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Dietary lipid levels requirements of hybrid yellow catfish (*Pelteobagrus fulvidraco* × *P. vachelli*)

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Key words: *Pelteobagrus fulvidraco* × *P. vachelli*, dietary lipid levels, growth performance, hematological index; serum biochemical index

Abstract

A 12-week feeding trial was conducted to determine the dietary lipid levels requirements of hybrid yellow catfish using pelleted diets containing six different lipid levels formulated to contain graded levels of 0.0% (control), 3.4%, 6.8%, 10.2%, 13.6%, and 17% of dry matter. A total of 504 juveniles with an average initial body weight of 8.77±0.012 g were randomly divided into 6 groups containing 3 triplicates with 28 fish per tank and fed each of the experimental diets. Results showed that maximal final body weight, weight gained and specific growth rate were significantly higher in the groups fed 3.4%, 6.8%, and 10.2% compared with that of groups fed 13.6% and 17.0% ($P < 0.05$). Feed conversion ratio and protein efficiency ratio were significantly lowered in the 6.8% and 10.2% groups ($P < 0.05$). The hepatosomatic index had no significant difference ($P > 0.05$) while the viscerosomatic index significantly decreased in 6.8% and 10.2% groups. White blood cells, red blood cells, hematocrit and platelets levels significantly increased in the decreased dietary lipids levels ($P < 0.05$) while hemoglobin had no observed significant difference ($P > 0.05$). Serum alanine aminotransferase, aspartate transaminase, total protein, total cholesterol, triglyceride and glucose were significantly influenced by the dietary lipid levels. Liver superoxide dismutase and catalase concentration decreased significantly in the 6.8% and 10.2% groups. Malondialdehyde increased in 6.8% and 10.2% groups. Glutathione peroxide showed insignificant results ($P > 0.05$). Study results suggested that, dietary lipid levels containing 3.4%,6.8% and 10.2% significantly influenced growth, blood function, antioxidant status and strengthened immune response in hybrid yellow catfish while higher dietary lipid levels (13.6% and 17.0%) decreased body weight and weakened immunity.

Introduction

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This study aimed at examining the possible effects of the dietary lipid levels on the growth, hematological index, serum biochemical index and the antioxidant property in the liver and muscle of hybrid yellow catfish (*Pelteobagrus fulvidraco* ×*Pelteobagrus Vachelli*). There is lack of detailed information on the dietary lipid requirements of many species of fish but there is plenty of information on the fatty acid composition of fish oil Lipids that are vital sources of energy and essential fatty acids subsequent to protein which is necessary for fish growth and development (Figueiredo-Silva et al., 2010). Lipids are well digested by fish and appear to be preferred over carbohydrate as an energy source (Houguo Xu et al., 2015). Special attention has been given by researchers, feed manufacturers and farmers to develop feeds, which maximize nutrient utilization with low nutrient loss. Excess body lipid buildup causes countless effects in fish mostly in the liver which reduces the harvest yields and causes health problems, hence causes burden to the sustainable development of aquaculture. Most carnivorous fishes do not utilize carbohydrates while herbivorous and omnivorous fish can utilize carbohydrate better. The lower growth performance and higher FCR in fish might be due to lack of lipids for structural uses but not digestible energy (Ghanawi et al., 2011). Moreover, excessive lipids in diets may depress growth in some fish species. Fish fed to satiation could regulate feed intake in order to satisfy their digestible energy requirements (Gomez-Requeni et al., 2013).

Increase in dietary lipids can significantly improve efficient utilization of dietary protein for fish growth purposes. Fat deposition can occur in weight gain as a result of protein retention and considered not an accurate predictor of true growth (Kanazawa et al., 1985). Fatty acids also have a pivotal role in the immune system in addition to their well-known role as an energy source for fish growth and development. The potential for dietary lipids to fulfill this function is limited and regulated by their degree of digestibility (Du et al., 2005). Dietary lipids have important physiological functions and significantly affect the fish flesh quality such as the FA profile of fish body lipids (Valente et al., 2009). Dietary lipids requirements of fish are influenced by some factors such as: dietary protein contents, carbohydrate contents, fish life stage and environmental temperature (Jin et al., 2013). Fatty acids have a decisive role in the fish immune system in addition to their well-known role as an energy source for fish growth and development. A supplementation of lipid rather than carbohydrate as a non-protein energy source is generally more effective for increasing dietary energy level because lipid is an energy-dense nutrient that is readily metabolized by fish, especially by carnivorous fish (NRC, 2011). Fish regulate metabolism and absorption of dietary lipids in the digestive system via digestive enzymes. Several studies have suggested that the activities of the main digestive enzymes are to respond to different dietary compositions, to determine how effective a given diet is and to optimize growth and food utilization in fish (Shi et al., 2013).

