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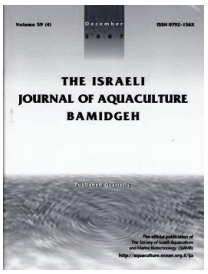

Dietary Protein Requirements for Juvenile Hybrid Culter (*Culter alburnus* ♀ × *Ancherythroculter nigrocauda* ♂)

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Keywords: juvenile hybrid culter; dietary protein level; growth performance; digestive enzyme activities

Abstract

This study was conducted to evaluate the effects of dietary protein levels on growth performance and digestive enzyme activities of juvenile hybrid culter (*Culter alburnus* ♀× *Ancherythroculter nigrocauda* ♂). Five isolipidic experimental diets were formulated to contain graded levels of protein ranging from 29.54-48.98%. These were fed to triplicate groups of fish (11.44 ± 0.7 g/fish) for 8 weeks. Results from the feeding trial indicated that growth performance, body composition, and digestive enzyme activities of the fish were significantly different ($P<0.05$) between the groups. Growth performance had a generally increasing trend with increasing dietary protein levels. Weight gain (160.76%) and specific growth rate (1.71) of fish in the group where feed contained 40.07% dietary protein was significantly higher than the group where feed contained 33.58% dietary protein (118.76% and 1.40, respectively), but there was no significant difference compared with 45.04% dietary protein. Based on the statistical significance, our results indicated that the dietary protein level should not be less than 40% for juvenile hybrid culter.

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Introduction

Protein in the diet is an important nutritional component for fish growth and reproduction as well as maintenance of a sustainable farming environment (Lee et al., 2000). Insufficient dietary protein retards growth, and causes low feed utilization, and low disease resistance in fish. On the other hand, excessive utilization of protein in diets may retard fish growth and also leads to water pollution caused by nitrogenous excretions (Boyd and Tucker 2014). Thus, the ratio of protein in fish diets should be carefully monitored.

Fish breeding scientists have successfully bred a new hybrid culter namely *Culter alburnus* ♀× *Ancherythroculter nigrocauda* ♂ which has been widely farmed in China. To date, there have been a few preliminary studies about the genetics of this hybrid fish published (Chen et al., 2014; Zhu et al., 2016). However, the available information on nutrient requirements (especially dietary protein requirement) of this fish is scarce. Thus, commercial feed for other fish species has been used for the culture of these fish. This has led to feed wastage, and water pollution that affects fish growth.

The objective of the present study was to determine the optimal dietary protein requirement by measuring growth performance, feed utilization, body composition, and digestive enzyme activities of juvenile hybrid culter.

Materials and Methods

Five experimental diets were formulated from ingredients containing 29.54, 33.85, 40.07, 45.04 and 48.98% crude protein (see Table 1). White fishmeal and corn gluten meal were used as protein sources and soybean oil as lipid source. All ingredients were thoroughly mixed with soybean oil and water, made into pellets (2mm, diameter) and dried in an oven at 45°C until the moisture was reduced to < 10%. The dry pellets were stored in a freezer at -20°C until used.

Table 1. Formulation and proximate composition of the experimental diets（%）

	Diet1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
Ingredients					
White fish meal[1]	25	32.5	40	47.5	55
Corn gluten meal[2]	15	15	15	15	15
Soybean oil	6.5	6	5.5	5	4.5
Wheat bran	20	17.5	15	12.5	10
α-Starch	31	26.5	22	17.5	13
Vitamin premix[3]	0.5	0.5	0.5	0.5	0.5
Mineral premix[4]	1	1	1	1	1
Mold inhibitor	0.5	0.5	0.5	0.5	0.5
Monocalcium phosphate	0.5	0.5	0.5	0.5	0.5
Proximate composition (dry matter basis)					
Crude protein	29.54	33.85	40.07	45.04	48.98
Crude lipid	20.15	19.94	20.18	20.21	20.11
Ash	9.5	10.39	11.05	12.48	13.33

