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Dietary lipid concentrations influence growth, liver oxidative stress, and serum metabolites of juvenile hybrid snakehead (*Channa argus* × *Channa maculata*)

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Abstract The study was conducted to evaluate the effect of dietary lipid levels on growth, liver oxidative stress, and serum metabolites of juvenile hybrid snakehead (*Channa argus* × *Channa maculata*). Five isonitrogenous (crude protein 420 g kg^{-1}) practical diets containing 58, 87, 115, 144, and 173 g kg^{-1} crude lipid (named L58, L87, L115, L144, and L173, respectively) were fed to triplicate groups of 30 fish (mean initial weight 24 g) for 8 weeks. The results showed that the final body weight (58.68–78.81 g), specific growth rate (1.41–1.75 % day^{-1}), and protein efficiency ratio (1.66–2.64) increased significantly with the increasing dietary lipid levels. Liver lipid contents (71.65–101.80 g kg^{-1}) and crude lipid (52.10–83.63 g kg^{-1}) of whole body increased with increasing dietary lipid levels and reached the highest values in fish of L173. Fish of L173 showed lower alkaline phosphatase (23.81 King Unit $gprot^{-1}$) and catalase activities (4.44 U $mgprot^{-1}$) but higher malondialdehyde content (0.69 nmol $mgprot^{-1}$) in liver than the other groups. Higher alanine transaminase activity (8.20 U L^{-1}), aspartate transaminase activity (63.65 U L^{-1}), and triglyceride (0.29 mmol L^{-1}) in serum were observed in fish of L173 compared to the other treatments. Fish of L144 showed higher superoxide dismutase activity and glutathione peroxidase activities in liver than that of fish fed diet L58. Fish fed diet L58 showed lower total cholesterol (3.61 mmol L^{-1}), high-density lipoprotein cholesterol (1.39 mmol L^{-1}), and low-density lipoprotein cholesterol (0.46 mmol L^{-1}) in serum. These results suggested that juvenile snakehead (*Channa*

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argus × *Channa maculata*) achieved good growth performance with dietary lipid level 173 g kg^{-1}. Diet with 143 g kg^{-1} lipid was more conductive to liver health. The appropriate dietary lipid supplementation needs to be determined in further studies.

Keywords Snakehead · Dietary lipid levels · Growth performance · Body composition · Liver health · Blood traits

Introduction

Lipid as a macronutrient is principally a source of energy and essential fatty acids and provides fundamental structural components in the membranes. An appropriate amount of lipid can spare protein and reduce feed cost, which is important for carnivorous fish with limited ability to utilize carbohydrate for energy (NRC 2011). However, diets with excessive lipid might negatively affect fish growth performance (Han et al. 2014), cause an increase of fat deposition (López et al. 2009), affect fish health and welfare (Sargent et al. 2002), and reduce the commercial value of fillets (Martino et al. 2002).

The hybrid snakehead fish, *Channa argus* × *Channa maculata*, is an air-breathing carnivorous fish with important economic value and widely cultured in China, especially in Guangdong Province. The total snakehead production of China in 2013 was over 500,000 tons (Fisheries Bureau of Ministry of Agriculture 2014). At present, commercial feed of juvenile snakehead in China commonly contains about 10 % crude lipid, which appears to be not appropriate. Furthermore, current farming of snakehead still partly depends on frozen trash fish and the absence of premium complete feed becomes one of the most serious constraints for the industrial development.