Hybrid Yellow catfish belongs to the Osteichthyes, Siluriformes, Bagridae and are presently famous aquaculture species in China. Hybrid yellow catfish are omnivorous fish and have the ability to adapt to the ecological environmental changes (Li et al., 2012). However, hybrid yellow catfish maintains a smaller body size (maximum body weight is less than 300 g) and a slow growth rate (reaching an average weight of 125 g after a 20-month growth) compared to the Pelteobagrus vachelli fish (Yan Chan et al., 2014). Hybrid yellow catfish have a delicious taste possessing tiny bones inside the muscle hence having high nutritional value and it can attain a relatively fast growth rate under cultivation environment (reaching an average weight of 125g after a 12-months growth) (M.F et al., 2011). Hybrid yellow catfish fish species are distributed in many rivers having large surface areas in China, such rivers are Liaohe, Huaihe, Yangtze, Xiangjiang, Minjiang and Pearl Rivers. Some researchers also suggested that, hybrid yellow catfish are not suitable for intensive pond farming system due to the high oxygen consumption rate and the oxygen threshold of Pelteobagrus vachelli are higher than Pelteobagrus fulvidraco. Moreover, hybrid yellow catfish fish habitats are widely distributed in the lakes and rivers of Southwest, northwest and the regions inhabited by some ethnic groups of China (Yan et al., 2014).

Materials and Methods

Ethical approval

All the methods applied in this study were performed in accordance with the Guidelines for Experimental Animals established by the National Ministry of Science and Technology (Beijing, China). The study proto-calls were approved by the Freshwater Fisheries Research Centre of the Chinese Academy of Fisheries Sciences, Wuxi-China (Jiangsu province).

Experimental diets

Experimental diets were formulated containing six graded levels of dietary lipid levels 0.0% (control), 3.4%, 6.8%, 10.2%, 13.6% and 17.0% Kg^{-1}) feed respectively. Fish meal, soybean meal, cotton seed meal and rapeseed meal were used as protein sources while fish oil and sunflower oil were utilized as the lipid sources and wheat flour was used as carbohydrate source (all diets were isoenergetic). All the dry ingredients were thoroughly mixed until homogenous in a Hobart-type mixer. The diets were produced as cold-extruded pellets (1.5 mm, 2.0 mm and 2.5 mm diameter) which were air-dried for about an hour to reduce the moisture, thereafter the feeds were collected and sealed in the vacuum- packed bags and stored in a freezer at -20°c temperature room until the experiment begun. The feed formulation and the proximate composition of the diets stored (−20 °C) until the experiment begun. Formulations are presented in **Table 1**. The ingredients were all crushed into fine or thin stage to pass through the 60-size mesh sieve (250um) and were weighed after. The dry ingredients were mixed one after another manually for about 10 minutes. Fish and soya beans oil was added into the diets mixtures and also mixed for about 10 minutes. Lastly, the mixtures were transferred to the mixer and were homogenized for further 10 minutes. Distilled water was added into the mixture to attain a suitable pelleting consistency. The diets were air dried close to an hour under the shade and some samples were collected for proximate analysis. Lipid stability in the diets was examined by measuring the total lipid content before and after being immersed in water for exactly 30 minutes and the results indicated that, diets reached 96.4% in total after been immersed in water for 30 minutes.

Table 1 Formulation and chemical composition of the experimental diets (g kg−1 dry matter)

Ingredients	Dietary Lipids levels (%)					
	0.0	3.4	6.8	10.2	13.6	17.0
Fish meal	25	25	25	25	25	25
Casein	7.6	7.6	7.6	7.6	7.6	7.6
Gelatin	1.9	1.9	1.9	1.9	1.9	1.9
Corn starch	32.3	26.53	20.769	14.006	7.243	0.48
Fish oil	0	3.4	6.8	10.2	13.6	17
[4]Soybeans meal[a]	10	10	10	10	10	10
[4]Cotton seed meal[b]	10	10	10	10	10	10
[4]Rapeseed meal[c]	10	10	10	10	10	10
[5]Multi-vitamins[a]	0.5	0.5	0.5	0.5	0.5	0.5
[5]Compound mineral salts[b]	0.5	0.5	0.5	0.5	0.5	0.5
Choline Chloride	0.5	0.5	0.5	0.5	0.5	0.5
VC sodium Phosphate	0.2	0.2	0.2	0.2	0.2	0.2
Calcium dihydrogen phosphate	1.5	1.5	1.5	1.5	1.5	1.5
Cellulose	0	2.37	4.731	8.094	11.457	14.82
Total	100	100	100	100	100	100
Proximate chemical composition (dry matter bases %)						
Crude Protein	38.60	37.98	37.97	37.95	37.93	37.91
Crude Lipids	2.26	5.65	9.04	12.43	15.01	19.20
Energy g/kg	15.45	15.79	16.12	16.29	16.45	16.61