[1] Gaolong Dietary Company, Wuhan, China, Imported from USA.
[2] Xinwang Dietary Company, Wuhan, China.
[3] Vitamin premix (mg/kg diet) thiamin 50 mg; riboflavin, 90 mg; pyridoxine HCl, 40 mg; vitamin B_{12}, 0.2 mg; vitamin K_3, 20 mg; inositol,1600 mg; pantothenic acid, 120 mg; niacin acid, 400 mg; folic acid, 40 mg; biotin, 2.40 mg; retinol acetate, 64 mg; cholecalciferol, 10 mg;alpha-tocopherol, 240 mg; ascorbic acid, 4000 mg; choline chloride, 5000 mg; ethoxyquin 300 mg, wheat middling, 36.78 g.
[4] Mineral premix (mg or g/kg diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2$-$6H_20$ (1%), 50 mg; $CuSO_4$-$5H_2O$, 10 mg; $FeSO_4$-H_2O, 80 mg; $ZnSO_4$-H_2O,50 mg; $MnSO_4$-H_2O,60 mg; $MgSO_4$-$7H_2O$, 1200 mg; NaCl,100 mg; Zoelite,15.45g.

Experimental fish were obtained from Wuhan Aquaculture Science Research Institute, Wuhan, China. Prior to beginning the experiment, they were acclimated to the experimental conditions for 2 weeks and fed a commercial diet twice a day. At the

beginning of the 8 week feeding trial, 450 uniform-sized healthy fish (11.44 ± 0.7g/fish) were stocked in 15 experimental net cages (1m×1m×1m), 30 fish per cage. Each diet was assigned to three cages in a completely randomized design. The fish were hand-fed gradually to satiation twice a day (08:00 and 17:00 h), and feed consumption was recorded daily. During the experimental period, the temperature ranged from 25-28°C, and dissolved oxygen was approximately 8.6 mg/L.

At the beginning of the feeding trial, a sample of 20 experimental fish and samples of their diets were collected and stored at -20°C for further chemical analysis. At the end of the feeding trial, all the fish in each cage were collectively weighed and counted to calculate mean weight.

Ten fish from each cage were selected for chemical analysis. The proximate compositions of the fish and diets were analyzed according to standard methods (AOAC, 1995). Samples were dried to a constant weight at 105°C for 12 hours to determine moisture. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl system method after acid digestion; crude lipid by ether extraction using Soxhlet; ash by combustion at 550°C for 24 hours.

Another six fish from each cage were selected for digestive enzyme activity analysis. Intestines of fish were separated and treated using the method described in our previous study (Yu et al., 2013) with some modifications. Individual intestines were washed thoroughly with chilled saline (0.69% NaCl, w/v), dried quickly on a piece of filter paper and weighed. The intestines were homogenized into 10 volumes (w/v) of 0.69% NaCl, and then centrifuged at 2500 rpm for 10 min. The supernatant was separated for enzyme analysis. Digestive enzyme activities in intestines (trypsin, amylase and lipase) were determined using trypsin assay kit (No. A080-2); amylase assay kit (No. A016-1) and lipase assay kit (No. A054-1) (Jiancheng Bioengineering Ltd., Nanjing, China). Protein concentration in homogenates was determined using the total protein assay kit (with standard: BCA method, No. A045-3). (Jiancheng Bioengineering Ltd., Nanjing, China).

Weight gain (WG), specific growth ratio (SGR), feed efficiency (FE), protein efficiency ratio (PER), condition factor (CF), viscerosomatic index (VSI) and hepatosomatic index (HSI) were calculated according to the following equations:

WG (g/fish) = final mean body weight - initial mean body weight.

SGR (%/day) = (ln final weight - ln initial weight) × 100/days of experiment.

FE = wet weight gain / dry feed intake.

PER = fish wet weight gain (g) / protein intake (g).

CF = 100 × (W / L3), W: wet body weight (g) and L: body length (cm).

VSI = 100 × (viscera weight / body weight).

HSI = 100 × (liver weight / body weight).

Results are presented as mean ± SD. All data were subjected to one-way ANOVA. Significant differences ($P < 0.05$) were assessed using Tukey's HSD test. All statistical analyses were conducted using SPSS 16.0 for Windows (SPSS, Michigan Avenue, Chicago, IL, USA).

