RESEARCH ARTICLE

EFFECTS OF DIETARY PROTEIN LEVEL ON GROWTH PERFORMANCE, MUSCLE COMPOSITION AND NITROGEN EXCRETION IN JUVENILE *ANCHERYTHROCULTER NIGROCAUDA* (YIH & WU 1964)

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ABSTRACT

Quantification of the optimum dietary protein level for juvenile *A. nigrocauda* in relation to nutrition is important for commercial fish culture operations, and to estimate the muscle composition and changes nitrogen excretion for the fish, the fishes were fed with changing protein levels. Six experimental diets containing 30.01%, 34.57%, 39.63%, 44.75%, 49.52%, and 54.47% protein with 17.24 MJ/kg diet, respectively, were fed to three-replicate six groups of forty fish (mean weight: 16.30±0.02 g) for 70 days. The results showed that the maximum specific growth rate (SGR) and the best protein efficiency ratio (PER) occurred at 44.75% dietary protein level ($p < 0.05$). The polynomial equations regression analysis indicated that for maximal SGR, *A. nigrocauda* optimal required 44.32% dietary crude protein and the potential for the culture. The feed conversion ratio (FCR) was significantly decreased with increasing dietary protein levels up to 44.75% ($p < 0.05$), but no significant differences were found between the groups with protein levels of 44.75% to 49.52% ($p > 0.05$), and thereafter FCR was increased with further increases of dietary protein. Moreover, the fish accumulated the more protein and the less lipid in the muscle with the more dietary protein level. Muscle composition did not show difference on moisture and ash content between the groups with 30.01% to 54.47% protein level ($p > 0.05$). The amount of Ammonia-N excreted by *A. nigrocauda* within 24 h was enhanced ($p < 0.05$) as increasing dietary protein level, whereas Urea-N increased ($p < 0.05$) except the highest dietary protein level of 54.47%.

INTRODUCTION

A. nigrocauda is a cyprinid fish endemic to the upper reaches of the Yangtze River. It is usually dominant in the fish assemblages of estuary habitats of small tributaries and also abundant in lentic habitats of most reservoirs located in the region of its original distribution. Unfortunately, due to long term overfishing, water pollution and habitat degradation, its habitat was reduced by approximately 25% after the completion of the Three Gorges Reservoir. Fortunately, aquaculture of the fish has been initiated in a few farms in the upper Yangtze River and it has gained more attention because of its market potential for intensive culture. As the intensification of fish culture has lead to dependence on artificial feeds, reducing the feeding costs could be a key factor for successful development of aquaculture. And for those costs, protein is the most expensive component in fish feeds and also the most important factor affecting growth performance of fish.

The protein requirement for optimum growth is the first nutritional parameter to be determined for formulated feed production for a newly established cultured fish species (Kim and Lee, 2009). Many studies have been carried out to determine protein requirements for fish, with estimated protein requirements ranging from 30 to 55% according to fish species, fish size, dietary protein sources and environmental conditions (NRC, 1993). In China, protein requirement studies for *Ancherythroculter* have mainly focused on *A. nigrocauda* (Ding *et al*., 2012) *and E. ilishaeformis* (Chen *et al*., 2005). A direct relation between protein metabolism and nitrogen excretion has been found in fish (Gelineau and Medale, 1998). Ammonia is the main end product of protein metabolism in teleosts, which accounts for at least 80% of total nitrogen excretion (Elliott, 1976). The measurements of Ammonia-N and Urea-N excretion have been used as indicators of the effects of nutritional factors on protein metabolism (Perera *et al*., 1995). Therefore, quantification of Urea-Nitrogen excretion for fish species in relation to nutrition is important for commercial fish culture operations. The present study was designed to estimate the optimum dietary protein level and changes in muscle composition for the juvenile *A. nigrocauda*

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based on a 70 days' growth experiment. The nitrogen excretion within 24 h was also used as an index of protein metabolism.

MATERIALS AND METHODS

Experimental diets

Six experimental diets were made is energetic (17.24 MJ/kg) by adjusting the dextrin and a-starch content (Table 1). Brown fish meal (Peru) and casein were used as the protein sources. Fish oil and soybean oil were used as lipid sources and dextrin and a-starch were used as carbohydrate sources. The experimental diets were formulated to six graded level of crude protein (30.01%, 34.57%, 39.63%, 44.75%, 49.52% and 54.47%). Diet ingredients were ground through 80 mesh sizes. Micro components were mixed with the progressive enlargement method. Distilled water (40%, W/W) and lipid were added to the premixed dry ingredients and thoroughly mixed until homogenous in a mixer (SLX - 80, Luoyang, China). The 1.5-mm diameter pellets were wet-extruded, and dried in a ventilated oven at 45°C for 12 h, sealed in plastic bags and stored at - 4°C until used.