1.White fish meal, obtained from Copeinca (Lima, Peru), crude protein 67.4 %, crude lipid 9.3 %
2.Casein, obtained from Hualing Casein Company Ltd. (Gansu, China), crude protein 91.2 %
3.Gelatine, obtained from Rousse lot Gelatin Company Ltd (Guangdong, China), crude protein 91.6 %
4. Tongwei Shihai Feed Corporation Ltd, Wuxi, China
[a] Soybean meal, crude protein 41.4 %

[b] Cottonseed meal, obtained from Tongwei Shihai Feed Corporation Ltd, Wuxi, China, crude protein 38.4 %, crude lipid 0.3 %
[c] Rapeseed meal, obtained from Tongwei Shihai Feed Corporation Ltd, Wuxi, China, crude protein 36.2 %, crude lipid 1.2 %
5. Vitamin mix and mineral mix were provided by Guangzhou Chengyi Aquatic Technology Ltd (Guangzhou, China)
[a] Per kg diet contains thiamine, 20 mg; riboflavin, 20 mg; pyridoxine, 10 mg; nicotinic acid, 100 mg; calcium pantothenate, 50 mg; biotin, 1 mg; folacin, 5 mg; inositol, 500 mg; vitamin E, 50 mg; vitamin A, 2 mg; vitamin B12, 0.02 mg; vitamin K3, 10 mg; vitamin D3,0.05 mg
[b] Per kg diet contain ZnSO47H2O, 525.5 mg; MnSO4H2O, 49.2 mg; KI, 5.23 mg; FeSO47H2O, 238.8 mg; MgSO47H2O, 4.62 g; CuSO45H2O, 11.8 mg; CoCl6H2O, 0.2 mg; Na2SeO4, 0.66 mg; KCl,600 mg; NaCl, 107.1 mg.

Fish and experimental conditions

Healthy juvenile hybrid yellow catfish were obtained from the hatchery at Freshwater Fisheries Research Centre of the Chinese Academy of Fisheries Sciences, Wuxi-China (Jiangsu province). Tanks used for fish culturing during the experiment were disinfected first by chlorine dioxide to prevent disease outbreak before fish stocking. The juveniles were stocked in indoor freshwater re-circulating system comprised of 18 fiber glass tanks (water volume,450 L and aerated) at the fish greenhouse, Wuxi Fisheries College of Nanjing Agricultural University. The juveniles were reacclimatized under laboratory conditions for 2 weeks before starting the experiment, the fish were fed with basal feeds (crude protein 29.0%, lipids 8.0%) twice daily by hand at 8:00-9:00am and at 16:00-17:00pm at the rate of 5% of the fish body weight**.** The amount of the diet consumed was recorded daily, the feeding rate was adjusted every week by weighing the total weight of the fish in each tank. All the fish were starved for 24hrs at the start of the experiment, 504 juveniles in total with the average similar size of 8.77±0.012g were randomly stocked into the 18 indoor fiberglass tanks (water volume,450 L) at a stocking density of 28 fish per a tank. The dissolved oxygen (DO) in the water was kept near satiation and was measured daily at (DO) > 8.09 mg L^{-1} with a HACH HQ30d oxygen meter (Hach Company, Loveland, USA). Temperature and pH of the water were measured once a week to understand changes in the water at 28 ± 0.5°C and pH 7.83 ± 0.2. No significant difference in initial body weight was observed amongst the fish groups. Fish were fasted once every week to ensure that, fish in each tank eat up all the feeds given in about 30 minutes. Uneaten feeds and faeces were collected by siphoning from the tanks after one hour of feeding daily to avoid pollution.

Samples collections and fish weighing

Samples were collected after 12 weeks of feeding trials; the fish were all fasted (starved) for 24 hours to evacuate the alimentary tract contents prior to sampling. Four (04) fish were selected at random for sampling from each tank, individual weight taken before and after fish evisceration and anesthetized with an overdose of tricaine sulfonate at 200 mg L−1 (MS-222; Argent Chemical Laboratories, Redmond, WA, USA).Four sets of Blood samples were obtained, approximately 1.5 ml of blood samples were collected from the caudal veins with heparinized (100 IU ml−1) tuberculin syringes for hematological assays (white blood cells, red blood cells, hemoglobin, hematocrit and platelets) and serum biochemical index, blood samples were also collected from the same four fish using a 1-ml syringe, sorted into 1.5 ml Eppendorf tubes. The serum was collected by centrifuging blood at 3000 x g for 15 min at 4 °C as previously described (Zhou et al.,2010). Supernatants were removed and stored at −80 °C, Hepatosomatic index (HSI) and visceromatic index (VSI) were determined from the four-individual fish per tank by obtaining tissues and expressing ratios as a percent of body weight.