Results

In this study, the growth performance and feed utilization of juvenile hybrid culter fed different dietary protein levels are presented in Table 2. Dietary protein levels significantly affected growth performance and feed utilization ($P<0.05$). WG and SGR showed an increasing trend in relation to the dietary protein level ($P<0.05$). WG (160.76%) and SGR (1.71) of fish in the group contained 40.07% dietary protein was significantly higher than those contained 33.58% dietary protein (118.76% and 1.40, respectively), but there was no significant difference compared with 45.04% dietary protein. In addition, FE also increased with increasing dietary protein level. There was no significant difference in the PER between the experimental groups ($P>0.05$).

Table 2. Growth and feed performance of juvenile hybrid culter fed the experimental diets

	Protein levels (%)				
	29.54	*33.58*	*40.07*	*45.04*	*48.98*
IBW (g/fish)	11.51±0.08	11.42±0.41	11.53±0.38	11.46±0.22	11.29±0.16
FBW (g/fish)	24.37±1.06a	25±1.68a	30.09±2.79b	32.72±1.04b	33.44±0.73b
WG (%)	111.43±5.23a	118.76±4.67a	160.76±11.84b	185.76±7.21bc	196.24±2.82c
SGR(%/ay)	1.34±0.08a	1.40±0.06 a	1.71±0.14b	1.87±0.08bc	1.94±0.03c
FE	0.57±0.06a	0.63±0.08 a	0.74±0.09b	0.77±0.05b	0.79±0.02b
PER	1.93±0.20	1.85±0.24	1.82±0.24	1.74±0.10	1.56±0.03

Different letters represent significant differences at P<0.05.
Abbreviations: IBW, Initial body weight; FBW, Final body weight; WG, Weight gain; SGR, Specific growth rate; FE, Feed efficiency; PER, Protein efficiency ratio.

Biometric parameters of juvenile hybrid culter are shown in Table 3. HIS, VSI, and CF were significantly affected by increasing dietary protein levels (P < 0.05). CF value in 29.54% protein diet (1.04) was significantly lower than those in 45.04-48.98% protein diets (P<0.05). The HSI and VSI values in fish fed 40.07% protein diet (1.06 and 8.57, respectively) were significantly higher than other groups (P<0.05).

Table 3. Biometric parameters of juvenile hybrid culter fed the experimental diets

Protein levels (%)	*CF*	*HSI*	*VSI*
29.54	1.05±0.01a	0.85±0.01a	6.32±0.87a
33.58	1.04±0.01a	0.94±0.13ab	6.24±0.34a
40.07	1.10±0.04ab	1.06±0.06b	8.57±0.98b
45.04	1.14±0.06b	0.79±0.04a	7.29±0.91ab
48.98	1.16±0.04b	0.84±0.07a	6.77±0.11ab

Different letters represent significant differences at P<0.05.
Abbreviations: CF, Condition factor; HIS, Hepatosomatic index; VSI, Viscerosomatic index

Body composition of the fish (wet basis) is shown in Table 4 and crude protein content as well as crude lipid content were significantly affected by dietary protein level (P<0.05), although no significant difference in ash content was found. Moisture content significantly decreased with increase of dietary protein levels (P<0.05). Fish fed diets containing 45.04% protein had the highest protein (17.2%) and lipid content (12.57%), which were significantly higher than those fed diets containing 29.54% dietary protein (16.09% and 10.58%, respectively).

Table 4. Proximate composition (%, wet basis) of juvenile hybrid culter fed the experimental diets.

Protein levels (%)	*Moisture*	*Crude protein*	*Crude lipid*	*Ash*
29.54	72.87±0.19b	16.09±0.12a	10.58±0.33a	3.14±0.08
33.58	73.20±0.71b	16.39±0.34a	10.33±0.60a	3.11±0.14
40.07	72.67±0.15b	16.15±0.19a	11.10±0.38ab	3.04±0.14
45.04	71.02±0.43a	17.20±0.21b	12.57±0.19b	2.97±0.08
48.98	71.94±0.72ab	17.00±0.35b	11.44±0.72ab	3.00±0.03

Different letters represent significant differences at P<0.05.