Table 1. Ingredient composition of experimental diets (% dry matter)

Ingredients /%	Dietary proteins level (%)					
	30.01	34.57	39.63	44.75	49.52	54.47
Brown fish meal (Peru)	35.36	40.51	48.36	56.29	63.69	70.38
Casein	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	1.56	1.73	1.42	1.45	1.81	1.22
Fish oil	4.61	3.83	3.56	3.53	1.91	1.84
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.5
Vitamin mixture[a]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral mixture[b]	2.50	2.50	2.50	2.50	2.50	2.50
Dextrin[c]	34.23	29.85	22.09	14.46	7.28	2.57
a-Starch[d]	8.45	9.08	9.57	9.93	10.55	8.99
Sodium alginate	0.50	0.50	0.50	0.50	0.50	0.50
Gelatin	0.50	0.50	0.50	0.50	0.50	0.50
Proximate composition (%)						
Crude protein	30.01	34.57	39.63	44.75	49.52	54.47
Crude lipid	9.13	9.08	9.14	9.11	9.10	9.06
Dry matter	91.52	91.33	90.21	90.44	90.57	90.49
Ash	11.33	11.02	11.65	11.89	12.35	12.49
Energy (MJ/kg)[d]	17.35	17.13	17.16	17.21	17.24	17.36
Protein/energy ratio (mg/kJ)	18.46	20.18	23.09	26.01	28.72	31.38

[a]Vitamin premix (IU or mg/kg diet): *DL*-alpha tocopheryl acetate, 60 IU; Na menadione bisulfate, 5 mg; retinyl acetate, 15 000 IU; *DL*-cholecalcifeol, 3000 IU; B_1, 15 mg; B_2, 30 mg; B_6, 15 mg; B_{12}, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1 000 mg; calcium pantothenate, 50 mg; choline, 2000 mg.
[b]Mineral premix (g or mg/kg diet): $CaCO_3$, 2.15 g; MgO, 1.24 g; $FeC_6H_5O_7 \cdot H_2O$, 0.2 g; KI, 0.4 mg; $ZnSO_4$, 0.4 g; $CuSO_4 \cdot 5H_2O$, 0.3 g; $MnSO_4 \cdot H_2O$, 0.3 g; $CaH_2PO_4 \cdot 2H_2O$, 5g; $CoC_{12} 6H_2O$, 2 mg; Na_2SeO_3, 3 mg; KC1, 0.9 g; NaC1, 0.4 g.
[c]Supplied by Qufu Tianli Medical Supplements Co., Ltd. (Shandong, China).
[d]Energy: calculated after NRC (1993) as 16.7, 16.7 and 37.7 MJ/kg for carbohydrate, protein and lipid, respectively.

Experimental procedures

Two thousand healthy juveniles *A. nigrocauda* were obtained from Yangqiao Fingerling Station (in Luzhou's research workstations of the Institute of Hydrobiology, Chinese Academy of Sciences). The fish were transported to the laboratory in Henan University of Technology, acclimated to laboratory conditions and fed a commercial feed (42.0% crude

protein, 9.1% crude lipid, 17.2 MJ/kg digestible energy; supplied by Kaifeng Bio-Tech Co., Ltd., Kaifeng, China) for two weeks. At the end of the acclimation period, fish with an average weight of approximately 16.30±0.02 g were randomly selected (720) and stocked in eighteen 800-L tanks (triplicate groups per dietary treatment) at a density of 40 fish/tank. The fish were fed twice daily (3% of their body weight) by hand as much as they would consume in 40 min at 08:30 AM and 17:30 PM for 70 days. Water was recirculated (0.05 L/min) with its temperature at 24.5±0.2°C maintained by a heating rod. During the experiment, all rearing tanks were provided with continuous aeration and the diurnal cycle was 12-h light (12-h dark) (in the period of 1 April - 24 May 2013). Any uneaten feed was collected 40 minutes after every feeding, and the dry matter content was determined for both supplied and uneaten diet, and the data used for feed consumption calculation. Feed consumption was recorded by subtracting the amount of uneaten diet from total amount of diet fed on a dry weight basis.

