Effects of Feeding Frequency on the Post-Feeding Oxygen Consumption and Ammonia Excretion of the Juvenile Snakehead

Dingcong He[1], Ge Li[1], Hang Xie[1], Shuting Liu[1], Yiping Luo[1,*]

[1] Southwest University, Key Laboratory of Freshwater Fish Reproduction and Development, Ministry of Education, 400715, Chongqing, China.

Abstract

The effects of feeding frequency on the specific dynamic action of the juvenile snakehead were examined. The fish were fed at four different frequencies with the same total meal intakes (4% body mass), and the postprandial oxygen consumption rate and ammonia excretion rate were determined for two days. The durations of oxygen consumption and ammonia excretion significantly increased with increasing feeding frequency. The specific dynamic action coefficient did not significantly change as the feeding frequency increased. The net energy gain of the fish fed 4 meals daily was lower than the fish fed at the other frequencies. No significant change was found for the post feeding ammonia quotient as the feeding frequency increased. The total post feeding ammonia excretion and its ratio to the total nitrogen intake of the fish fed 4 meals daily were higher than those of the fish fed at any other frequency. These results suggest that frequent feedings result in a prolonged digestion process and reduce net energy gain and dietary protein efficiency, and very frequent feedings with smaller meals may be less beneficial for food energy utilization in the sedentary ambush foraging snakehead fish.

Keywords: Specific dynamic action, ammonia quotient, *Channa argus*.

Introduction

Specific dynamic action (SDA) refers to the accumulated energy expended from the physiological and biochemical processes occurring during ingestion, digestion, absorption, and assimilation of a meal (Jobling, 1981; Beamish and Trippel, 1990; McCue, 2006; Secor, 2009). The major proportion of the SDA can stem from different sources depending on meal composition (Andrade *et al.*, 2004; Secor, 2003, 2009). Ammonia excretion, the primary excretion product of protein catabolism in freshwater fish, generally increases after feeding matching the increase in oxygen consumption (Wood, 1993; Médale *et al.*, 1995; Leung *et al.*, 1999). The ratio of ammonia excretion to oxygen consumption, termed the ammonia quotient (AQ), is used to estimate the percentage of protein respired as a substrate (Kutty, 1978). A number of factors affect the metabolic response post-feeding, such as meal size (Fu *et al.*, 2005a, 2005b; Jordan and Steffensen, 2007; Secor and Faulkner, 2002; Secor *et al.*, 2007; Wang *et al.*, 2012), meal composition (Swennen *et al.*, 2006; Fu *et al.*, 2005c; Luo and Xie, 2008; McCue *et al.*, 2005), feeding frequency (Guinea and Fernandez, 1997; Fu *et al.*, 2005b), temperature (Secor and Faulkner, 2002;

Rosen and Trites, 2003; Wang *et al.*, 2003; Peck *et al.*, 2005; Kaseloo and Lovvorn, 2006; McCue, 2006; Secor *et al.*, 2007; Luo and Xie, 2009; Greene *et al.*, 2013), dissolved oxygen concentration (Jordan and Steffensen, 2007; Zhang *et al.*, 2010), and body size (Chappell *et al.*, 1997; Secor and Faulkner, 2002; Secor, 2007; Peck *et al.*, 2005; Luo and Xie, 2008; McGaw *et al.*, 2013; Crocker-Buta and Secor, 2014).

Animals in nature may face different food distributions and have options between foraging frequently on small meals or infrequently on a large meal, which have differences in energy utilization efficiency related to fitness (Verlinden and Wiley, 1989). Feeding frequency is also an important issue in fish culture in the laboratory or farm because of its possible influence on dietary efficiency and water quality management for ammonia excretion (Priede, 1985). However, compared to other well-studied factors, there are only a limited number of studies for the effect of feeding frequency on SDA in several species of fish, the carp *Cyprinus carpio* (Hamanda and Maeda, 1983), the walleye *Stizostedion vitreum* (Beamish and MacMahon, 1988), the gilthead sea bream *Sparus aurata* (Guinea and Fernandez, 1997), and the southern catfish *Silurus meridionalis* (Fu *et al.*, 2005b). Controlling for total food intake, multiple

feedings may have cumulative effects on post-feeding peak oxygen consumption (Guinea and Fernandez, 1997) or may reduce the peak oxygen consumption for relatively smaller meal sizes per feeding (Fu *et al.*, 2005b). Whether feeding frequency influences energy efficiency during SDA and ammonia excretion remains unclear.