As far as we know, several researches have been published concerning dietary lipid levels for a few snakehead species. Maximum growth was reported at a dietary lipid level about 60 g kg^{-1} for *Channa striatus* fingerlings (Bloch, 1793) (Boonyaratpalin 1981; Aliyu-Paiko et al. 2010) and 130 g kg^{-1} for *Channa striata* (10.0–13.8 g) (Samantaray and Mohanty 1997). Diet supplemented with 120 g kg^{-1} lipid was optimal for *Channa argus* (Zhu and Wang 2011). However, to our knowledge, no information is available about dietary lipid levels in artificial diets for *Channa argus* × *Channa maculata.* Apart from the growth performance, other secondary indices reflecting the biological functions should also be considered in dietary nutrition investigation. Liver is a solid organ and an early stress indicator in the body, carrying a complex array of functions (Antonopoulou et al. 2014; Ebrahimkhani et al. 2014). Several data obtained from different fish species showed that liver functions could be impaired by improper level of dietary lipid inclusion (Lin et al. 1990; Hevrøy et al. 2004; Li et al. 2012; Jin et al. 2013). Therefore, the objective of the present study was to evaluate the effect of dietary lipid concentrations on growth, liver oxidative stress, and serum metabolites of juvenile hybrid snakehead (*Channa argus* × *Channa maculata*).

Materials and methods

Experimental diets

The formulation and proximate composition of the experimental diets are given in Table 1. Five isonitrogenous (crude protein 420 g kg^{-1}) practical diets were formulated to contain

Table 1 Formulation and proximate composition of the experimental diets (g kg^{-1} dry matter)

	Diet				
	L58	L87	L115	L144	L173
Ingredients					
Fish meal[a]	400	400	400	400	400
Soybean meal[a]	300	300	300	300	300
Wheat middlings	250	220	190	160	130
Menhaden fish oil	0	30	60	90	120
Soybean lecithin oil	10	10	10	10	10
Choline chloride	2	2	2	2	2
Mineral premix[b]	15	15	15	15	15
Vitamin premix[c]	20	20	20	20	20
$Ca(H_2PO_4)_2{\cdot}H_2O$	20	20	20	20	20
Analyzed proximate composition[d]					
Moisture	87.1	80.6	75.6	71.5	70.0
Crude protein	428	427	425	422	419
Crude lipid	58	87	115	144	173
Crude ash	103.6	108.5	106.8	110.7	106.4
GE (MJ kg^{-1})	17.54	18.19	18.86	19.52	20.18

[a] Fish meal (Peru steam-treated): crude protein 675 g kg^{-1}, crude lipid 92 g kg^{-1}; soybean meal (solvent extracted): crude protein 475 g kg^{-1}

[b] Mineral premix (mg kg^{-1} of diet): Na, 30; K, 50; Mg, 100; Cu, 4; Fe, 25; Zn, 35; Mn, 12; I, 1.6; Se, 0.2; Co, 0.8

[c] Vitamin premix (mg kg^{-1} of diet): VA, 18; VD_3, 5; VE, 150; VC (350 g kg^{-1}), 500; VB_1, 16; VB_6, 20; VB_{12}, 6; VK_3, 18; riboflavin, 40; inositol, 320; calcium-D-pantothenate, 60; niacinamide, 80; folic acid, 5; biotin, 2; ethoxyquin, 100

[d] Crude protein, lipid, ash, gross energy are expressed on a dry matter basis and given as means ($n = 2$)

graded levels of fish oil (0, 30, 60, 90, and 120 g kg^{-1}; named L58, L87, L115, L144, and L173, respectively). All ingredients were ground through a 320 μm mesh before final mixing and then blended with the oil. The experimental diets were prepared using a cooking extruder (TSE65S, Beijing Modern Yanggong Machinery S&T Development CO., Ltd., China) in Tongwei Fishery Science and Technology Park, then air-dried and stored at 4 °C until used.

Experimental animals

Snakehead (*Channa argus* × *Channa maculata*) used in this experiment were obtained from a commercial farm (Yongchuan, Chongqing, China). Prior to the trial, fish were acclimated and fed a commercial feed (Zhejiang Zhongda Group Feed CO., Ltd., China) for 1 week. The experimental design and procedure were approved by the Animal Care and Use of Committee of Southwest University following the requirements of the Regulations for the Administration of Affairs Concerning Experimental Animals of China (The State Science and Technology Commission 1988).

