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Effect of protein levels on growth, feed utilization, nitrogen and energy budget in juvenile southern flounder, *Paralichthys lethostigma*

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Abstract

A feeding trial with five crude protein levels (549, 513, 472, 445 and 399 g kg^{-1}) was conducted to investigate the optimum protein level in diets of juvenile southern flounder (*Paralichthys lethostigma*). Budgets of nitrogen and energy were discussed. Fish (initial weight 32.9 ± 0.5 g $fish^{-1}$, mean ± SD) were fed the experimental diets to satiation twice daily for 61 days. Protein levels affected specific growth rate in wet weight (SGR_W) and protein (SGR_P) significantly. SGR_W and SGR_P were highest at 512.5 g kg^{-1} protein level. SGR_W was positively correlated to growth nitrogen (GN), growth energy (GE), nitrogen digestibility, energy digestibility, amount of digestible nitrogen and amount of digestible energy. Faecal nitrogen (FN) and faecal energy (FE) were affected significantly with trends contrary to SGR_W. The nitrogen budget was described by the equation 100CN = 2.1FN + 34.4UN + 63.5GN (CN, nitrogen intake; UN, excretion nitrogen). The energy budget was 100IE = 4.04FE + 3.32UE + 54.35GE + 38.30ME (IE, gross energy intake; UE, excretion energy; ME, metabolizable energy). The average proportion of GE and ME in assimilated energy (AE) was described by the equation 100AE = 58.65GE + 41.35ME.

KEY WORDS: budget, feed utilization, fish, growth, protein, southern flounder (*Paralichthys lethostigma*)

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Introduction

Protein is an important component in the diet because it supplies amino acids to organisms for growth. Protein can also be metabolized as an energy source. Many studies have been carried out to determine protein requirements for fish, with estimated protein requirements ranging from 400 to 550 g kg^{-1} for carnivorous fish [National Research Council (NRC) 1983].

The southern flounder, *Paralichthys lethostigma*, is native to the mid-Atlantic and Gulf of Mexico coasts of the United States (Powell & Swartz 1977). The species can grow well in seawater, freshwater and estuaries (Smith *et al.* 1999; Benetti *et al.* 2001). Its landings have declined, leading to interest in culturing native flatfishes for stock enhancement or food fish production (Arnold *et al.* 1977; Waters 1996; Jenkins & Smith 1999). In the international marketplace, increasing demand for southern flounder makes it an important candidate for commercial culture (Waters 1999). So far diets for southern flounder have been mostly natural diets (Burke 1995; Kamermans *et al.* 1995; Denson & Smith 1997). An artificial diet is required to support its commercial culture. The aim of the present study was to determine the optimum protein level of the southern flounder's diet and explore its nitrogen and energy budgets.

Materials and methods

The trial was carried out in 15 net cages (1.5 × 0.7 × 0.7 m^3, water height 0.4 m). About 50 cm height from every cage's top, a PVC-pipe poured filtered, fresh sea water into the cage. The cycle volume was more than three times every day. Air was provided through two PVC pipes that were fixed in the bottom corners of the net cage. A nylon film was fixed on the bottom of the fishing netcage and overlapped the four sides to a height of 15 cm. Water temperature was recorded at 6:20 and 14:50 hours daily and fluctuated from 26.5 to 31.5 °C. Other water chemical factors were examined weekly. Salinity ranged from 31 to 33 g L^{-1}, dissolved oxygen was more than

4.5 mg L^{-1}, light intensity near the cage top ranged from 50 to 200 Lux at noon and pH ranged from 7.8 to 8.2.

Five experimental diets (D1–D5) contained 549.2, 512.5, 471.7, 455.6 and 399.1 g kg^{-1} protein level respectively (Table 1). The moist diets (1000 g diet: 400 mL water) were made into pellets and stored at −20 °C. The diameter of pellets was adjusted to 0.2, 0.3 and 0.5 cm according to fish size.