The snakehead (*Channa argus*), a piscivorous fish, is widely distributed in China and is a recently invasive species in North America (Wang *et al.*, 2012). The life history and ecological characteristics of this species have been reported (Liu *et al.*, 1998, 2000; Herborg *et al.*, 2007; Landis *et al.*, 2011). This fish has a sedentary ambush foraging style in the field, and the effects of meal size on SDA have been assessed (Wang *et al.*, 2012). The sedentary ambush fish is hypothesized to have metabolic characteristics optimal for digesting less-frequent but larger meals (Priede, 1985; Fu *et al.*, 2009). As a sedentary ambush predator, this species is expected to have a relatively higher energy utilization efficiency and faster digestion following less-frequent but larger meals. We hypothesize that infrequent feeding on large meals may be more profitable to the snakehead with higher energy and nutrition efficiency after feeding, such that there is less SDA energy consumption, a faster SDA process, and less protein oxidation. The objective of this study was to exam the variation of the parameters of postprandial oxygen consumption and ammonia excretion responses among the snakeheads fed at different frequencies.

Materials and Methods

Experimental Animals

Juvenile snakeheads were obtained from a hatchery in Guangdong Province, China. They were acclimated at $25.0 \pm 0.2°C$ in a rearing system with continuous aeration for one month prior to the experiment. During the acclimation period, the fish were fed once daily to satiation with cutlets of freshly killed loach (*Misgurnus anguillicaudatus*) without viscera, head and tail, and the uneaten food was removed after 15 minutes. The photoperiod was set at 12L:12D, and the ammonia-N concentration was maintained lower than 0.015 mg L^{-1}. Experiments were conducted in according with the ethical requirements of the Animal Care of the Fisheries Science Institution of Southwest University, China and requirements of environment and housing facilities for laboratory animals of China (Gb/T14925-2001).

Measuring Oxygen Consumption and Ammonia Excretion

We measured the rates of oxygen consumption and ammonia excretion using a 15-chamber, continuous-flow respirometer, as described by Wang *et al.* (2012). One chamber without fish was used as the control. The water was maintained at the same temperature as during the acclimation period. The dissolved oxygen concentration was measured at the outlet of the chamber using an oxygen meter (HQ30, Hach Company, Loveland CO, USA). The ammonia concentration of the outlet water was measured using the salicylate-hypochlorite method (Verdouw *et al.*, 1978). The flow rate of the water through the respirometer chamber was measured by collecting the water outflow from each tube into a 50 mL beaker for varying periods of time in minutes. The flow rate of each chamber was adjusted to assure greater than 70% saturation of the dissolved oxygen concentration in the outlet water (Blaikie and Kerr, 1996; Wang *et al.*, 2012). To enhance the accuracy of the oxygen readings, a difference of at least 16 mM was maintained between the outflow water of the experiment chamber and the control chamber (Wang *et al.*, 2012).

The following formulas were used to calculate the oxygen consumption rate (M_{O2}, mmol O_2 h^{-1}) and ammonia excretion rate (TAN, mmol NH_3-N h^{-1}) of the individual fish:

$$M_{O2} = \Delta O_2 \times v$$
$$TAN = \Delta am \times v$$

where ΔO_2 and Δ_{am} represent the absolute values of the concentration difference of the oxygen (mM O_2) and ammonia (mM NH_3-N) between the experimental chamber and the control chamber, and v (L h^{-1}) is the velocity of flow in the chamber.