Samples collection techniques and chemical analyses

At the end of the growth trial, *A. nigrocauda* were starved for 24 h and then weighed. Total number and mean body weight of fish in each tank were measured to calculate the weight gain (WG), SGR, FCR and PER. A portion of dorsal muscle from three fishes, respectively, in each tank, was hold together, homogenized and frozen (- 20°C) for subsequent proximate analysis. Crude protein, crude lipid, moisture and ash in diets and muscle tissue were determined by the standard methods (AOAC, 2005). Moisture was determined by oven-drying at 105°C for 24 h. Crude protein (N × 6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030 - Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid was determined by ether-extraction using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Ash was determined by muffle furnace at 550°C for 24 h.

Nitrogen test

Admeasurements of Ammonia-N and Urea-N excretion were carried out in a thermo regulated (24.5±0.2°C), ultraviolet-sterilized, closed system consisting of nineteen 10 L round bottom buckets (containing 6 L water) designed according to the original layout described by Cho *et al.* (1992). Six groups, three-replicate per group, each subgroup consisting of 1 *A. nigrocauda* juvenile (initial mean body mass, 49.62±0.56 g) and one bucket without fish was used as a control check, underwent a preliminary 48 h adaptation period before being fed the corresponding six protein levels in isoenergetic (i.e. one meal, approximately 3.50% body mass). Ammonia-N and Urea-N concentration were measured using the urease Berthlot method (Shanghai Institute of Biotechnology, kit) within 24 h, respectively.

The method of calculating indicators

Weighing and counting the test fish were used to statistically evaluate the total amount of feed. Weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio were calculated as follows:

Specific growth rate (SGR, %/day) = $100 \times (\ln W_t - \ln W_0) / days$;
Feed conversion ratio (FCR) = (total feed consumed, g) / (total weight gain, g);
Protein efficiency ratio (PER) = (weight gain, g) / (protein fed, g);
$U = V \times [(N_2 - n_2) - (N_1 - n_1)] / (24 \times m)$.

Where: W_t is final body weight (g), W_0 is initial body weight (g). U is excretion rate (mg/(kg×h)), V is volume of water in the bucket (L), N_1 and N_2 represent Ammonia-N and urea nitrogen mass concentration of initial and terminal water in the bucket (mg/L); n_1 and n_2 represent Ammonia-N and urea nitrogen mass concentration of initial and terminal water in the control check bucket (mg/L), respectively. m is weight of fish (kg), 24 is the interval of one day (terminal and initial water samples, h).

Statistical analysis

All growth data were subjected to analysis using one-way analysis of variance (ANOVA). Statistical significance was set at the 5% probability level and means were separated using Tukey-HSD. To predict more accurate responses to the dietary protein intake, the optimum level was estimated using second-degree polynomial regression analysis $(Y = aX^2 + bX + c)$ as described by Zeitoun *et al.* (1976). The software SPSS, version 13.0 (SPSS, Richmond, USA) and Matlab (version 7.1) were used as described by Siddiqui and Khan (2009).

RESULTS

Growth performance and feed utilization

The final weight, SGR, PER and FCR for the juvenile *A. nigrocauda* after the 70-day feeding trial are presented in Table 2. Survival was 100% in all treatments.

protein levels diet. The PER was also significantly increased when dietary protein levels increased from 34.57% to 44.75% and then significantly decreased (p<0.05). The best PER was found in fish groups fed the 44.75% diet.

Muscle composition

Muscle protein content of the fish was noted with the increase in protein content of the diet from 30.01% to 49.52% (Table 3). However, in the period as the 44.75%, 49.52%, 54.47% protein groups displayed significantly higher (p<0.05) muscle protein compared to fish from the 30.01% and 34.57% protein groups. The higher protein diet significantly decreased the contents of fat (1.16%) as compared to the lower protein diet. There were no differences in muscle ash and moisture concentration among dietary groups (p>0.05).

Table 3. Proximate composition (% wet weight) of the muscle of juvenile A. nigrocauda fed diets with different protein levels (%)

Dietary protein level	Moisture	Crude protein	Crude lipid	Ash
30.01	79.14±0.13	15.69±0.04[b]	1.25±0.04[a]	0.92±0.04
34.57	79.12±0.08	15.71±0.11[b]	1.24±0.02[a]	0.92±0.07
39.63	79.09±0.11	15.90±0.06[ab]	1.20±0.03[ab]	0.93±0.05
44.75	79.06±0.25	16.11±0.14[a]	1.16±0.06[b]	0.94±0.01
49.52	78.98±0.05	16.01±0.07[a]	1.12±0.05[bc]	0.95±0.02
54.47	78.95±0.14	15.96±0.11[ab]	1.10±0.01[c]	0.95±0.02

Data are means of three replicate groups; values in the same row with different superscripts (a, b, c) are significantly different (p<0.05).