The experiment was conducted from 2 May 2004 to 1 July 2004 at the Wan Fang Aquaculture Co. Ltd located in the South China Sea tropical region (109.8°E, 19.8°N). Fish were transferred into the cages 2 weeks prior to the trial for acclimatization. After fish were weighed, they were sorted and diets were randomly assigned. Each diet had three replications with each cage containing 15 fish (initial weight 32.9 ± 0.5 g $fish^{-1}$, mean ± SD). Feeding was at 6:40 and 15:10 hours daily. Thirty minutes after the fish finished feeding, uneaten feed was collected and dried. Weight changes in diet pellets were determined in control cages without fish for correcting the estimates of amounts intake.

Table 1 Composition of the experimental diets used for protein levels study

Ingredient (g kg^{-1})	Experimental diets D1	D2	D3	D4	D5
White fishmeal	350	350	350	350	350
Protein mixture[1]	320	280	240	200	160
Fish oil	82	82	82	82	82
α-starch	135	135	135	135	135
Dextrin	0	40	80	120	160
Vitamin mixture[2]	50	50	50	50	50
Mineral mixture[3]	50	50	50	50	50
Betaine	8	8	8	8	8
Sodium alginate	5	5	5	5	5
Proximate composition					
Crude protein (g kg^{-1})	549.2	512.5	471.7	445.6	399.1
Digestible protein (g kg^{-1})	538.3	503.4	460.8	444.7	391.4
Gross energy (MJ kg^{-1})	20.24	19.85	20.02	19.39	19.06
Digestible energy (MJ kg^{-1})	19.53	19.21	19.12	18.39	18.34
CP/GE (g MJ^{-1})	27.1	25.8	23.6	23.5	20.9
DP/DGE (g MJ^{-1})	27.6	26.2	24.1	24.2	21.3

CP/GE, crude protein/gross energy; DP/DGE, digestible protein/digestible energy.

[1] Casein : gelatin = 6 : 1.

[2] Vitamin mixture (g kg^{-1}): thiamin 0.2; riboflovin 0.7; pyridoxine 0.185; cobalamin 1.1; retinol 0.642; cholecalciferol 0.03; phylloquinone 0.158; folic acid 0.05; calcium patotheniate 0.935; inositol 13.35; niacin 2.670; tocopherol 2.0; choline 26.5; ascorbic acid 0.865; ρ-amino benzoic acid 0.6; biotin 0.017.

[3] Mineral mixture (g kg^{-1}): NaCl 1.422; $MgSO_4 \cdot 7H_2O$ 6.8; $KH_2PO_4 \cdot 2H_2O$ 12; $Ca(H_2PO_4)_2$ 7.5; Fe-citrate 1.485; $ZnS0_4 \cdot 7H_2O$ 0.185; $MnSO_4 \cdot 4H_2O$, 0.04; $CuCl_2$ 0.002; $CoC1_2$, 0.05; KI 0.01; $AlCl_3$ 0.006; Ca-lactate 16; $NaH_2PO_4 \cdot 2H_2O$ 4.5.

Faeces were also collected and dried. Dead fish were collected and body weights recorded immediately. When the feeding trial was terminated, all fish were starved for 24 h and three fish from each cage were selected randomly for assay. Five fish were also selected for assay at the start of the experiment.