At the start of the experiment, the fish were fasted for 24 h and weighed after being anesthetized with 0.01 % MS-222 (Sigma, USA). Snakehead (mean initial weight: 24 g)

were randomly allocated into 15 cylindrical plastic tanks (capacity: 177L) with screened covers for the growth trial (30 fish per tank). Each dietary treatment was randomly assigned to three tanks. Fish were fed to apparent satiation by hand three times (08:30, 12:30, and 18:00) daily for 8 weeks. During the experiment, wasted feed were collected and corresponding weight was calculated. Dead fish were weighed and taken into account in the growth performance calculation. Photoperiod was held to a constant 12-h light/12-h dark cycle. The static water in each tank was monitored daily for temperature (24.5 ± 2.5 °C), pH (7.4 ± 0.4), dissolved oxygen content (6–7 mg L^{-1}), ammonia nitrogen content (<0.10 mg L^{-1}), nitrite nitrogen (0.005–0.010 mg L^{-1}), and sulfide (<0.05 mg L^{-1}).

Sample collection and chemical analysis

At the termination of the experiment, fish were fasted for 24 h before harvest. Total numbers were counted, and mean body weight of fish was measured. Three fish per tank were anesthetized with overdose of MS-222 to assess the whole-body composition. Another six fish from each tank were randomly selected and anesthetized with 0.01 % MS-222 (Sigma, USA), and blood sample was collected from the caudal vein using a 1-mL syringe with a 27-gauge needle and allowed to clot at 4 °C. Following centrifugation (3000 rpm, 10 min) at 4 °C (Sorvall ST 16R, Thermo Fisher Scientific Inc., Germany), serum was separated, immediately frozen in liquid nitrogen and stored at −20 °C until analyzed. The bloodless fish were then dissected to obtain viscera, liver, and intraperitoneal fat for calculating morphological parameters. Pooled livers of another three fish per tank were also immediately frozen in liquid nitrogen and stored at −20 °C until used for malondialdehyde determination and enzymatic analysis.

Feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), survival, viscera ratio (VR), hepatosomatic index (HSI), and intraperitoneal fat ratio (IPF) were measured using the following formulae: FI = feed consumption (g)/[(initial weight + final weight (weight of dead fish included))/2 × 56 days], SGR = [ln (mean final weight)–ln (mean initial weight)/56 days] × 100, FCR = total feed intake in dry basis (g)/weight gain (g), PER = total weight gain (g)/protein intake (g), survival rate (%) = (final number of fish/initial number of fish) × 100, VR = 100 × viscera weight (g)/body weight (g), HSI = 100 × hepatic weight (g)/body weight (g), IPF = 100 × intraperitoneal fat weight (g)/body weight (g).

All chemical composition analyses of diets and body were conducted by standard methods (AOAC 2005). Moisture was determined by oven drying to a constant weight at 105 °C in DHG-9240A (Keelrein instrument Co., Ltd., China). Protein was determined by measuring nitrogen ($N \times 6.25$) using the Kjeldahl method in FOSS Kjeltec 2300 (Foss Analytical Instruments Co., Ltd., Sweden); lipid by ether extraction (without acid hydrolysis) using Soxtec; ash by combustion at 550 °C for 12 h in a muffle furnace (Shenyang Energy-Saving Electric Furnace Factory, China). The gross energy content of feed was determined by direct combustion in an adiabatic calorimeter GR3500 (Changsha Instrument Factory, P. R. China). Total lipid of liver was measured following the method of Bligh and Dyer (1959). Serum enzymes and biochemical indexes including alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assayed using automatic biochemical analyzer HITACHI 7100 (ISE) (Hitachi, Ltd., Japan) and attached kit (Sichuan Maker Biotechnology Co., Ltd., China).