Nitrogen excretion rate within 24 h

As presented in Table 4, the Ammonia-N excretion was significantly increased with the increase in dietary protein levels (p<0.05). Significant differences (p<0.05) in Urea-N excretion was evident in fish fed 30.01%, 34.57%, 39.63% and 44.75% protein diets whereas it remained non-significant

Table 2. Growth performance of juvenile A. nigrocauda fed diets with different protein levels for 70-day

Protein levels (%)	Initial weight (g)	Final weight (g)	SGR (%/d)	FCR	PER	Survival (%)
30.01	16.31±0.02	48.52±0.22[d]	1.56±0.02[d]	1.97±0.06[a]	1.69±0.03[c]	100
34.57	16.29±0.01	53.89±0.16[c]	1.71±0.04[c]	1.77±0.03[b]	1.64±0.02[c]	100
39.63	16.28±0.03	65.56±0.39[b]	1.99±0.01[b]	1.41±0.01[c]	1.79±0.02[b]	100
44.75	16.30±0.02	70.81±0.33[a]	2.10±0.03[a]	1.16±0.03[e]	1.92±0.03[a]	100
49.52	16.29±0.02	64.71±0.18[b]	1.97±0.02[b]	1.14±0.04[e]	1.77±0.02[b]	100
54.47	16.31±0.02	57.40±0.30[c]	1.80±0.02[c]	1.26±0.02[d]	1.46±0.01[d]	100

Values are presented as means (SEM, n = 3. Means in the same column with different superscripts (a, b, c, d, e) are significantly different from each other (p<0.05).

The final weight significantly increased when dietary protein levels increased from 30.01% to 44.75% (p<0.05) and then significantly decreased (p<0.05). The SGR showed a similar trend as the final weight. The highest final weight or SGR of juvenile *A. nigrocauda* was obtained at 44.75% protein levels. On subjecting the SGR data to second-degree polynomial regression analysis (Zeitoun *et al.*, 1976), the optimum dietary protein level was found to be 44.32%. The relationship of protein level (Y) and SGR (X) was described by the following equations: $Y = - 0.0025X^2 + 0.2216X - 2.9010$ ($R^2 = 0.944$). FCR was significantly decreased with increasing dietary protein levels up to 44.75% (p<0.05), but there were no significant differences for protein levels from 44.75% to 49.52% (p>0.05), and increased thereafter with further increases in dietary protein level. The poorest FCR was obtained when juvenile *A. nigrocauda* were fed the 30.01%

(p>0.05) for the groups receiving other diets (49.52% and 54.47%).

Table 4. Effect of dietary protein levels on daily nitrogen excretion rates in juvenile A. nigrocauda

dietary protein (%)	Body mass (g)	NH₃-N mg/(kg·h)	Urea-N mg/(kg·h)
30.01	49.95±0.22	14.54±0.08[f]	7.64±0.35[e]
34.57	50.36±0.16	15.85±0.08[e]	9.50±0.23[d]
39.63	48.89±0.39	18.03±0.24[d]	10.32±0.21[c]
44.75	49.09±0.33	19.87±0.11[c]	11.17±0.16[b]
49.52	49.81±0.18	20.89±0.14[b]	11.68±0.25[a]
54.47	50.03±0.30	21.28±0.19[a]	9.84±0.45[d]

Data are means of three replicate groups; values in the same row with different superscripts (a, b, c, d, e, f) are significantly different (p<0.05).

DISCUSSION

The 44.75% crude protein produced maximum final weight and SGR of juvenile *A. nigrocauda* (16.30 g initial weight) in this study. Comparable studies in other *Acherythroculter* species are very limited so comparisons are studied *Erythroculter ilishaeformis* or carnivores. The different grades of protein requirements according to species/weight were changed from 35.7% to 41.35% in *A. nigrocauda* (35.7% protein level, 40.52 g initial weight, Ding *et al*., 2012), *E. ilishaeformis* (41.35% protein level, 2.88 g initial weight, Chen *et al*., 2005). Later on, a relation between protein level and species/weight was decreased with increasing fish weight and age in *Oreochromis niloticus* (Mohsen *et al*., 2010). Variations in dietary crude protein needs related to fish initial weights may be attributed to different protein needs at different life history stages. In other word, the protein requirements of *A. nigrocauda* depended on the body mass. The optimal dietary protein content for maximal SGR of *A. nigrocauda*, analyzed by second-order polynomial regression, is estimated to be 44.32% (Table 2) dietary protein for the culture conditions used in this study.