Crude protein (CP) (N × 6.25) and energy of the experimental diets, fish and faeces were determined by the Kjeldahl method and an adiabatic bomb calorimeter respectively. Data were subjected to a one-way analysis of variance (ANOVA) to test the effect of dietary protein level. When significant differences ($P < 0.05$) were detected, Duncan's multiple range tests were used to compare mean values among treatments. Data were also analysed by a linear regression model. The statistical software was SPSS 11.0. (SPSS Inc., Chicago, Illinios, USA) Specific growth rate in wet weight [SGR_W = 100 (ln final weight/fish–ln initial weight/fish)/growth days, % day^{-1}] was calculated. Specific growth rate in protein (SGR_P) and energy (SGR_E) were similar to SGR_W (Xie *et al.* 1997a). The nitrogen budget was described by the equation CN =FN + GN + UN (CN, nitrogen intake; FN, faecal nitrogen; GN, growth nitrogen; UN, excretion nitrogen). CN, FN and GN were determined directly. UN was calculated by difference. The energy budget (Brett & Groves 1979) was described by the equation IE = FE + UE + GE + ME (IE, gross energy intake; FE, faecal energy; UE, excretion energy; GE, growth energy; ME, metabolizable energy). IE, FE and GE were determined directly. The absolute value of UE was calculated according to the equation UE =(CN − FN − GN) × 24.8 (Cui *et al.* 1992), where 24.8 (MJ kg^{-1}) is a heat constant for ammonia nitrogen. ME was calculated by equation ME = IE − FE − UE − GE (Cui & Wootton 1988a; Cui & Liu 1990a). Assimilated energy (AE) = GE + ME = IE − FE − UE.

Results

The performance of southern flounder fed various diets is shown in Table 2.

Specific growth rate in wet weight (SGR_W) and protein (SGR_P) were affected by protein levels ($P < 0.01$). SGR_W and SGR_P were highest at 512.5 g kg^{-1} protein level. SGR_W and SGR_P decreased when the protein level was higher or lower than 512.5 g kg^{-1}; 399.1 g kg^{-1} was the lowest protein level, but the corresponding SGR_W and SGR_P were higher than the 471.7 g kg^{-1} and 445.6 g kg^{-1} protein levels. SGR_E was not found to be significantly different, but its trend was similar to SGR_W and SGR_P. Broken line analysis showed that SGR_W would attain the highest value at 514.6 g kg^{-1} protein level (Fig. 1).

Table 2 Growth, feed utilization, nitrogen budget and energy budget of southern flounder (*Paralichthys lethostigma*) fed various protein level diets

Diet	D1	D2	D3	D4	D5
Mortality (%)	0^{a}	0^{a}	4.45 ± 3.85^{b}	11.11 ± 7.70^{b}	0^{a}
SGR$_W$ (% day^{-1})	2.03 ± 0.05^{ab}	2.10 ± 0.07^{a}	1.82 ± 0.09^{cd}	1.73 ± 0.05^{c}	1.92 ± 0.09^{bd}
SGR$_P$ (% day^{-1})	2.21 ± 0.05^{a}	2.25 ± 0.05^{a}	1.98 ± 0.11^{b}	1.83 ± 0.02^{c}	2.04 ± 0.09^{b}
SGR$_E$ (% day^{-1})	2.41 ± 0.16	2.48 ± 0.15	2.23 ± 0.22	2.13 ± 0.09	2.39 ± 0.08
FCR	1.86 ± 0.04^{a}	1.75 ± 0.06^{b}	1.74 ± 0.06	1.48 ± 0.04^{c}	1.46 ± 0.03^{c}
PER	3.16 ± 0.07	3.16 ± 0.11	3.37 ± 0.12	3.04 ± 0.09	3.39 ± 0.04
CR (% day^{-1})	0.97 ± 0.03^{ab}	1.06 ± 0.06^{b}	0.95 ± 0.07^{a}	1.07 ± 0.05^{b}	1.08 ± 0.05^{c}
GN (g)	40.90 ± 4.10^{a}	40.17 ± 3.74^{a}	33.07 ± 1.36^{b}	27.83 ± 1.10^{b}	32.20 ± 2.79^{b}
ADN (g)	63.07 ± 6.74^{a}	63.40 ± 8.61^{a}	47.73 ± 4.07^{b}	46.87 ± 4.38^{b}	48.47 ± 2.976^{b}
GE (MJ)	8.64 ± 1.51	8.66 ± 1.48	7.32 ± 1.15	6.43 ± 0.61	7.66 ± 0.25
ADE (MJ)	14.30 ± 1.52	15.11 ± 2.01	12.38 ± 1.06	12.38 ± 1.23	14.00 ± 1.13
As percentage of CN					
FN (%)	1.98 ± 0.04^{ab}	1.77 ± 0.10^{a}	2.31 ± 0.24^{bc}	2.39 ± 0.13^{c}	1.93 ± 0.32^{a}
GN (%)	63.57 ± 1.60	62.53 ± 3.61	67.87 ± 3.87	58.23 ± 3.26	65.40 ± 7.70
UN (%)	34.43 ± 1.70	35.73 ± 3.49	29.87 ± 3.84	39.40 ± 3.35	32.67 ± 7.97
DN (%)	98.02 ± 0.04^{a}	98.23 ± 0.10^{a}	97.69 ± 0.24^{b}	97.61 ± 0.13^{c}	98.07 ± 0.32^{ac}
As percentage of IE					
FE (%)	3.49 ± 0.23^{a}	3.23 ± 0.33^{a}	4.51 ± 0.75^{bc}	5.17 ± 0.59^{c}	3.79 ± 0.17^{ab}
UE (%)	3.71 ± 0.18	3.66 ± 0.36	2.79 ± 0.36	3.60 ± 0.31	2.83 ± 1.08
GE (%)	58.02 ± 5.38	55.38 ± 4.34	56.18 ± 3.96	49.34 ± 3.66	52.81 ± 3.38
ME (%)	34.79 ± 5.12	37.73 ± 4.16	36.52 ± 4.49	41.89 ± 3.77	40.57 ± 4.39
DE (%)	96.51 ± 0.23^{a}	96.77 ± 0.33^{a}	95.49 ± 0.75^{b}	94.83 ± 0.59^{c}	96.21 ± 0.17^{a}
As percentage of AE					
GE (%)	62.50 ± 5.61	59.47 ± 4.55	60.62 ± 4.59	54.09 ± 4.08	56.59 ± 4.22
ME (%)	37.50 ± 5.61	40.53 ± 4.55	39.38 ± 4.59	45.91 ± 4.08	43.41 ± 4.22