Sample collections and chemical analysis

Growth performance

Feed consumption was recorded daily, after 8 and 12-weeks experiment, fish were individually counted and weighed to calculate the body weight gain (BWG), specific growth rate (SGR) and the feed conversion ratio (FCR) for each treatment were also calculated.

Blood biochemical analysis

Hematological index: white blood cells (WBCs) and red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT) and platelets (PLT) were measured using an automatic blood cell analyzer (bc-5300: Shenzhen MINDRAY Bio Medical Co., Ltd., Shenzhen, China). All reagents were purchased from Shenzhen MINDRAY Bio Medical. Meanwhile Serum biochemical index: triglyceride (TG),

total cholesterol (TC), total protein (TP), glucose concentrations (GLU-GOD), alanine aminotransferase (ALT) and aspartate transaminase (AST) activities were assayed after centrifuging the blood samples (3000 rpm, at 4 °C for 15 minutes). All of these indicators were measured using a fully automatic biochemical Analyzer (bs-400, MINDRAY). All the testing kits were purchased from MINDRAY Medical International Co. Ltd P.R. China.

Hepatic antioxidant function analysis in the liver and muscle

Liver tissues were weighed and homogenized in ice-cold phosphate buffer saline (PBS & Ethanol, pH7.4) at 1:9 (tissue: PBS and Ethanol and the muscle at also 1:9 (PBS & pH7.4) ratio using the bead homogenizers (Scientz-48, Ningbo Scientz Biotechnology Co. Ltd, China). The homogenates were centrifuged at 3000 rpm 4°c for 15 minutes. Liver and muscle rude supernatants were stored at -20° c for further biochemical analysis. The liver and muscle concentrations in superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and the malondialdehyde (MDA) were assayed using commercial test kits (Nanjing Jiancheng Bioengineering institute, China).

Proximate chemical composition analysis

Crude protein was evaluated and estimated by multiplying the nitrogen concentration by 6.25. The crude lipids were measured by the Ether extraction using Soxhlet method. The experimental diets gross energy was analyzed by an adiabatic bomb calorimeter (PARR1281, USA) shown in **Table 1**.

Statistical Analysis and Calculations

Statistical analysis conducted by using SPSS version 20 (SPSS, Chicago, IL, USA). ALL the data were subjected to one-way analysis of variances (ANOVA) followed by S.K.N, LSD and Duncan multiple comparisons. The significant differences ($P < 0.05$) among the groups means were further compared using Duncan's multiple range tests. The optimum dietary lipids levels requirement was estimated using second-degree polynomial regression analysis as described by (ZetrouN et al., 1976). All the results were expressed as means ± SE.

Growth parameters were calculated according to the following equations below.

Weight Gained Rate = (Final Body Weight – Initial Body Weight)/Initial Body Weight ×100

Specific Growth Rate = 100 × (Ln (Final Body Weight) - Lin (Initial Body Weight)/Days of experimental work).

Feed Conversion Ratio = (Fish feed intake) / (Final Body Weight – Initial Body Weight).

Protein Efficiency Ratio = (Final Body Weight – Initial Body Weight) / Feed Protein Intake

Hepatosomatic Index = (Liver weight(g) × 100) / Eviscerated fish Final Weight

Viscerosomatic index = (Viscera weight (g) × 100) / Eviscerated fish Final Weigh

Results

Growth performance and feed utilization

No disease outbreak witnessed throughout the experimental practices. The dietary lipid levels effects on the growth performance and feed utilization of hybrid yellow catfish (*Pelteobagrus fulvidraco* × *Pelteobagrus vachelli*) are shown in **Table 2**. FBW, WG and SGR were significantly affected by the dietary lipid's levels from 3.4% up to 10.2% dietary lipids levels ($P < 0.05$) and decreased significantly from 13.6% to 17% dietary lipid levels. FCR and PER significantly indicated similar results in the fish treatments fed 3.4% and 6.8% followed by 10.2% dietary lipid levels compared to the other groups ($P < 0.05$). HSI was not significantly affected by the dietary lipid levels ($P > 0.05$) statistically, its high value was observed in the group fed 10.2% dietary lipid level compared to the rest of the groups. VSI significantly shown similar results in all the fish groups fed dietary lipid levels compared to the control group ($P < 0.05$).

Table 2 Growth Parameters of Hybrid Yellow Catfish fish for 12 weeks.