Hepatic malondialdehyde (MDA) contents and enzyme activities

Samples of liver were homogenized into 10 volumes (w/v) of ice-cold, 0.85 % physiological saline at 10,000 rpm for 1 min using fluko superfine homogenizers (FLUKO Equipment Shanghai Co., Ltd. China). Homogenates were centrifuged at 4000 rpm for 10 min at 4 °C (Sorvall ST 16R, Thermo Fisher Scientific Inc., Germany). The supernatant was used to determine the malondialdehyde (MDA) contents, alkaline phosphatase (AKP; EC 3.1.3.1), catalase (CAT; EC 1.11.1.6), superoxide dismutase (SOD; EC 1.15.1.1), and glutathione peroxidase (GPx; EC 1.11.1.9) activities spectrophotometrically using diagnostic reagent kits (Nanjing Jiancheng Bioengineering Institute, China). Thiobarbituric acid reactive substances (TBARS) were determined as described by Rueda-Jasso et al. (2004). MDA undergoes a condensation reaction in the presence of thiobarbituric acid and generates a red product with a maximum absorption peak at 532 nm. The results were expressed as nmol of MDA mg^{-1} protein. AKP activity was assayed based on the method of Bessey et al. (1946), and one unit of AKP was defined as the quantity of enzyme producing one milligram phenol in 15 min at 37 °C. The results were expressed as King Unit of AKP mg^{-1} protein. CAT activity assayed at 37 °C according to Beers and Sizer (1952), and one unit of the enzyme is defined as 1 μmol of H_2O_2 consumed per minute. SOD activity determined at 37 °C according to Panchenko et al. (1975) and one unit of SOD was defined as the amount of protein needed to decrease the reference rate to 50 % of maximum inhibition. GPx activity was assayed at 37 °C according to Wheeler et al. (1990). One GPx unit is defined as 1 μmol of NADPH consumed per minute. The specific activities of CAT, SOD, and GPx were represented as units per mg protein. The protein concentration of the enzyme extracts was determined according to Bradford (1976). All enzyme assays were performed in triplicate.

Statistical analysis

All data were accord with normal distribution and subjected to one-way ANOVA (analysis of variance) using SPSS 22.0 for Windows (International Business Machines Corporation, Armonk, NY, USA). Differences between the means were tested by Duncan's multiple range tests. Overall significance level = 0.05 and the results are presented as mean ± -SEM (standard error of the mean) of three replicate groups.

Results

Growth

The growth performance of snakehead is shown in Table 2. The survival ranged from 92.22 to 98.89 % and showed no significant differences among all groups ($P > 0.05$). The final body weight, SGR, and PER increased significantly with the increasing dietary lipid levels ($P < 0.05$), whereas FI and FCR showed a contrary trend ($P < 0.05$).

Morphological parameters and body composition

The morphological parameters and body composition of snakehead are presented in Table 3. VR and IPF were significantly affected by the dietary lipid levels and were higher

Table 2 Growth performance of hybrid snakehead fed diets with different lipid levels for 8 weeks (Mean ± SEM)[a]

	Diet				
	L58	L87	L115	L144	L173
Initial body weight (g)	24.18 ± 0.18	24.20 ± 0.14	24.15 ± 0.12	24.06 ± 0.14	24.12 ± 0.18
Final body weight (g)	58.68 ± 0.88^{e}	63.42 ± 0.41^{bd}	65.48 ± 0.62^{bc}	68.18 ± 1.02^{b}	72.81 ± 0.61^{a}
FI (g 100 g^{-1} BW/d)[b]	1.74 ± 0.07^{a}	1.60 ± 0.06^{ab}	1.49 ± 0.02^{bc}	1.40 ± 0.03^{c}	1.42 ± 0.02^{c}
SGR (% day^{-1})[c]	1.41 ± 0.02^{e}	1.53 ± 0.01^{d}	1.58 ± 0.02^{c}	1.65 ± 0.02^{b}	1.75 ± 0.01^{a}
FCR[d]	1.44 ± 0.04^{a}	1.21 ± 0.04^{b}	1.06 ± 0.01^{c}	0.94 ± 0.02^{d}	0.90 ± 0.02^{d}
PER[e]	1.66 ± 0.05^{d}	1.97 ± 0.06^{c}	2.24 ± 0.02^{b}	2.54 ± 0.05^{a}	2.64 ± 0.07^{a}
Survival[f]	92.22 ± 4.01	93.33 ± 5.09	95.56 ± 1.11	97.78 ± 1.11	98.89 ± 1.11

