower case; dietary lipid = upper case) in the same column are not significantly different ($P > 0.05$).

Table 6
Intestinal enzyme activities of northern snakehead juveniles fed diets of varying protein and lipid levels for 8 weeks.

Item	Protease, U/mg	Amylase, U/mg	Lipase, U/g
Individual treatment means[1]			
P45L9	118.0	99.5	86.3^{z}
P45L12	119.3	98.9	194.4^{wx}
P45L15	120.4	98.6	145.1^{xyz}
P48L9	120.3	99.3	104.1^{yz}
P48L12	119.0	97.4	162.7^{xyz}
P48L15	121.4	99.0	389.3^{v}
P51L9	121.9	97.2	177.9^{xy}
P51L12	119.1	98.0	215.5^{wx}
P51L15	120.4	98.4	268.9^{w}
Pooled SEM[2]	1.78	1.14	17.60
Means of main effects[3]			
Protein			
45	119.2	99.0	141.9^{b}
48	120.2	98.6	218.7^{a}
51	120.4	97.5	220.8^{a}
Lipid			
9	120.1	98.7	122.8^{C}
12	119.1	98.1	190.9^{B}
15	120.7	98.4	267.8^{A}
Two-way ANOVA (*P*-value)			
Protein	0.97	0.904	<0.001
Lipid	0.953	0.986	<0.001
Protein × Lipid	0.997	0.997	<0.001

P45L9 = 45% crude protein, 9% crude lipid; P45L12 = 45% crude protein, 12% crude lipid. P45L15 = 45% crude protein, 15% crude lipid, etc.
[1] Treatment means represent the average values for 3 tanks per treatment. Within the same column, means with the same superscript letter (v, w, x, y, z) are not significantly different ($P > 0.05$).
[2] Standard error of the mean (pooled).
[3] Main effect means followed by the same superscript letter (dietary protein = lower case; dietary lipid = upper case) in the same column are not significantly different ($P > 0.05$).

(*Channa punctatus*) also had the highest WG with dietary CP of 45% (Zehra and Khan, 2012).

Dietary lipid has considerable influence on the effect of protein on growth performance of fish. In this study, there was significant improvement in WG and SGR at both 45% and 48% dietary protein levels as dietary lipid level increased from 9% to 12%. This trend can be suggested to be due to protein-sparing effect, which was also corroborated by the significant increase in PR as dietary lipid increased from 9% to 12%. This could mean that increasing lipid content of diet from 9% to 12% at 45% and 48% dietary protein levels improves protein utilization in this species. Protein-sparing effect was also observed in several fish species including blunt snout bream (*Megalobrama amblycephala*) (Li et al., 2010), Atlantic salmon (*Salmo salar*) (Hillestad and Johnsen, 1994), totuava (*Totoaba macdonaldi*) (López et al., 2006) and gilthead sea bream (*Sparus aurata*) (Vergara et al., 1996). However, just as in the current research, other studies also proved that this phenomenon could not be sustained beyond certain levels of dietary lipid (Davis and Arnold, 1997; Jover et al., 1999; Kim and Lee, 2005). It is therefore imperative to ensure a suitable ratio of protein and non-protein energy sources in determining optimum protein level of a diet so as to mitigate the catabolism of protein for energy in order to reduce feed cost. It can be surmised from the results of this study that dietary lipid requirement for practical diet for growth of *C. argus* can be ranged from 12% to 15% at 48% protein based on growth performance and feed utilization. This implies that lipid level in the diet of this species could be reduced from 15% to 12% at 48% protein level without negatively affecting performance. The result is similar to 13% dietary lipid level reported by Samantaray and Mohanty (1997) for *C. striatus* but higher than 6.5% reported by Aliyu-Paiko et al. (2010a; 2010b) for *C. striatus*. In the current study, slight variations in amino acid and fatty acid profiles of test diets due to minor differences in ratios of some ingredients may have influenced growth performance to some extent. Future research is therefore suggested for better understanding of the effects of fatty acid and amino acid profiles on this species.