This typical analyzed by second-order polynomial regression to change dietary protein level in isoenergetic diets had been observed in many other species irrespective if rearing strategies (Chou *et al*., 2001). In general, carnivores have a maintenance protein requirement in the range of 40% - 55% (Wilson, 1989). In natural habitats, the *A. nigrocauda* is a pelagic fish and carnivore, and feeding habits are also reflected in its dietary need for protein. Overall, the optimum dietary protein requirements is higher compared with the same small species but lower levels of protein requirement compared those carnivores, such as *Silurus meridionalis* (51.0%, Zhang *et al*., 2000) and snakehead (52%, Wee and Tacon, 1982). The discrepancies in protein requirement are perhaps due to fish species, fish size, environmental conditions of fish *et al*. Juvenile *A. nigrocauda* fed lower protein diets (30.01% - 34.57% protein) had significantly higher FCR than those fed the other diets, similar to that trended for juvenile fish of many other species (Anderson *et al*., 1981; Shyong *et al*., 1998). Low dietary protein levels produced high FCRs, possibly due to the intake of nutritionally inadequate nutrient levels to promote growth, or which can be attributed to the increased protein content of diet with resultant effect of enhanced weight gain (Deng *et al*., 2011).

In contrast, higher dietary protein level produced higher FCR in the present study can be attributed to the fact that the fish body cannot utilize more dietary protein once the optimum level has been reached. Similar results have been reported in the same fish (Ding *et al*., 2012). However, the values of the FCR showed lower when dietary protein concentration varying from 44.75 - 49.52%. In addition, the lower PER in fish fed diet containing 49.52% crude protein compared to fish fed diet containing 44.75% crude protein indicates that excess protein was used for metabolic purposes other than growth (Lee *et al*., 2001), which showed that the optimum dietary protein level was about 44.75%. The PER increased with increasing dietary protein to a 44.75%, beyond which PER was depressed. It is generally known that fishes cannot utilize excess dietary protein for protein synthesis but can utilize it for energy source (Santinha *et al*., 1996). A direct relationship between protein

intake and nitrogen excretion has been found in other fish (Chakraborty and Chakraborty, 1998). From a metabolic point of view, excess amount of dietary protein intake leads to the released of excess amino groups, which must be excreted at the expense of much energy resulting to lesser utilization of energy for enhance PER purposes. The results of Ammonia-N and Urea-N excreted also supported the above explanation in the present study. Generally, carnivorous fish tend to have a low ability to utilize carbohydrate as an energy source and no more than 20% of diet is recommended (Hemre *et al*., 2002). Webb and Gatlin (2003) found similar results for red drum. In the present study, the crude lipid content of the diets was similar (9.10%), but carbohydrate levels (11.56% – 42.68%) decreased with increasing dietary protein level. Previous studies had shown that increasing non-protein energy sources from carbohydrate or lipid in the diet could increase protein synthesis thereby decreasing the nitrogen excretion result a reduction in environmental output (Viola and Rappaport, 1979; Engin and Carter, 2001). The reasons might be suppressing the activity of amino acid-degrading enzymes in *hepatopancreas* by high-starch diets (Shimeno *et al*., 1981).

In this study, protein content of juveniles *A. nigrocauda* increased at increasing protein levels of (30.01% - 44.75%) and then remained substantially constant when fish were fed with higher protein levels (49.52% - 54.47%, Table 3). A similar trend for protein content had been reported to other aquatic animals by Kim and Lee (2009) in *Takifugu rubripes* and Zhang *et al*. (2010) in *Sparus macrocephalus*. However, other researchers had reported no significant changes in fish muscle protein content when fish were fed with various levels of dietary protein (Moore *et al*., 1988; Hernandez *et al*., 2001). Furthermore, protein content of some other aquatic animals was found to differ slightly when dietary protein level increased (Monentcham *et al*., 2009; Abdel-Tawwab *et al*., 2010). The reason for this phenomenon was that both endogenous factors such as fish size, sex as well as exogenous factors such as diet composition and culture environment influenced the proximate composition of fish (Shearer, 1994). This maybe partly explained by the lack of agreement concerning the influence to different dietary protein concentrations on the protein content of the fish muscle. The muscle lipid content of the juveniles decreased obviously with increasing dietary protein level, a relationship which has also been reported with other aquatic species (Yang *et al*., 2003; Kim *et al*., 2004).