[a] Values in each row with different superscripts have significant differences ($P < 0.05$)

[b] Feed intake (FI) = feed consumption (g)/[(initial weight + final weight (weight of dead fish included))/2 × 56 days]

[c] Specific growth rate (SGR) = [ln (mean final weight) − ln (mean initial weight)/56 days] × 100

[d] Feed conversion ratio (FCR) = total feed intake in dry basis (g)/weight gain (g)

[e] Protein efficiency ratio (PER) = total weight gain (g)/protein intake (g)

[f] Survival rate (%) = (final number of fish/initial number of fish) × 100

Table 3 Morphological parameters and body composition of hybrid snakehead at the end of growth trial (Mean ± SEM)[1]

	Diet				
	L58	L87	L115	L144	L173
Morphological parameters (*g* kg^{-1})					
VR[2]	70.65 ± 1.58^{c}	81.92 ± 1.07^{b}	86.78 ± 3.03^{b}	89.68 ± 3.67^{ab}	96.78 ± 4.33^{a}
HSI[3]	21.42 ± 0.18^{b}	23.77 ± 0.58^{ab}	24.40 ± 1.93^{ab}	25.19 ± 0.26^{a}	26.04 ± 0.96^{a}
IPF[4]	15.23 ± 1.63^{c}	20.35 ± 0.64^{bc}	24.79 ± 2.36^{ab}	25.42 ± 3.18^{ab}	30.05 ± 3.60^{a}
Body composition (*g* kg^{-1} *wet weight*)					
Liver lipid contents	71.65 ± 5.35^{b}	73.20 ± 1.90^{b}	81.15 ± 3.55^{ab}	87.73 ± 5.39^{ab}	101.80 ± 8.88^{a}
Moisture	719.43 ± 2.92^{a}	708.93 ± 5.87^{ab}	702.90 ± 4.57^{abc}	688.10 ± 4.45^{c}	696.83 ± 7.66^{bc}
Crude protein	178.83 ± 5.17^{a}	171.7 ± 4.97^{ab}	171.13 ± 4.94^{ab}	165.60 ± 4.79^{ab}	160.00 ± 4.62^{b}
Crude lipid	52.10 ± 2.35^{c}	64.87 ± 8.90^{bc}	69.53 ± 2.81^{ab}	78.20 ± 3.98^{ab}	83.63 ± 3.32^{a}
Crude ash	52.60 ± 0.85	51.93 ± 2.09	53.13 ± 0.78	52.67 ± 1.42	51.50 ± 2.27

[1] Values in each row with different superscripts have significant differences ($P < 0.05$). Data are presented as means from three replicate tanks (morphological parameters: 6 fish per tank; liver lipid contents, 6 fish; moisture, crude protein, crude lipid, and crude ash, 3 fish per tank)

[2] Viscera ratio (VR) = 100 × viscera weight (g)/body weight (g)

[3] Hepatosomatic index (HSI) = 100 × hepatic weight (g)/body weight (g)

[4] Intraperitoneal fat ratio (IPF) = 100 × intraperitoneal fat weight (g)/body weight (g)

of L173 group than those of other groups ($P < 0.05$). HSI of L144 and L173 groups were significantly higher than that of L58 group ($P < 0.05$). Liver lipid contents and crude lipid of whole body increased with the increase of dietary lipid levels and achieved the highest level in fish fed diet L173, whereas moisture and crude protein decreased with the increase of the dietary lipid levels ($P < 0.05$). No significant differences were observed in crude ash among all groups ($P > 0.05$).