Experimental Procedures

The SDA responses were determined at four different feeding frequencies by equally dividing the same total meal amount (4% body mass) into 1 meal every other day (0.5 meal daily), 1 meal daily, 2 meals daily (at 18:00 and 06:00), and 4 meals daily (at 18:00, 24:00, 06:00, 12:00). For each feeding frequency, 14 fish were used and allowed to acclimate in the respirometer chamber for one week, and one chamber without fish was used as the blank control. After 24 hours of fasting, the fish were weighed and placed back into the chamber overnight. The M_{O2} and TAN were measured at 2-h intervals over an 8-h period before the experimental feeding. The values were then averaged as the resting M_{O2} and TAN. A predetermined quantity of cutlets of freshly killed loach without viscera, head and tail were offered as the food. Immediately after the fish finished the meal, the chambers were closed, and the M_{O2} and TAN were measured continuously every two hours until the values returned to pre-feeding levels. The fish that were fed were used as the experimental fish, and the final sample numbers were 7, 11, 11 and 9 for the feeding frequencies of 0.5, 1, 2, and 4 meals daily,

respectively. The cutlets of loach were sampled and dried to constant mass at 70°C for 5 to 7 days, and the energy content was analyzed using an oxygen bomb calorimeter (Model 1281, Parr Instrument Company, Moline, IL, USA). The average energy content used as the meal energy content was 4.34 kJ g^{-1}. The protein content was measured using the Kjeldahl method. The total N intake (g) was calculated as the dietary protein intake (g) divided by 6.25. The light was left on for the duration of the experiment to minimize the influence of circadian rhythms on the metabolic rate (Tandler and Beamish, 1980; Blaikie and Kerr, 1996; Iglesias *et al.*, 2003; Fu *et al.*, 2005a).

Parameters in SDA

The following post-feeding parameters for oxygen consumption and ammonia excretion of individual fish were quantified: (1) resting metabolic rate (resting M_{O2}, mmol O_2 h^{-1}) and resting ammonia excretion rate (resting TAN, mmol NH_3-N h^{-1}), the average rates measured in fasting fish; (2) peak metabolic rate (peak M_{O2}, mmol O_2 h^{-1}) and peak ammonia excretion rate (peak TAN, mmol NH_3-N h^{-1}), the maximum rates measured after feeding; (3) factorial scope, calculated as the peak rate divided by the resting rate; (4) duration (h), the time from feeding until the rate returned to within the standard error of the resting rate for a given fish; (5) SDA (mmol O_2), the accumulated oxygen consumption above the resting rate; (6) SDA coefficient (%), energy of SDA quantified as the percentage of the energy content of the meal; (7) total energy consumption (kJ), calculated by the energy consumption including SDA and resting metabolism during the SDA response; (8) net energy gain (kJ), calculated by the energy obtained from the food minus the total energy consumption; (9) SDA ammonia excretion (SDA TAN, mmol NH_3-N), the accumulated ammonia excretion above the resting rate; (10) resting ammonia quotient (AQ$_{resting}$), calculated as the resting ammonia excretion (mmol NH_3-N h^{-1}) divided by the resting oxygen consumption (mmol O_2 h^{-1}); (11) ammonia quotient during SDA (AQ$_{during SDA}$), calculated as the total ammonia excretion (mol NH_3-N) divided by the total oxygen consumption (mol O_2) during SDA. and (12) Ex$_{NH3–N}$/In$_{–N}$ (%), the ratio of the total postprandial ammonia-N excretion (mol) to the total N intake (mol). The oxygen consumption was also converted to energy using a conversion factor of 13.84 J mg $O_2$$^{-1}$ (442.88 J mmol $O_2$$^{-1}$) (Guinea and Fernandez, 1997). The mass specific values of these parameters were also calculated.

Statistical Analysis

The data analysis was performed using SPSS, version 19.0. The SDA parameters of the fish fed at different feeding frequencies were compared using a general linear model (GLM), with body mass as a covariate. A *P*-value less than 0.05 was considered statistically significant. All data are presented as the mean ± SE.