Parameters	Dietary lipids levels (%)					
	0.0 (control)	3.4	6.8	10.2	13.6	17.0
IBW (g/fish)[1]	8.77±0.012	8.75±0.01	8.74±0.012	8.76±0.013	8.74±0.006	8.64±0.12
FBW(g/fish)[2]	30.43±4.07^{c}	38.04±1.76^{ab}	41.45±1.61^{a}	35.26±2.2^{abc}	34.15±1.43^{abc}	31.81±2.59^{bc}
WG (%)[3]	160.04±44.88^{b}	234.97±2.94^{ab}	255.49±30.52^{a}	225±15.98^{ab}	211.34±13.22^{ab}	180.53±13.19^{ab}
SGR(%/day)[4]	1.09±0.21^{b}	1.44±0.01^{ab}	1.5±0.1^{a}	1.4±0.05^{ab}	1.35±0.05^{ab}	1.22±0.05^{ab}
FCR[5]	3.45±0.59^{a}	2.48±0.06^{b}	2.43±0.29^{b}	2.37±0.09^{b}	2.39±0.05^{b}	2.97±0.2^{ab}
PER(g/g)[6]	0.97±0.16 ^{a}	0.69±0.01^{b}	0.68±0.08^{b}	0.66±0.02^{b}	0.67±0.01^{b}	0.83±0.05^{ab}
HIS (%)[7]	1.1±0.09	1.08±0.07	0.97±0.04	1.13±0.22	0.8±0.03	0.82±0.09
VSI (%)[8]	11.01±0.66^{b}	12.53±1.32^{ab}	12.35±0.33^{ab}	12.37±0.64^{ab}	12.67±0.59^{ab}	15.31±2.05^{a}

Note: Data are Means ± SE (n=12). Values in the same row with different superscripts are significantly different by Duncan's test ($P < 0.05$) [1]IBW (initial body weight g/kg) [2]AFBW (Average final body weight g/kgs) [3]WG (weight gained) = (final body weight-initial body weight/initial weight)*100% [4]SGR (specific growth rate) = 100%*(ln final body weight (g) – ln initial body weight (g) / feeding days) [5]FCR (feed conversion ratio) **= (**feed intake/ final body weight -initial body weight (g)) [6]PER (Protein efficiency ratio) = (final body weight – initial body weight /protein intake) [7]HIS (hepatosomatic index) = (liver weight /final weight) * 100% [8]VSI (viscerosomatic index) = (visceral weight /final weight) *100%.

Hematological constituents

Hematological WBC, RBC, HCT and the PLT activity significantly showed similar results in the fish treatment fed 17.0% dietary lipids levels compared to the control group ($P < 0.05$) indicated in **Table 3**. HGB showed no any significant difference amongst all the groups ($P > 0.05$).

Table 3 Effects of dietary lipid levels on the hematological Parameters of Hybrid Yellow catfish.

Parameters	Lipids Levels (%)					
	0.0 (control)	3.4	6.8	10.2	13.6	17.0
WBC(×10^{12}/L) [1]	120.37±3.52^{a}	129.04±3.85^{a}	131.23±6.29^{ab}	130.52±4.92^{ab}	123.14±4.22^{a}	120.16±4^{b}
RBC(×10^{12}/L) [2]	2.03±0.05^{ab}	2.33±0.05^{ab}	2.92±0.08^{ab}	2.84±0.08^{ab}	2.08±0.08^{a}	2.05±0.08^{b}
HGB(g/L) [3]	75.33±2.98	83.83±2.74	83.33±2.75	84.25±3.78	80.33±4.67	80.58±3.52
HCT (%) [4]	24.8±1.14^{ab}	28.75±1^{a}	28.9±1.01^{ab}	29.88±1.24^{ab}	27.31±1.39^{ab}	26.07±1.1^{b}
PLT(g/L) [5]	35±2.08^{a}	28.08±1.35^{bc}	33.41±1.34^{a}	30.25±2.2^{ab}	25.25±1.53^{bc}	24.08±1.45^{b}

Note: Data are Means ± SE (n=12). Values in the same row with different superscripts are significantly different by Duncan's test ($P < 0.05$). [1] WBC: (White blood cells) [2] RBC: (Red blood cells) [3] HGB: (Hemoglobin) [4] HCT: (Hematocrit) and [5] PLT: (Platelet).

Serum biochemical analysis

Dietary lipid levels have significantly affected the serum ALT, AST, TP, GLU-GOD, TC and TG activities and contents ($P < 0.05$) as shown in **Table 4**. ALT and GLU-GOD indicated similar results in fish treatment fed 3.4% dietary lipids levels compared to the control group. AST and TP showed similar results in group fed 6.8% dietary lipids levels compared to the control treatment. TC exhibited similar values amongst the groups fed 3.4%, 10.2%, 13.6% and 17% dietary lipids levels compared to the control group. TG higher values observed in fish groups fed 3.4%, 6.8% and 10.2% dietary lipids levels respectively.