Excess of dietary lipid above the required level could lead to fat deposition in the peritoneal cavity, liver and other tissues (NRC, 2011). The VSI and HSI are important traits directly affecting fish yield and are largely influenced by body lipid deposition. In the present study, 12% and 15% dietary lipid caused significantly higher VSI and HSI values. High dietary lipid level was reported to increase VSI of fish such as juvenile cobia, *Rachycentron canadum* (Wang et al., 2005) and rainbow trout *Oncorhynchus mykiss* (Chaiyapechara et al., 2003). Similar trend of HSI and dietary lipid was also portrayed in Atlantic cod, *Gadus morhua* (Jon et al., 2008); rockfish, *Sebastes schlegeli* (Lee et al., 2002); and Eurasian perch, *Perca fluviatilis* (Xu et al., 2001). The higher VSI and HSI might be due to higher lipid deposition in the viscera, which can be partly explained by the fact that increasing dietary lipid increased whole-body and hepatic lipid content. So excessive dietary lipid for northern snakehead could compromise quality of the fish and affect shelf life of its products. Increasing dietary protein and lipid resulted in high liver lipid deposition in the current study, comparable with some findings (Luo et al., 2005; Han et al., 2014) but in disagreement with other findings (Jin et al., 2015; Rahimnejad et al., 2015). This result may be indication that liver contributes significantly to lipid storage in northern snakehead. Protein content of liver is highly dependent on protein intake (Kosterlitz, 1947), which was contradicted by this study as dietary lipid, rather than protein had significant effect on liver protein content. Liver moisture content increasing with increasing dietary protein in this study may be related to dietary protein being unable to increase hepatic protein content, which was similar to findings by Jin et al. (2015) and differed from other studies (Wang et al., 2016; Jiang et al., 2015). Increase in whole-body lipid content with increment of dietary lipid in the present study was in line with other reports (Shapawi et al., 2014; Jiang et al., 2015; Rahimnejad et al., 2015). Highest whole-body protein found in fish fed the highest protein diet was comparable with reports by Chen et al. (2010) and Wang et al. (2016) but contradicted other findings (Tuan and Williams, 2007; Rahimnejad et al., 2015). Whole-body moisture content decreased with increasing dietary protein, similar to findings by Wang et al. (2016) and contrary to that of Chen et al. (2010). Result of decreased whole-body moisture content with increasing dietary lipid was in accordance with other studies (Du et al., 2005; Luo et al., 2005; Lim et al., 2009).

Growth involves several processes and is highly influenced by the digestive as well as absorptive physiology of an organism, which affect the rudimentary dynamic of utilization of ingested nutrients and control the extent of a fish's response. The ability of fish to effectively utilize a given diet is probably determined by the activity of digestive enzymes and their response to different diet compositions (Pérez-Jiménez et al., 2009). However, this field of research seems largely unexplored in this species and warrants more investigation for better insight into the relationship between digestive enzyme activity and growth performance. In this study, increasing dietary protein and lipid levels were inversely proportional to dietary carbohydrate content but had no significant effect on the intestinal α-amylase activity. This result was in accordance with research on African catfish *C. gariepinus* (Ali and Jauncey, 2004) and contradicted outcomes of studies on juvenile golden pompano, *Trachinotus ovatus* (Zhou et al., 2015) and spotted snakehead *C. punctatus* (Moitra and Bhattacharya, 1975). Intestinal protease activity was not significantly affected by dietary protein levels, similar to findings in gibel carp, *Carassiusauratus gibelio* (Ye et al., 2015) and contrary to outcome of research on hybrid clarias

catfish (*C. batrachus* × *C. gariepinus*) (Giri et al., 2003). The lack of significant differences in protease and amylase activities in this study could be attributed to differences in protein and carbohydrate substrate concentrations in the diets not being vast enough to trigger much disparity in enzymatic activities. Intestinal lipase activities increased with increasing dietary lipid level according to this study, which was comparable with studies on European sea bass (*Dicentrarchus labrax*) (Zambonino-Infante and Cahu, 1999) but in contrast to findings in striped snakehead (*C. striatus*) (Aliyu-Paiko et al., 2010a). Meanwhile, significant difference in lipase activity in this study could be evidence of efficient use of lipid in the experimental fish.

5. Conclusions

Northern snakehead fish fed diets P48L12 and P48L15 had best growth, FCR, and PR. Therefore it is suggested that dietary lipid range of 12% to 15% at 48% protein is suitable for formulation of practical feed for the culture of juvenile northern snakehead, under the conditions of this study.

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