Results

There was no significant difference for body mass, resting metabolic rate, resting TAN, AQ$_{resting}$, or AQ$_{during SDA}$ among the four feeding frequency treatments (Table 1). For each treatment M_{O2} increased abruptly after feeding to a peak before declining gradually back to pre-feeding levels (Figure 1). The multiple peak values of M_{O2} were not significantly different from each other for the fish fed 1, 2, or 4 meals daily (Figure 1). The post-feeding TAN showed a similar response pattern as the M_{O2}, with larger fluctuations (Figure 2). The duration of ammonia excretion was longer than the duration of oxygen consumption, except for the fish fed 0.5 meals daily (Table 1). The SDA durations of oxygen consumption (P=0.000004) and ammonia excretion (P=0.000186) significantly increased with an increase in feeding frequency. Both SDA and the SDA coefficient did not significantly vary among feeding frequency treatments (Table 1). The SDA ammonia excretion tended to increase as the feeding frequency increased, but no significant difference was observed because of larger variation for each group. The net energy gain of the fish fed 4 meals daily was lower than the fish fed other frequencies (P=0.000091) (Figure 3). The Ex$_{NH3-N}$/In$_{-N}$ of the fish fed 4 meals daily was higher than that the fish fed any other frequency (P=0.034). No significant variation was found in the AQ$_{during SDA}$ among feeding frequency treatments, and as a result, the percentage of oxygen consumed for protein metabolism was maintained within a narrow range (36.19% to 41.68%).
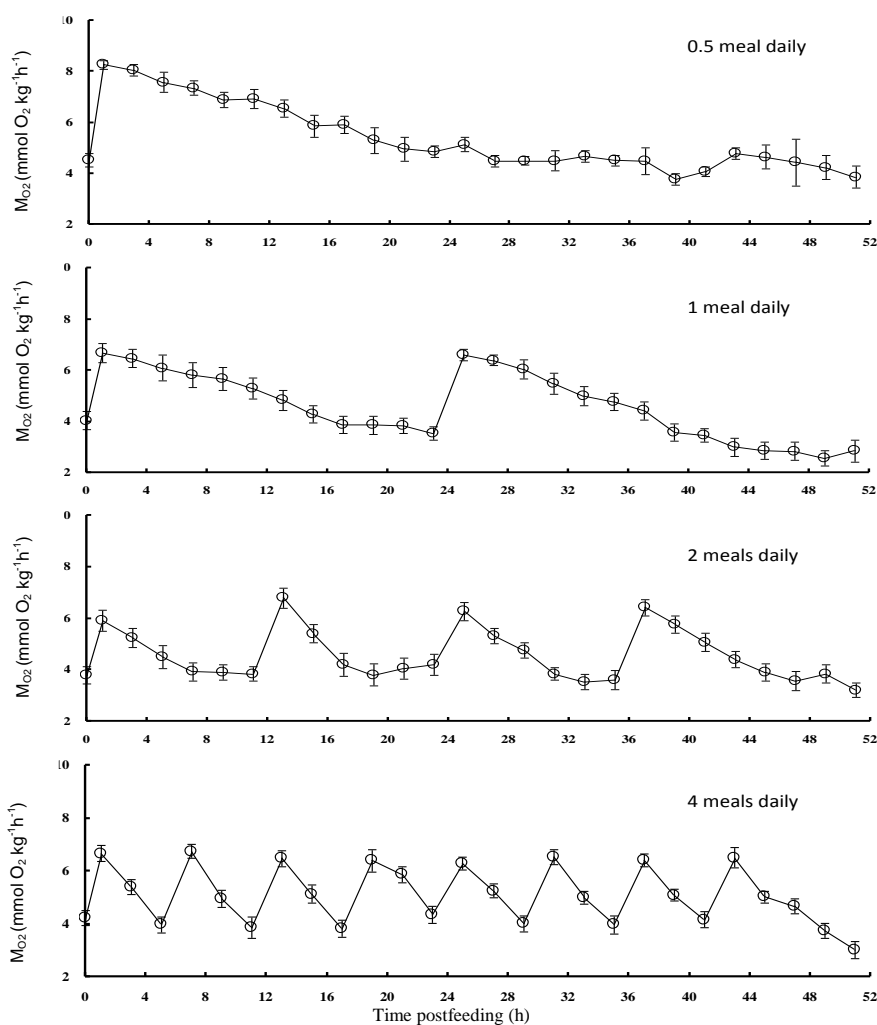
The metabolic parameters after the first feeding were compared among the four groups (Table 2). A single feeding rate at 0.5, 1, 2, and 4 meals daily were 4%, 2%, 1%, and 0.5% of body mass daily, respectively. The highest peak M_{O2} was observed in the 0.5 meals daily fish, but no significant difference existed for the fish fed 1 meal, 2 meals, or 4 meals daily. The SDA durations, SDA, and SDA ammonia excretion increased significantly with increasing meal size (P<0.002). The SDA coefficient, peak time and factorial scope for M_{O2} or TAN did not significantly vary among feeding treatments.