Table 4 Effects of dietary lipid levels on the serum biochemical Parameters of Hybrid Yellow catfish.

Note: Data are Means ± SE (n=12). Values in the same row with different superscripts are significantly different by Duncan's test ($P < 0.05$). [1] ALT: (Alanine transaminase) [2] AST: (Aspartate transaminase) [3] TP: (Total protein) [4] TC: (Total cholesterol) [5] TG (Triglyceride) and [6] GLU-GOD (Glucose).

Parameters	Lipids Levels (%)					
	0.0 (Control)	3.4	6.8	10.2	13.6	17.0
ALT(UL^{-1}) [1]	2.01±0.37^{b}	3.37±0.55^{a}	3.11±0.39^{b}	3.88±0.58^{ab}	1.72±0.15^{b}	1.84±0.25^{b}
AST(UL^{-1}) [2]	59.65±7.06^{b}	70.72±6.55^{ab}	77.99±4.25^{a}	83.89±7.82^{ab}	66.08±2.93^{ab}	63.16±3.13^{ab}
TP (g L^{-1}) [3]	59.65±7.06^{b}	66.72±6.55^{ab}	67.99±4.25^{a}	67.89±7.82^{ab}	63.08±2.93^{ab}	62.16±3.13^{ab}
TC (g L^{-1}) [4]	1.1±0.04 ^{a}	1.36±0.1^{b}	1.32±0.04^{ab}	1.43±0.13^{b}	1.38±0.04^{b}	1.51±0.06^{b}
TG (mmol L^{-1}) [5]	1.16±0.16^{b}	1.62±0.31^{ab}	1.34±0.11^{b}	1.74±0.42^{ab}	1.29±0.16^{b}	2.28±0.33^{a}
GLU_GOD (mmol L^{-1}) [6]	1.57±0.08^{b}	1.96±0.09^{a}	1.93±0.08^{a}	1.8±0.09^{ab}	2.05±0.11^{a}	1.77±0.11^{ab}

Hepatic antioxidant function in the liver

Effects of dietary lipids on the hepatic antioxidant function in the liver as shown in **Table 5**. Superoxide dismutase (SOD) showed similar results in the group fed 6.8%, 10.2% and 13.6% dietary lipid levels compared to the control group ($P < 0.05$) however, the highest value was observed in the control group. Glutathione peroxidase (GSH-Px) was significantly different in the group fed 6.8% dietary lipid levels compared to the other groups ($P < 0.05$) and have the lowest figure observed in the same treatment 6.8% followed by 10.2% dietary lipid levels. Catalase (CAT**)** was significantly different in the groups fed 6.8% and 10.2% followed by 13.6% dietary lipid levels than the other groups and the lowest value observed was in fish group fed 6.8% dietary lipid level. Malondialdehyde (MDA) showed significant difference in the group fed 6.8% and 10.2% dietary lipid levels than other groups and the lowest (best) value exhibited in the group fed 3.4% dietary lipid levels.

Table 5 Effects of dietary lipid levels on the antioxidant enzymes function in the Liver of Hybrid Yellow catfish.

Parameters	Lipids Levels (%)					
	0.0 (Control)	3.4	6.8	10.2	13.6	17.0
SOD (Umg^{-1} protein) [1]	67.24±2.2^{a}	63.04±1.49^{ab}	54.63±2.1^{c}	59.53±1.72^{bc}	59.79±1.65^{bc}	63.29±1.53^{ab}
GSH-Px (Umg^{-1}prot) [2]	594.41±12.5^{a}	574.85±14.79^{ab}	485.36±17^{c}	531.29±13.99^{b}	543.37±16.5^{b}	547.44±17.16^{b}
CAT (Umg^{-1} protein) [3]	6.43±0.18^{a}	6.3±0.15^{ab}	5.55±0.19^{c}	5.61±0.13^{c}	5.92±0.13^{bc}	5.99±0.15^{abc}
MDA (Umg^{-1} protein) [4]	17.32±0.92^{b}	16.91±1.1^{b}	22.57±1.03^{a}	20.8±1.17^{a}	20.01±1.27^{ab}	19.27±0.99^{ab}

Note: Data are Means ± SE (n=12). Values in the same row with different superscripts are significantly different by Duncan's test ($P < 0.05$). [1] SOD: (superoxide dismutase) [2] GSH-Px: (glutathione peroxidase) [3] CAT: (catalase) and [4] MDA: (malondialdehyde).