All values are mean ± SD of three replicates. Values in each row not sharing a common superscript are significantly different ($P < 0.05$, ANOVA). Specific growth rate in wet weight (SGR$_W$, % day^{-1}) = 100 (ln final weight − ln initial weight)/growth days. Specific growth rate in protein (SGR$_P$, % day^{-1}) = 100 (ln final protein − ln initial protein)/growth days. Specific growth rate in energy (SGR$_E$, % day^{-1}) = 100 (ln final energy − ln initial energy)/growth days. Feed conversion ratio (FCR) = 100 × (wet weight gain/dry diet fed). Protein efficiency ratio (PER) = 100 × (wet weight gain/protein fed). Those, including faecal nitrogen (FN), growth nitrogen (GN), faecal energy (FE) and growth energy (GE) were determined directly. Digestibilities of nitrogen (DN, %) and energy (DE, %) were 100 − FN (%) and 100 − FE (%) respectively. Urea nitrogen (UN, %) = 100 − FN (%) − GN (%). Excretion energy (UE, J) = (CN − FN − GN) × 24.8 × 10^{3} MJ kg^{-1}. Metabolizable energy (ME, %) = 100 − FE (%) − GE (%) − UE (%). Consumption rate (CR, % day^{-1}) = 100 dry diet intake/growth days/(initial wight/2 + final weight/2). Amount of digestible nitrogen (ADN, g) = dry diet intake × DN. Amount of digestible energy (ADE, MJ) = dry diet intake × DE.

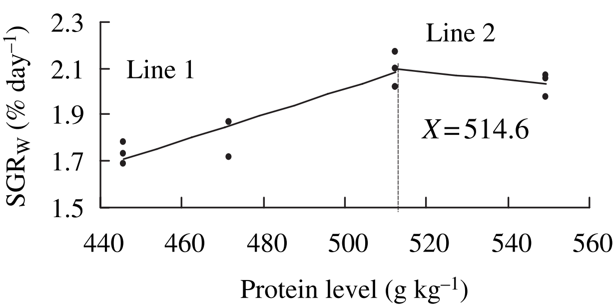

Figure 1 Broken line analysis of specific growth rate in wet weight (SGR$_W$) in juvenile southern flounder (*Paralichthys lethostigma*) fed diets containing various protein levels except D5. Line 1: $Y = 2.094 - 0.00556(514.6 - X)$ for $X < 514.6$ ($r^2 = 0.8470$) and Line 2: $Y = 2.094 - 0.00175(X - 514.6)$ for $X > 514.6$ ($r^2 = 0.2561$).