In the present study, Lipid decreased with the increase of dietary protein and concomitantly a decrease of dietary carbohydrate might have stimulated several tissue lipogenic enzyme activities and converted dietary carbohydrate into fat. Moveover, the increase in Ammonia-N and Urea-N excretion with increasing dietary protein was in agreement with previous findings for the juvenile rainbow trout (Alsop and Wood, 1997). The rate of nitrogen release to the water is closely related to the production of nitrogen by the fish. The major source of Ammonia-N and Urea-N in fish is protein catabolism; thus, it appears that protein catabolism closely follows protein intakes in fish. In fact, previous studies on the juvenile Australian short-finned eel had reported positive relationship between dietary protein intake and ammonia excretion (Guo *et al*., 2012). Similarly, in Bidyanus bidyanus, increasing dietary protein levels (13 to 55%) resulted in a steady increase in ammonia nitrogen excretion from 47.2 to

468.88 mg/(kg×d) (Yang *et al*., 2002). This suggested that the increase of amino acids were deaminated and excreted as ammonia for energy purpose rather than deposited for tissue growth when fish was fed high protein diets (Mohanta *et al*., 2008). Nitrogen efflux rates, notably ammonia, showed a pattern of excretion that was directly related to the protein content of the diet, but nitrogen metabolism could also be influenced by the source of dietary protein, dietary energy and the total available energy (Louise *et al*., 1995). Porter *et al*. (1987) proposed a dependence of fish total nitrogen production upon their feeding regimes-including the source, amino acid balance of the proteins and the proportion of protein to carbohydrate and lipid present. Ammonia is the principal, but not necessarily the only, nitrogenous metabolic end-product excreted by teleost fish. Tulli *et al*. (2007) found, Urea-N represented a variable proportion of total N-excretion (from 8.2 to 21.0%) and accounted for 3.6 to 9.2% of the nitrogen intake for the juveniles sea bass.

This agrees with Brett and Zala's (1975) for findings sockeye salmon in which the Urea-N fraction accounted for only 21% of the total nitrogen excreted (urea + ammonia). In the present study, postprandial Urea-N excretion rates of the juvenile *A. nigrocauda* (from 29.13% to 37.04%) were significantly higher than those of the juvenile sea bass and sockeye salmon when they all were fed with similar conditions, reflecting the higher metabolic rate in *A. nigrocauda*. Tulli *et al*. (2007) reported that Urea-N excretion was not affected by the quality of the dietary protein sources, but there was a significant effect of the dietary arginine level. The strong relationship between arginine intake and Urea-N excretion confirmed the direct implication of dietary arginine degradation in urea formation in sea bass similarly to what was observed in rainbow trout and turbot (Fournier *et al*., 2003). In the present study, brown fish meal (Peru) and casein were used as the protein source. The high fish meal content may increase arginine level, resulting in high excretion of Urea-N in fish. In addition, general arginase is often closely related to the metabolism of casein. The high casein content in diet can increase arginase activities and digestibility of arginine, resulting in higher Urea-N excretion in the fish.

Conclusions

In summary, the optimal protein level for juvenile *A. nigrocauda* is 44.32% based on SGR polynomial equations analysis. To formulate protein balanced diets for aquaculture of *A. nigrocauda*, further studies should focus on the role of P/E ratios and other nutrients *et al* for the intensive culture of the young *A. nigrocauda*.

Acknowledgements

REFERENCES

Abdel-Tawwab, M., M. H. Ahmad, Y. A. E. Khattab, and A. M. E. Shalaby. 2010. Effect of dietary protein level, initial bodyweight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture 298:267–274.

Alsop, H. D. and C. M. Wood. 1997. The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol*. 200:2337–2346.

Anderson, R. J., E. W. Kienholz, and S. A. Flickinger. 1981. Protein requirements of smallmouth bass and largemouth bass. *J. Nutr*. 111:1085–1097.

AOAC (Association of Official Analytical Chemists). 2005. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Brett, J. R. and Zala, C. A. 1975. Daily pattern of nitrogen excretion and oxygen consumption of the sockeye salmon, (*Oncorhynchus nerka*) under controlled conditions. *J. Fish. Res. Board Can*. 32:2479–2486.

Chakraborty, S. C. and S. Chakraborty. 1998. Effect of dietary protein level on excretion of ammonia in Indian major carp, *Labeo rohita*, fingerlings. *Aquacult. Nutr*. 4:47–51.