Trypsin and lipase activities in the intestine of juvenile hybrid culter improved significantly (P<0.05) with increased dietary protein level, although there was no significant difference in amylase activity between experimental groups (Table 5). Fish fed 29.54% dietary protein diet had the lowest trypsin activity (505.8 U/mg protein) and lipase activity (82.57 U/g protein) in the intestine and these were significantly lower (P<0.05) than fish fed 40.07% dietary protein diets. However, trysin, lipase, and amylase activities were not significantly enhanced when the dietary protein level increased from 40.07 to 48.98%.

Table 5. Digestive enzyme activity in intestine of juvenile hybrid culter fed experimental diets.

Protein levels (%)	*Protease activities (U/mg protein)*	*Lipase activities (U/g protein)*	*Amylase activities (U/g protein)*
29.54	505.80±65.56a	82.57±11.67a	224.86±20.87
33.58	573.66±40.68a	160.97±12.59b	232.11±16.79
40.07	740.48±35.13b	166.54±11.12b	285.74±34.98
45.04	543.66±15.04a	142.00±18.16b	202.29±34.59
48.98	590.17±13.29a	137.59±12.29b	256.45±38.60

Different letters represent significant differences at $P<0.05$.

Discussion

In this study, dietary protein levels in diets significantly affected growth performance and feed utilization of juvenile hybrid culter. Based on our results we concluded that dietary protein levels in diets should not fall below 40% for this fish. This was similar to its maternal parent *Culter alburnus*, about 42%, (Wang et al., 2004) and other fish such as juvenile *Sarotherodon mossambicu*, 40% (Jauncey 1982), juvenile *Myxocyprinus asiaticus, 46.5%* (Zhang et al., 2009) and *Lepomis macrochirus,* 41.51-42.37% (Yang et al., 2016).

In the present study, crude protein of fish showed a general increasing trend with increased dietary protein levels and similar results were found in other fish species (Zhang et al., 2009). Whole body protein was often measured as an index of protein utilization. However future investigation is needed to determine whether dietary protein level was the only parameter which contributed to this variation, since dietary protein levels increased however the amount of wheat bran and α–starch was reduced. Crude lipid content of fish also showed a general increasing trend with increasing dietary protein levels. In some fish species, excess dietary protein is deposited as body fat (Deng et al., 2011) or utilized as energy fuel in metabolism (Wu and Gatlin 2014). Our results indicated that dietary protein was not only used for protein deposition, but also deposited as body fat for juvenile hybrid culter.

Digestive enzymes were responsible for the digestion of nutrients in the diets. Digestive enzyme activity, which could be affected by species of fish, fish health, water temperature, feed composition, and other factors, was the most important indicator of digestive ability (Yu et al., 2013). In the present study, increased dietary protein levels caused up-regulation of trypsin activity, but this activity did not improve when dietary protein levels increased from 40.07 to 48.98%. Similar results were reported in *Culter alburnus* (Qian et al., 2004) and *Cyprinus carpio* (Kawai and Ikeda 1972). In general, digestion of dietary protein in fish seems to be controlled by two different kinds of protease: pepsin and trypsin. However, as a stomachless fish, trypsin may be the major enzyme to digest the dietary protein in diets for hybrid culter. It appears that as the dietary protein levels increased, trypsin activity in the juvenile hybrid culter was enhanced to improve digestion and absorption of dietary protein. This result is in accordance with optimal digestion theory: metabolism is enhanced when the secretion of digestive enzymes by animals is positively correlated with substrate concentration (Sibly 1981; Penry and Jumars 1986).

The first response of animals fed deficient macronutrients is to increase the secretion of enzymes to digest macronutrient, while animals decrease the secretion of enzyme for excess macronutrients (Clissold et al., 2010). In the present study, the increase in dietary protein content was counteracted by a subsequent reduction in dietary carbohydrates, in the form of wheat bran and α-Starch. Amylase activity of juvenile hybrid culter was slightly up-regulated, although this was not significant. This result suggests that when juvenile hybrid culter are fed carbohydrate deficient diets this could cause up-regulation of amylase activity.

In conclusion, the results of this study indicated that dietary protein levels improved growth performance, feed utilization, and digestive enzyme activities. Based on the results we concluded that dietary protein for juvenile hybrid culter should not fall below 40%.

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