There were significant differences in feed conversion ratios (FCR, $P < 0.01$). FCR decreased as the CP level decreased. Protein efficiency ratio (PER) showed no significant difference. There were significant differences in consumption rates (CR, $P < 0.01$). CR of D5 was significantly higher than the other diets. CR of D3 was the lowest and significantly lower than that of D2 and D4. Significant differences were detected in GNs and the amount of digestible nitrogens (ADN) ($P < 0.01$). GNs of D1 and D2 were significantly higher than the other diets. ADN trends were similar to that of GN. No significant differences were found in GE and the amount of digestible energies (ADE).

Proportions of GN and UN in CN were not different. UE, GE, ME in energy intake (IE) did not differ significantly. Digestibilities of nitrogen (DN, $P < 0.05$) and energy (DE, $P < 0.01$) were different with a trend similar to SGR$_W$. SGR$_W$ was positively correlated to GN (Fig. 2, $r^2 = 0.7991$, $n = 15$, $P < 0.01$), GE (Fig. 3, $r^2 = 0.6848$, $n = 15$, $P < 0.01$), ADN (Fig. 4, $r^2 = 0.6856$, $n = 15$, $P < 0.01$), ADE (Fig. 5, $r^2 = 0.6988$, $n = 15$, $P < 0.01$), DN (Fig. 6,

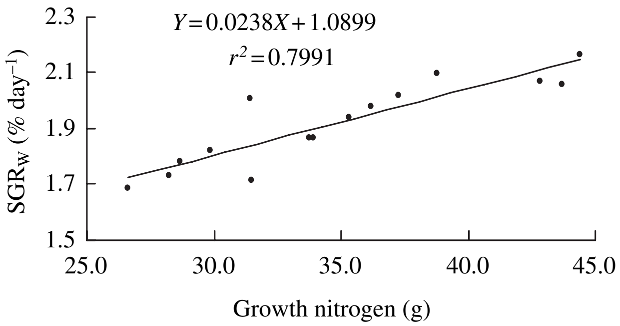

Figure 2 Relationship between specific growth rate in wet weight (SGR_W) and growth nitrogen ($n = 15$, $P < 0.01$).

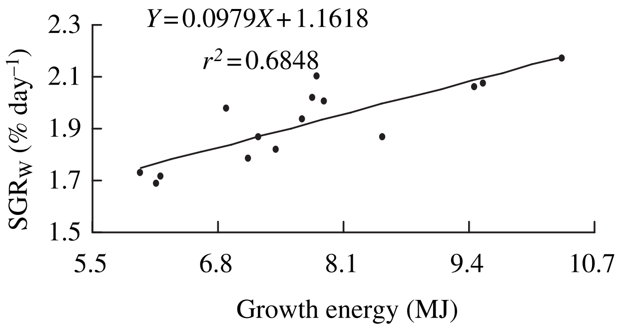

Figure 3 Relationship between specific growth rate in wet weight (SGR_W) and growth energy ($n = 15$, $P < 0.01$).

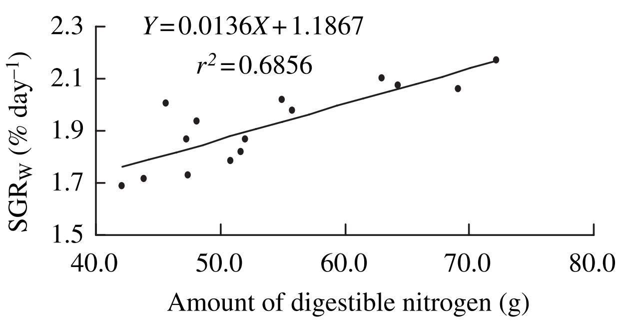

Figure 4 Relationship between specific growth rate in wet weight (SGR_W) and amount of digestible nitrogen ($n = 15$, $P < 0.01$).