Liver malondialdehyde (MDA) contents and enzyme activities

Liver MDA contents and enzyme activities were significantly affected by the dietary lipid levels ($P < 0.05$) (Table 4). AKP activity significantly decreased with the increasing dietary lipid levels ($P < 0.05$). Fish fed diet L173 showed lower CAT activity, but higher MDA contents compared to the other treatments ($P < 0.05$), while no significant differences were observed in CAT activity and MDA contents of L58, L87, L115, and L144 groups, respectively. Fish fed diet L144 showed higher SOD activity than that of fish fed diet L58 ($P < 0.05$). GPx activities of L144 and L173 groups were higher than those of the other groups ($P < 0.05$).

Serum enzymes activities and serum lipid metabolism indexes

The serum enzymes and biochemical indexes of snakehead are presented in Table 5. The ALT activity of group L173 was higher than those of other groups ($P < 0.05$). The AST activities increased with the dietary lipid levels and achieved the highest value in fish fed diet L173 ($P < 0.05$). TC of fish fed diet L58 were significantly lower than those of L115, L144, and L173 groups ($P < 0.05$). TG increased with increasing dietary lipid levels and achieved the highest activities in fish fed diet L173 ($P < 0.05$). HDL-C and LDL-C of fish fed diet L173 and diet L144, respectively, were significantly higher than that of fish fed diet L58 ($P < 0.05$), but HDL-C/TC and LDL-C/TC showed no differences among all groups.

Discussion

In the present study, survival was over 90 % in all dietary treatments, and increasing dietary lipid levels improved the growth performance of snakehead. Best growth performance was observed at the highest lipid level, suggesting that 173 g kg^{-1} lipid could be effectively utilized by snakehead. Similarly, maximum growth was observed at a dietary lipid level of 180 g kg^{-1} in *Channa striatus* with an initial weight of 30 g (Ghaedi et al. 2016). However, some previous studies showed relatively lower lipid levels in snakehead feed, such as 60 g kg^{-1} lipid for *Channa striatus* fingerling (Boonyaratpalin 1981; Aliyu-Paiko et al. 2010), 130 g kg^{-1} lipid for *Channa striata* (10.0–13.8 g) (Samantaray and Mohanty 1997) and 120 g kg^{-1} lipid for *Channa argus* (Initial weight approximately 11.0 g) (Zhu and Wang 2011). The discrepancy might be resulted from the varietal difference with distinct sizes. On the other hand, this study suggests that diet with lipid level over 173 g kg^{-1} is acceptable for better growth of snakehead. Further study would be needed to investigate the higher dietary lipid supplementation of snakehead feed.

The decreasing FCR indicates that the increasing lipid level provided fish more energy for fish growth and snakehead could utilize high-energy feed well. High-energy feed reduced the feed consumption of fish, which is the reason for lower FI in groups with

Table 4 Liver malondialdehyde (MDA) contents and enzyme activities of hybrid snakehead fed diet with different lipid levels for 8 weeks (Mean ± SEM)[1]

	Diet				
	L58	L87	L115	L144	L173
AKP (King Unit gprot^{-1})[2]	59.05 ± 3.48^{a}	50.92 ± 2.88^{b}	33.13 ± 0.77^{c}	28.06 ± 1.52 ^{cd}	23.81 ± 0.71^{d}
CAT (U mgprot^{-1})[3]	7.12 ± 0.32^{a}	7.22 ± 0.05^{a}	7.05 ± 0.27^{a}	7.55 ± 0.08^{a}	4.44 ± 1.12^{b}
SOD (U mgprot^{-1})[4]	595.32 ± 24.60^{b}	657.62 ± 31.13^{ab}	738.46 ± 15.54^{ab}	759.83 ± 7.80^{a}	671.73 ± 106.07^{ab}
GPx (U mgprot^{-1})[5]	1.23 ± 0.09^{c}	1.55 ± 0.15^{c}	2.20 ± 0.15^{b}	2.80 ± 0.19^{a}	2.76 ± 0.24^{a}
MDA (nmol mgprot^{-1})[6]	0.44 ± 0.03^{b}	0.46 ± 0.06^{b}	0.50 ± 0.08^{b}	0.56 ± 0.03^{ab}	0.69 ± 0.02^{a}