Discussion

No fish disease outbreak or pathogenic stress was detected throughout the experiment. According to the present study results, WG, SGR, FCR and PER of hybrid yellow catfish were best in the fish groups fed 3.4%, 6.8%and 10.2% dietary lipid levels and then decreased as the dietary lipid levels increased to 13.6% and 17.0% indicating that the growth benefit had an evident improvement in the feed utilization. Our results correspond with (Huang et al.,2009)

Dietary lipids levels greater than 10.2% have no significant benefits for improvement of hybrid yellow catfish growth. This study is reasonably in agreement with a similar report that dietary Lipid levels in feed composition of 6–12% were found to boost the optimal growth of 5.7-g of Pelteobagrus vachelli juveniles (Qiang et al. 2017). The increase in dietary lipid levels in quantity higher than 10.2 % did not lead to better growth performance, suggesting that dietary fish oil around 10.2 % is sufficient to provide essential FAs and lipid-derived energy to hybrid yellow catfish. Other studies revealed reduced growth of fish fed on high-lipid diets, having excessive energy could cause reduced feed intake (Martins et al., 2007). A further study reported that high lipid levels in feed could cause imbalances in the protein to lipid ratio and digestible energy, leading to reduced lipid utilization and fatty acid synthesis, hence hampering fish growth (Mohanta et al. 2008). Similar trends were also observed in Atlantic halibut by (Martins et al., 2007). In this study, the optimal lipid levels requirements for hybrid yellow catfish were within the range of values reported for omnivorous fish lipids requirements established (Chou et al., 1990).

The dietary lipid levels have not significantly influenced HSI; statistically, it showed low values with increased dietary lipids levels while VSI significantly increased with increased dietary lipid levels. Our study suggested that, there was a low energy reserve in the ratio between lipids and carbohydrates. The results of the present study are in agreement with those findings in juvenile Chu's croaker (*Nibea coibor*) (Yisheng et al.,2016). HSI indicated the status of energy reserve, a high value of HSI means high energy reserve. High HSI and VSI are often related to weak growth and fish health due to increased levels of dietary carbohydrate. Other studies revealed that, the factors favoring animals' growth include diet composition, culturing conditions, age, and the experimental period taken while young animals are more sensitive to nutrient deficiency compared to the ones in the later growth levels (Huang et al.,2009). It has been reported that excessive dietary lipids resulted in excessive accumulation of fat in the visceral cavity and tissues. Fish prefer to store fats in the visceral cavity and liver under higher-lipid diets (Chou et al., 2001).

In the present study, WBC, RBC, HCT, and PLT increased significantly with the increasing dietary lipid levels from 3.4%, 6.8%, and 10.2%, and decreased inversely with the increasing dietary lipid levels from 13.6% to 17%. The increase in blood cells may be due to the excellent health conditions in culturing with well-balanced diets and in agreement with previous findings in channel catfish I. punctatus (Wang et al. 2014). Blood is an essential component of the immune system, and changes in blood parameters can be used to evaluate the physiological health of fish. Other studies have shown that blood health conditions can be indicative of dietary manipulations, and are good indicators of nutrition, stress, and the overall health of fish (Zhao et al. 2015). Hemoglobin exhibited no significant difference amongst the groups but, it increased with a decrease in dietary lipids in the groups fed 3.4%, 6.8%, and 10.2% then declined with the increase in dietary lipid levels in groups fed 13.6% and above. These results demonstrate that, dietary lipid levels have a positive effect on the hematological function of the hybrid yellow catfish, and environmental and anti-nutritional factors cause the variation in the values. Other studies showed that, factors such as essential nutrients deficiency, growth, anti-nutritional and the environment could cause differences in the contents of HCT and HGB. contents differ due to factors such as essential nutrients deficiency, growth, anti-nutritional and environmental factors (Kim et al., 2005).

Nevertheless, dietary lipids levels have significantly affected the serum alanine transaminase (ALT) aspartate transaminase (AST), total protein (TP), total cholesterol (TC), triglyceride (TG) and glucose (GLU_GOD) respectively. AST and TP constituents significantly increased with the low lipids trend in the groups fed 3.4%, 6.8% and 10.2% dietary lipid levels and then decreased with the increased dietary lipid levels from 13.6% to 17.0% hence, indicating healthy or no damage to the liver and improvement in the natural immunity as well as dietary lipids metabolism efficiency. Plasma ALT and AST are vital indicators that show the liver functions and its principal activities in evaluating the levels of its damage. Trenzado et al. (2009) and Wang et al. (2014) results indicated that ALT and AST are specifically known as the key

indicators of the liver or cellular damages in both mammals and fish, revealing toxicity levels via the diets. Also, ALT and AST are commonly known as the crucial aminotransferases in fish and usually are used as helpful indicators of liver damage (Owolabi et al., 2011). TP level is an indicator of diet metabolism, and natural immunity considered a vital defense mechanism of the fish. A similar result was also indicated in the fingerlings of the Japanese flounder (*Paralichthys olivaceus*) fed on the soya bean meal (Shi et al.,2013). A further study reported that TP is a vital indicator of diet metabolism; its decrease is likely to cause a decrease in digestion and metabolism of the diet. (Hrubec et al., 2000).