$r^2 = 0.3139$, $n = 15$, $P < 0.05$) and DE (Fig. 7, $r^2 = 0.5395$, $n = 15$, $P < 0.01$). No differences were detected in the proportion of GN or metabolism energy (ME) in AE.

Although significant differences were found for FN and FE, these were relatively small differences. Average values were calculated to be 2.08% for FN and 3.32% for FE. The nitrogen budget equation was 100CN = 2.08FN + 34.41UN + 63.52GN. The energy budget equation was 100IE = 4.04FE + 3.32UE + 54.35GE + 38.30ME. The average

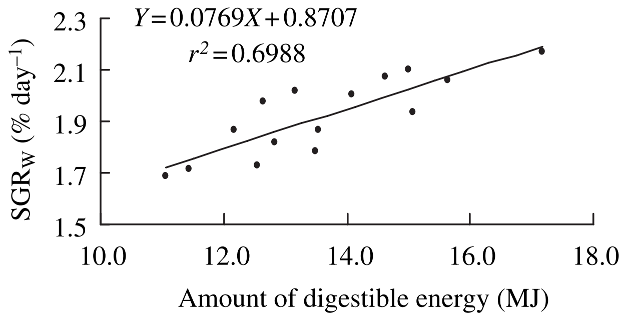

Figure 5 Relationship between Specific growth rate in wet weight (SGR_W) and amount of digestible energy ($n = 15$, $P < 0.01$).

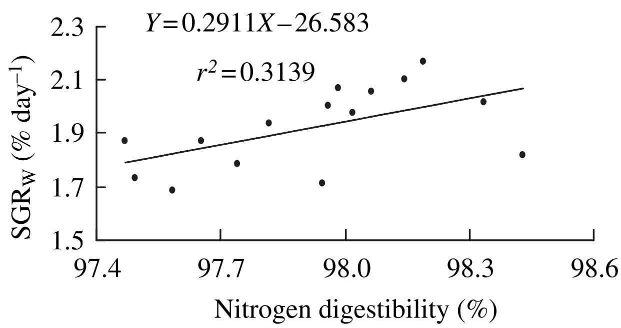

Figure 6 Relationship between specific growth rate in wet weight (SGR_W) and nitrogen digestibility ($n = 15$, $P < 0.05$).

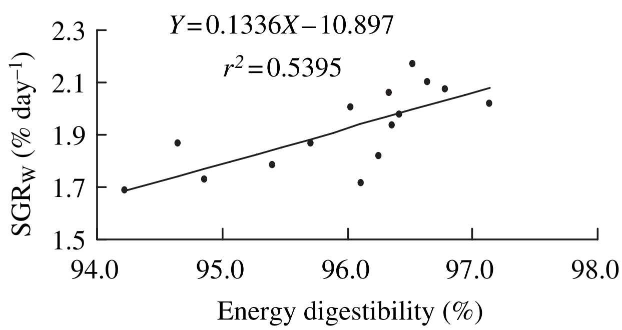

Figure 7 Relationship between specific growth rate in wet weight (SGR_W) and energy digestibility ($n = 15$, $P < 0.01$).

proportions of GE and ME in AE were described by the equation 100AE = 58.65GE + 41.35ME.