Chen, J. M., J. Y. Ye, Y. H. Wang, and X. Pan. 2005. Dietary protein requirement of *Erythroculter ilishaeformis. J. Fishery Sci. Chin*. 29:83–86 (in Chinese).

Chen, J. M., J. Y. Ye, Y. H. Wang, and Q. Pan. 2005. Dietary protein requirement of *Erythroculter ilishaeformis* juvenile. *J. Fisheries. Chin*. 19:83–86 (in Chinese).

Cho, C. Y. 1992. Feeding systems: energy and protein requirements. *Aquaculture* 100:107–123.

Chou, R. L., M. S. Su, and H. Y. Chen. 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). *Aquaculture* 193:81–89.

Deng, J. M., X .Zhang, B. L. Bi, L. F. Kong, and B. Kang. 2011. Dietary protein requirement of juvenile Asian red-tailed catfish *Hemibagrus wyckioides. Anim. Feed. Sci. Technol.* 170:231–238.

Ding, L. Y., Y. H. Chao, Q. B. Zhou, X. D. Xu, Y. X. Chen, G. He, and Y. Rao. 2012. A primary study on the optimal protein requirement in juvenile *Ancherythroculter nigrocauda. Jiangsu Agric. Sci*. 40:216–218 (in Chinese).

Elliott, J. M. 1976. The energetics of feeding, metabolism and growth of brown trout, *Salmo trutta*, in relation to body weight, water temperature and ration size. *J. Anim. Ecol*. 45:923–948.

Engin, K. and C. G. Carter. 2001. Ammonia and urea excretion rates of juvenile Australian short-finned eel (Anguilla australis australis) as influenced by dietary protein level. *Aquaculture* 194:123–136.

Fournier, V., M. F. Gouillou-Coustans, R. Metailler, C. Vachot, J. Moriceau, D. H. Le, C. Huelvan, E. Desbruyeres, and S. J. Kaushik. 2003. Excess dietary aginine affects urea excretion but does not improve N utilisation in rainbow trout *Oncorhynchus mykiss* and turbot *Psetta maxima. Aquaculture* 217:559–576.

Gelineau, A. and F. Medale. 1998. Effect of feeding time on postprandial nitrogen excretion and energy expenditure in rainbow. J. Fish Biol. 52:654–654.

Guo, Z. Q., X. M. Zhu, J. S. Liu, D. Han, Y. X. Yang, Z. Q. Lan, and S. Q. Xie. 2012. Effects of dietary protein level on growth performance, nitrogen and energy budget of juvenile hybrid sturgeon, *Acipenser baerii* ♀ × *A. gueldenstaedtii* ♂. *Aquaculture* 338–341:89–95.

Hernandez, M. D., M. A. Egea, F. M. Rueda, F. Aguado, F. J. Martinez, and B. Garcia. 2001. Effects of commercial diets with different P/E ratios on sharpsnout sea bream (*Diplodus puntazzo*) growth and nutrient utilization. *Aquaculture* 195:321–329.

Hemre, G. I., T. P. Mommsen, and A. Krogdahl. 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquacult. Nutr.* 8:175–194.

Kim, K. W., X. J. Wang, S. M. Choi, G. J. Park, and S. C. Bai. 2004. Evaluation of optimum dietary protein-to-energy ratio in juvenile olive flounder *Paralichthys olivaceus* (Temminck et Schlegel). *Aquac. Res.* 35:250–255.

Kim, S. S. and K. J. Lee. 2009. Dietary protein requirement of juvenile tiger puffer (*Takifugu rubripes*). Aquaculture 287:219–222.

Lee, H. Y. M., K. C. Cho, J. E. Lee, and S. G. Yang. 2001. Dietary protein requirement of juvenile giant croaker, *Nibea japonica* Temminck and Schlegel. *Aquac. Res.* 32:112–118.

Louise, G. B., F. U. Rogcr, and G. C. Ian. 1995. Effect of dietary protein on the nitrogen excretion and growth of the African catfish, *Clarias gariepinus.* Aquat Living Resour. 8:407–414.

Mohanta, K. N., S. N. Mohanty, J. K. Jena, and N. P. Sahu. 2008. Protein requirement of silver barb, *Puntius gonionotus*, fingerlings. *Aquacult. Nutr.* 14:143–152.

Mohsen, A. T., H. A. Mohammad, A. E. Khattab. Yassir, and M. E. Shalaby. Adel. 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture* 298:267–274.