The lowest TC concentration was observed in the fish group fed 6.8%, followed by 3.4%dietary lipids, which indicated the positive impact of the dietary lipids on the health and immunity of hybrid *Pelteobagrus fulvidraco*. In comparison, TG concentration increased higher in the fish fed 17% dietary lipids than other groups suggesting that there was a buildup of fat tissues with low oxidation of fatty acids in the liver, which might cause fatty liver. This study corresponds with (Nanton et al.,2001) study that, high TG concentration is associated with fat tissue build up causing fatty liver and when there is increased oxidation of fatty acid in the liver, serum TG levels normalize. Fish exposed to the toxic environments would result in a series of the pathological phenomenon, such as liver damaged (Zhao et al., 2015). In the present study, plasma GLU-GOD significantly increased with the decrease in dietary lipid levels in fish fed 3.4% and 6.8%, followed by 13.6%. Eventually, declining with the increased dietary lipids level fed 17%, suggesting health hepatic function and the activation of glycogen synthesis. Some studies on the Japanese flounder showed that low blood GLU of plant based diets exhibited the stimulation of glycogen synthesis and liver health functioning. The serum biochemical assays can reflect the health status, nutrition status, and adaptability to the environment once fish undergo physiological or pathological changes affected by external factors (Shi et al., 2013).

In our present study, the SOD, CAT, MDA, and GSH-Px enzymes were significantly influenced by the dietary lipid levels, and the antioxidant status in fish can be accurately reflected by the activity of SOD, CAT, and MDA. Hepatic SOD and CAT concentration showed low values in the fish group fed 6.8% and 10.2% than the other groups that increased with the increase in dietary lipid levels suggesting that there was a standard oxidation rate under low dietary lipid levels. Serum SOD and CAT are common antioxidant enzymes that can protect the organism against damage by reactive oxygen species (ROS), which may lead to many disorders through macromolecules attack. SOD removes the damaging ROS by the process of catalyzing the dismutation of the two superoxide radicals to the hydrogen peroxide and oxygen concentrations. The decrease in dietary lipid levels resulted in higher MDA concentration in the liver of fish fed 6.8%, followed by 10.2%, which suggests that increased susceptibility to fatty acid occurred per-oxidation in the fish liver. (Li et al., 2012).

Increased antioxidant capacity of the hybrid yellow catfish might increase the antioxidant enzymes to decrease the ROS and reducing the MDA contents. Moreover, MDA is formed as a typical product of lipid peroxidation, but overdose MDA can damage cell structure and function (Wang et al., 2014) found that environmental toxin exposure provoked an increase in hepatic MDA levels, which can generate tissue damage via the induction of lipid peroxidation (Antonopoulou et al., 2014).

The significant lowest GSH-Px concentration was observed in the group fed 6.8% and 10.2% than the other groups that increased with the increase in dietary lipid levels which indicate that increasing the dietary lipid levels may have stimulated an increase in antioxidant enzyme activities to neutralize stress-induced oxidative damage, such as lipid peroxidation. Similarly, (Qiang et al. 2017) reported that Antioxidant enzymes such as GSH-Px could provide information regarding the first line of cellular defense against toxic free radicals that cause oxidative stress. Under normal conditions, the antioxidant defenses of fish prevent the uncontrolled generation of ROS through the antioxidant system (Sun et al., 2013).

Conclusion

This study was the first to indicate that dietary lipid levels affected growth performance, hematological assays, serum biochemical index, and the antioxidant defense in the liver and muscle of the hybrid yellow catfish. Our results revealed that dietary lipid levels of 3.4, 6.8% and–10.2% increased weight gained, improved feed utilization, and balanced diet metabolism, and well antioxidant defense mechanism under culture conditions. Higher dietary lipid levels (13.6% & 17%) possibly will reduce growth and survival rate, causing a stress response in hybrid

yellow catfish, causing inhibited antioxidant defense that weakens the liver function leading to reduced immune response. Future studies can help to determine the optimum dietary lipid levels requirements of hybrid yellow catfish (P*elteobagrus fulvidraco* × *P. Vachelli*).

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