Discussion

The optimum protein level in diets for southern flounder, defined by the SGR and values for wet weight, protein and energy, was 512.5 g kg^{-1}. This is consistent with the theoretical optimum (514.6 g kg^{-1}) derived from the broken line model. The protein level, whether 512.5 or 514.6 g kg^{-1}, was similar to those used in cold-water fish diet, e.g. *Paralichthys olivaceus*, from 480 to 514 g kg^{-1} (Kikuchi 1999; Alam *et al.* 2002; Kim & Lee 2004), *Pleuronectes americanus*, 502 g kg^{-1} (Hebb *et al.* 2003), *Platichthys stellatus*, 488 g kg^{-1} (Lee *et al.* 2003). It was higher than those for warm-water fish, e.g. *Catla catla*, 350 g kg^{-1} (Seenappa & Devaraj 1995), *Bidyanus bidyanus*, 421.5 g kg^{-1} (Yang *et al.* 2002), *Rachycentron canadum*, 445 g kg^{-1} (Chou *et al.* 2001). This indicates that the southern flounder's protein requirement is closer to cold-water fish than to warm water fish although southern flounder lives in tropical regions; 514.6 (512.5) g kg^{-1} is near to the top of the range from 400 to 550 g kg^{-1} reported for a variety of carnivorous fish species (NRC 1983), which demonstrates that protein requirement for southern flounder is relatively high. Juvenile southern flounder eat more active epifaunal prey: mysids, amphipods and calanoid copepods (Burke 1995). In the estuary, 25–45% of the flounder stomachs contained only mysids, while 85–100% of the stomachs contained both mysids and other food categories. Individual growth rate of the flounder was only significantly related to the number of mysids in the stomachs, and not to any of the other food categories (Kamermans *et al.* 1995). Adult southern flounder eat shrimp and fish (Reid *et al.* 1956). These publications demonstrate that southern flounder belongs to carnivorous fish and that its feed habit supports the results we present.

Increases in dietary protein have often been associated with higher growth rates in many species. However, there is a protein level beyond which further growth is not supported, and may even decrease (Mohanty & Samantaray 1996; Shiau & Lan 1996; McGoogan & Gatlin 1999; Gunasekera *et al.* 2000; Kim & Lall 2001; Yang *et al.* 2002). The broken line method was used to determine the theoretical optimum protein level from the data. In the present study, because specific growth rate of D5 was higher than that of D4, the slope of Line 1 (Fig. 1) was decreased and theoretical optimum protein level (551.1 g kg^{-1}) could be evaluated. It was contrary to the trend that specific growth rate of D1 was lower than that of D2. SGR_W of D5 was excluded in broken line analysis and the 514.6 g kg^{-1} was more reasonable.

Specific growth rate in wet weight was positively correlated to GN and GE. This means that improved growth rate should follow with more GN and energy. SGR_W was positively correlated to ADN and ADE, because the proportions of GN in ADN and GE in ADE were not affected significantly and trends of ADN and ADE were similar to those of GN and GE. Improved ADN (ADE) could be realized by improved DN (DE) and (or) CR. It was perhaps higher GN, GE and PER of D5 than those of D4 that caused SGR_W of D5 to be higher than that of D4. A similar result was reported by Tibbetts *et al.* (2000) using diets containing 350, 390, 430, 470 and 510 g kg^{-1} CP in growth experiments. Though the highest specific growth rate was achieved at 470 g kg^{-1}, it did not decrease linearly from 430 to 350 g kg^{-1}. SGR and PER (calculated indirectly) of 390 g kg^{-1} were higher than those of 430 g kg^{-1}. Another similar result has been reported in bagrid catfish, *Mystus nemurus* (Ng *et al.* 2001). It may be relative to dietary protein to energy ratio in fish diets. The top growth rates were gained at the ratio optimum (Einen & Roem 1997; Tibbetts *et al.* 2001; Ali & Jauncey 2005). If the ratio is lower than optimal, fish will eat until GN requirement is satisfied. CP/GE and digestible protein (DP)/DGE in D5 were lowest, so fish in D5 increased its CR, DN and PER to meet its ADN and GN requirements. This benefited its growth rate to be higher than that in D4. Too low CP/GE and DP/DGE could alter some biological result, which was another reason to exclude SGR_W of D5 in broken line analysis.