[1] Values in each row with different superscripts have significant differences ($P < 0.05$). Data are presented as means from three replicate tanks (3 fish per tank)

[2] *AKP* alkaline phosphatase

[3] *CAT* catalase

[4] *SOD* superoxide dismutase

[5] *GPx* glutathione peroxidase

[6] *MDA* malondialdehyde

Table 5 Serum enzymes and lipid metabolism indexes of snakehead fed diets with different lipid levels for 8 weeks (Mean ± SEM)[1]

	Diet				
	L58	L87	L115	L144	L173
Serum enzymes					
ALT (U L^{-1})[2]	4.45 ± 0.35^{b}	4.95 ± 0.85^{b}	5.20 ± 0.40^{b}	6.10 ± 0.70^{b}	8.20 ± 0.20^{a}
AST (U L^{-1})[3]	38.90 ± 3.70^{c}	49.80 ± 3.60^{bc}	53.70 ± 5.60^{ab}	50.35 ± 1.65^{ab}	63.65 ± 9.95^{a}
Lipid metabolism indexes					
TC (mmol L^{-1})[4]	3.61 ± 0.35^{b}	4.34 ± 0.32^{ab}	4.47 ± 0.18^{a}	4.70 ± 0.26^{a}	4.95 ± 0.17^{a}
TG (mmol L^{-1})[5]	0.12 ± 0.01^{b}	0.14 ± 0.01^{b}	0.19 ± 0.01^{ab}	0.20 ± 0.02^{ab}	0.29 ± 0.05^{a}
HDL-C (mmol L^{-1})[6]	1.39 ± 0.06^{b}	1.59 ± 0.17^{ab}	1.75 ± 0.09^{ab}	1.67 ± 0.09^{ab}	1.79 ± 0.11^{a}
LDL-C (mmol L^{-1})[7]	0.46 ± 0.06^{b}	0.60 ± 0.02^{ab}	0.59 ± 0.01^{ab}	0.69 ± 0.09^{a}	0.65 ± 0.05^{ab}
HDL-C/TC[8]	0.39 ± 0.03	0.34 ± 0.03	0.38 ± 0.02	0.35 ± 0.01	0.36 ± 0.00
LDL-C/TC[9]	0.12 ± 0.01	0.13 ± 0.00	0.13 ± 0.00	0.14 ± 0.01	0.13 ± 0.00

[1] Values in each row with different superscripts have significant differences ($P < 0.05$). Data are presented as means from three replicate tanks (6 fish per tank)

[2] *ALT* alanine transaminase

[3] *AST* aspartate transaminase

[4] *TC* total cholesterol

[5] *TG* triglyceride

[6] *HDL-C* high-density lipoprotein cholesterol

[7] *LDL-C* low-density lipoprotein cholesterol

[8] *HDL-C/TC* high-density lipoprotein cholesterol (mmol L^{-1})/total cholesterol (mmol L^{-1})

[9] *LDL-C/TC* low-density lipoprotein cholesterol (mmol L^{-1})/total cholesterol (mmol L^{-1})

higher lipid levels. Besides, PER significantly increased with elevated lipid levels and remained constant in treatments containing 144 and 173 g kg^{-1} lipid. This suggests that lipid has the effect of sparing dietary proteins within certain range and a further increase does not have any further advantages (Samantaray and Mohanty 1997; NRC 2011).