Monentcham, S. E., V. Pouomigne, and P. Kestemont. 2009. Influence of dietary protein levels on growth performance and body composition of African bonytongue fingerlings *Heteriostis niloticus* (Cuvier, 1829). *Aquacult. Nutr.* 16:144–152.

Moore, B. J., S. S. O. Hung, and J. F. Medranno. 1988. Protein requirement of hatchery produced juvenile white sturgeon *(Acipenser transmontanus)*. *Aquaculture* 71:235–245.

Ng, W. K., S. C. Soon, and R. Hashim. 2001. The dietary protein requirement of a bagrid catfish, *Mystus nemurus* (Cuvier & Valenciennes), determined using semipurified diets of varying protein level. *Aquacult. Nutr.* 7:45–51.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academy Press, Washington, D.C. pp 114.

Perera, W. M. K., C. G. Carter, and D. F. Houlihan. 1995. Feed consumption, growth and growth efficiency of rainbow trout (*Oncorhynchus mykiss* Walbaum) fed on diets containing bacterial single-cell protein. *Br. J. Nutr.* 73:591–603.

Porter, C. B., M. D. Krom, L. Kobbins, L. Bricknell, and A. Davidson. 1987. Ammonia excretion and total N budget for gilthead seabream *(Sparus aurata)* and its effect on water quality conditions. *Aquaculture* 66:287–297.

Santinha, P. J. M., E. F. S. Gomes, and J. O. Coimbra. 1996. Effects of protein level of the diet on digestibility and growth of gilthead sea bream, *Sparus auratus* L. *Aquacult. Nutr.* 2:81–87.

Shearer, K. D. 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture* 119:63–88.

Shimeno, S., M. Takeda, S. Takayama, and H. Sasaki. 1981. Response of nitrogen excretion to change of dietary composition in carp. *Bull. Japn. Soc. Sci. Fish.* 47:191–195.

Shyong, W. J., C. H. Huan, and H. C. Chen. 1998. Effect of dietary protein concentration on growth and muscle composition of juvenile *Zacco barbata.* *Aquaculture* 167:35–42.

Siddiqui, T. Q and M. A. Khan. 2009. Effects of dietary protein levels on growth, feed utilization, protein retention efficiency and body composition of young *Heteropneustes fossilis* (Bloch). *Fish Physiol. Biochem.* 35:479–488

Tulli, F., C. Vachot, E. Tibaldi, V. Fournier, and S. J. Kaushik. 2007. Contribution of dietary arginine to nitrogen utilisation and excretion in juvenile sea bass (*Dicentrarchus labrax*) fed diets differing in protein source. *Comp. Biochem. Physiol.* 147:179–188.

Viola, S. and U. Rappaport. 1979. The extra-caloric effect of oil in the nutrition of carp. Isr. *J. Aquac.-Bamidgeh.* 35:44–52.

Webb, K.A. and III. D. M. Gatlin. 2003. Effects of dietary protein level and form on production characteristics and ammonia excretion of red drum *Sciaenops ocellatus.* *Aquaculture* 225:17–26.

Wee, K.L. and A. G. J. Tacon. 1982. A preliminary study on the dietary protein requirement of juvenile snakehead. *Bull. Jpn. Soc. Sci. Fish.* 48, 1463–1468.

Wilson, R. 1989. Amino acids and proteins. In: Halver, J.E. (Ed.), Fish Nutrition. Academic Press, San Diego, CA, pp. 112–153.

Yang, S. D., C. H. Liou, and F. G. Liu. 2002. Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture* 213:363–372.

Yang, S. D., T. S. Lin, C. H. Liou, and H. K. Peng. 2003. Influence of dietary protein levels on growth performance, carcass composition and liver lipid classes of juvenile *Spinibarbus hollandi* (Oshima). *Aquac. Res.* 34:661–666.

Zeitoun, I. H., D. E. Ullrey, W. T. Magee, J. L. Gill, and W. G. Bergen. 1976. Quantifying nutrient requirements of fish. J. Fish. Res. Board Can. 33:167–172.

Zhang, W., X. Xie, S. Fu, and Z. Cao. 2000. The nutrition of *Silurus meridionalis*: optimum dietary protein level. *Acta Hydrobiologica Sinica* 24:603–609 (in Chinese).

Zhang, J., F. Zhou, L. Wang, Q. Shao, and Z. Xu. 2010. Dietary protein requirement of juvenile black sea bream, *Sparus macrocephalus. J. World Aquac. Soc.* 41:151–164.

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