Improved growth and FCR with increasing protein levels is well known in carnivorous fish [NRC 1993]. Higher FCR following improved growth rate is profitable because of decrease in feed cost. An increase in protein level caused a decrease in PER, which was similar to results from Bromly (1980); Pongmaneerat & Watanabe (1991) and Kim & Lall (2001). But linear regression was between SGR_W and ADN. This indicated that CN could meet growth requirement and excess protein was catabolized to provide energy for growth (Adron *et al.* 1976; Lied & Braaten 1984). Why PER of D4 was lowest remains to be investigated. Mortalities of D3 and D4 were higher than the others significantly, but there were no obvious causes to explain it.

Because of low water current, faeces existed as contamination on the bottom of the cages and were cleared away in the present study. The placid nature of the fish made collection of faeces feasible. So faeces were collected for assay as much as possible. Protein and energy in faeces are inevitably lost and lead to over estimation of protein digestibility (Nitrogen digestibility from 97.6% to 98.2%) and energy digestibility (from 94.8% to 96.8%). Protein and energy digestibility, with the protein source based on herring meal, whey power and blood meal, were from 84.9% to 92.1% and from 85.2% to 91.7% respectively (Tibbetts *et al.* 2000). Moreover, protein digestibility was 92.3% (Médale *et al.* 1998) and 94% (Schmitz *et al.* 1984) respectively when the protein source was based on fish meal. Another higher digestibility was from 93.5% to 94.5% for protein and from 90.1% to 91.0% for energy (Peres & Oliva-Teles 1999) when protein source was fish meal and soluble fish protein, and even from 96.8% to 97.1% for protein (Arzel *et al.* 1995) when protein source was fish meal, soluble fish protein and casein. These former digestibilities were determined by the indicator method. The present protein and energy digestibility could be from 92.3% to 97.1% (FN from 7.7% to 2.9%) and near 90% (FE 10%) respectively if determination was by an indicator.

When diet intake was not restricted, energy budget was not affected by body size (Xie *et al.* 1997b) and temperature (Cui & Wootton 1988b; Xie & Sun 1993). Similar results were observed in the present study. Distribution patterns of amount of DP and DGE were not affected. Nitrogen budget and energy budget may show constant patterns when diet intake is not restricted (Cui & Wootton 1988b).

The proportion of energy intake lost in faeces ranged from 3.23% to 5.17% that is lower than results from six other species (6.18–10.96%) reported by Cui & Liu (1990b). FE was reduced when determined directly rather than by an indicator. Southern flounder fed to satiation allocated 41.35% of AE to ME, and 58.65% to GE. The two proportions could be adjusted by estimation. Protein digestibility was assumed to be 92.3% (faecal protein 7.7%) and energy digestibility was assume to be 90% (FE 10%) if digestibility determination was by an indicator. Average CN and energy intake were calculated to be 55.03 g and 14.2 MJ respectively.

$$\mathrm{UN} = 100\mathrm{CN} - 7.7\mathrm{FN} - 63.52\mathrm{GN} = 28.78(\%)$$

$$\mathrm{UE} = 55.03\ \mathrm{g} \times 28.78\% \times 24.8\ \mathrm{MJ\ kg^{-1}}/14.2\ \mathrm{MJ} = 2.77\ (\%)$$

$$\mathrm{AE} = 100\mathrm{IE} - 10\mathrm{FE} - 2.77\mathrm{UE} = 87.23\ (\%)$$

$$\mathrm{GE/AE} = 54.35/87.23 = 62.31\ (\%)$$

$$\mathrm{ME/AE} = 100 - 62.31 = 37.69\ (\%)$$

The mean combined losses in faeces and excretion of energy intake was 12.77% that was in the range from 10.5% to 16.0% reported for six species (Cui & Liu 1990b). Based on energy budgets for 14 fish species (Cui & Liu 1990a), average energy budget for fish was calculated to 100AE = 60ME + 40GE. Either 58.65% or 62.31% could suggest that the southern flounder have high growth efficiency and low metabolic expenditure. This is perhaps because the southern flounder spends a high proportion of its time resting on the water bottom.

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