In the present study, highest liver lipid content in fish fed diet containing the highest lipid (173 g kg^{-1}) suggests that high lipid diet could induce high lipid deposition in the liver, which could be a main reason for highest HSI. Low AKP in liver of fish fed 173 g kg^{-1} lipid indicates that this enzyme might be released into the blood stream, since the liver, bile ducts or gallbladder system possibly were not functioning properly or were blocked (Brain and Kay 1927; Kaplan and Righetti 1970). The enzymes ALT and AST are also two important indicators of hepatocellular injury (Boone et al. 2005). Significantly, higher ALT and AST activities in serum when fish were fed diet containing highest lipid indicate that the liver cells might be damaged to a certain extent (Jeon et al. 2013). Diet containing high lipid could possibly increase the burden of liver and cause liver dysfunction of snakehead.

Reactive oxygen species (ROS) could affect cellular integrity by causing oxidation reaction of fatty acids (Flood et al. 1996; Rueda-Jasso et al. 2004). As one of secondary oxidation products (El-Aal 2012), MDA levels in the liver of snakehead remained at relevantly equal levels when fish were fed diets containing 58–144 g kg^{-1} lipid. However,

higher MDA content in fish fed the diet containing 173 g kg^{-1} lipid implies that high dietary lipid level could impose a peroxidation burden on the snakehead to increase the risk of production of ROS. SOD, CAT, and GPx have been used as effective biomarkers to evaluate the effects of dietary lipid on fish species in many studies (Mourente et al. 2002; Zhang et al. 2009; Jin et al. 2013). The upward trend of CAT, SOD, and GPx suggests that the antioxidant defense of snakehead was enhanced to protect the liver cells from deleterious effects of endogenous ROS. On the other hand, the decreasing CAT and SOD activities in fish fed diet containing 173 g kg^{-1} lipid suggested that the balance between generation and removal of ROS would be in danger of breaking (Halliwell 2015) and oxidative stress would be induced if fish was fed diet containing higher lipid levels.

Up to now, no comparable information is available for the effect of dietary lipid on the blood characteristics of snakehead. In this study, TG significantly increased with increasing dietary lipid, which was similar to the report for *Takifugu rubripes* (Kikuchi et al. 2009). TC increased significantly with increasing dietary lipid levels, but reached a steady level in fish fed the diet with lipid over 115 g kg^{-1}, which suggests that dietary lipid might have limited effect on serum TC of snakehead. Similarly, in *Solea solea* (Bonvini et al. 2015) and *Argyrosomus regius* (Chatzifotis et al. 2010), both TG and TC were not affected by the dietary lipid. In addition, HDL-C and LDL-C were slightly influenced by the dietary lipid. The unchanged HDL-C/TC and LDL-C/TC seemed independent of the dietary lipid.

In this study, the positive correlation of whole-body lipid content and IPF with dietary lipid levels indicated excess lipid deposition in visceral cavity and tissues. The decreasing trend of moisture content with an increment of dietary lipid in the whole body in our study was in agreement with those previously reported in some carnivorous species (Pei et al. 2004; Kim et al. 2012). However, several researchers reported that no significant difference was observed in the value of whole-body moisture among treatments with different lipid levels (Ahmad 2008; Aliyu-Paiko et al. 2010). In addition, low crude protein of fish fed diet containing 173 g kg^{-1} lipid might be partly in relation to the dilution effect of lipid (Page and Andrews 1973). Although good growth performance was observed at high lipid levels, excess fat deposition could potentially adversely affect marketability and shelf life of fish (Cowey 1993; Ghanawi et al. 2011).

In conclusion, the results of this trial suggest that juvenile snakehead (*Channa argus* × *Channa maculata*) is capable of achieving good growth performance with dietary lipid level ranging from 58 to 173 g kg^{-1} and the appropriate dietary lipid level needs to be determined in further studies, while diet with lipid level over 144 g kg^{-1} may have a risk of introducing the oxidative stress in fish liver. To be safe, diet including 144 g kg^{-1} lipid is recommended considering the liver health of fish.

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