Discussion

The present results of the SDA and SDA coefficient are similar to our previous studies on the same species of snakehead fed the same meal size of 4% (SDA: 51.9 mmol O_2 kg^{-1}; SDA coefficient: 11.1%) (Wang *et al.*, 2012), suggesting a stable SDA response for this species. Daily multiple feeding induced cumulative effects on post-feeding M_{O2},

Table 1. Oxygen consumption and ammonia excretion variables in *Channa argus* fed at different frequencies (Mean±SE)

Feeding Frequency (meals daily)	0.5	1	2	4
Number	7	11	11	9
Body mass (g)	1.59±0.13	1.86±0.13	1.78±0.14	1.69±0.15
Total meal size (g)	0.07±0.01	0.09±0.01	0.08±0.01	0.07±0.01
Total meal energy (kJ)	0.29±0.02	0.37±0.03	0.34±0.03	0.32±0.02
Oxygen				
Resting M_{O2} (mmol O_2 kg^{-1} h^{-1})	4.50±0.22	4.02±0.37	3.78±0.36	4.22±0.30
Duration (h)	26.18±2.53[a]	32.52±1.69[ab]	39.23±2.51[bc]	45.14±1.18[c]
SDA (mmol O_2 kg^{-1})	50.50±5.26	46.63±6.47	44.94±9.14	51.17±3.93
SDA (kJ)	0.04±0.00	0.04±0.01	0.04±0.04	0.04±0.00
SDA (kJ kg^{-1})	22.15±2.18	19.95±2.74	21.25±4.03	22.45±1.81
SDA coefficient (%)	11.98±1.20	10.02±1.41	11.04±2.07	11.9±0.98
Total energy consumption (kJ)	0.11±0.01[a]	0.14±0.01[a]	0.15±0.02[ac]	0.18±0.01[bc]
Total energy consumption (kJ kg^{-1})	73.06±4.84[a]	76.01±4.94[a]	84.49±6.45[ac]	105.89±4.11[bc]
Net energy gain (kJ kg^{-1})	112.15±5.66[a]	123.68±4.49[a]	107.01±6.41[a]	83.13±3.84[b]
Ammonia				
Resting TAN (mmol NH_3-N kg^{-1} h^{-1})	0.48±0.03	0.40±0.04	0.31±0.02	0.40±0.33
Duration (h)	24.42±2.73[a]	38.90±1.48[b]	46.07±1.56[c]	49.03±0.51[c]
SDA TAN (mmol NH_3-N kg^{-1})	9.87±1.46	12.94±1.78	12.96±2.06	14.67±1.87
Ex_{NH3-N}/In_{-N} (%)	29.94±3.28[a]	25.98±1.84[a]	28.36±2.76[a]	40.21±1.42[b]
$AQ_{resting}$	0.11±0.01	0.10±0.01	0.09±0.01	0.10±0.01
Oxygen consumed for prefeeding protein metabolism (%)	32.58±2.71	31.820±3.93	27.38±3.23	29.62±2.68
$AQ_{during\ SDA}$	0.13±0.01	0.14±0.01	0.12±0.01	0.13±0.00
Oxygen consumed for protein metabolism during SDA (%)	39.67±2.62	41.68±1.97	36.19±1.87	40.38±1.11

Figure 1. The effect of feeding frequency on the postprandial oxygen consumption rate (M_{O2}, mmol O_2 kg^{-1} h^{-1}) of *Channa argus*. The total meal intake for each frequency group was 4% body mass.
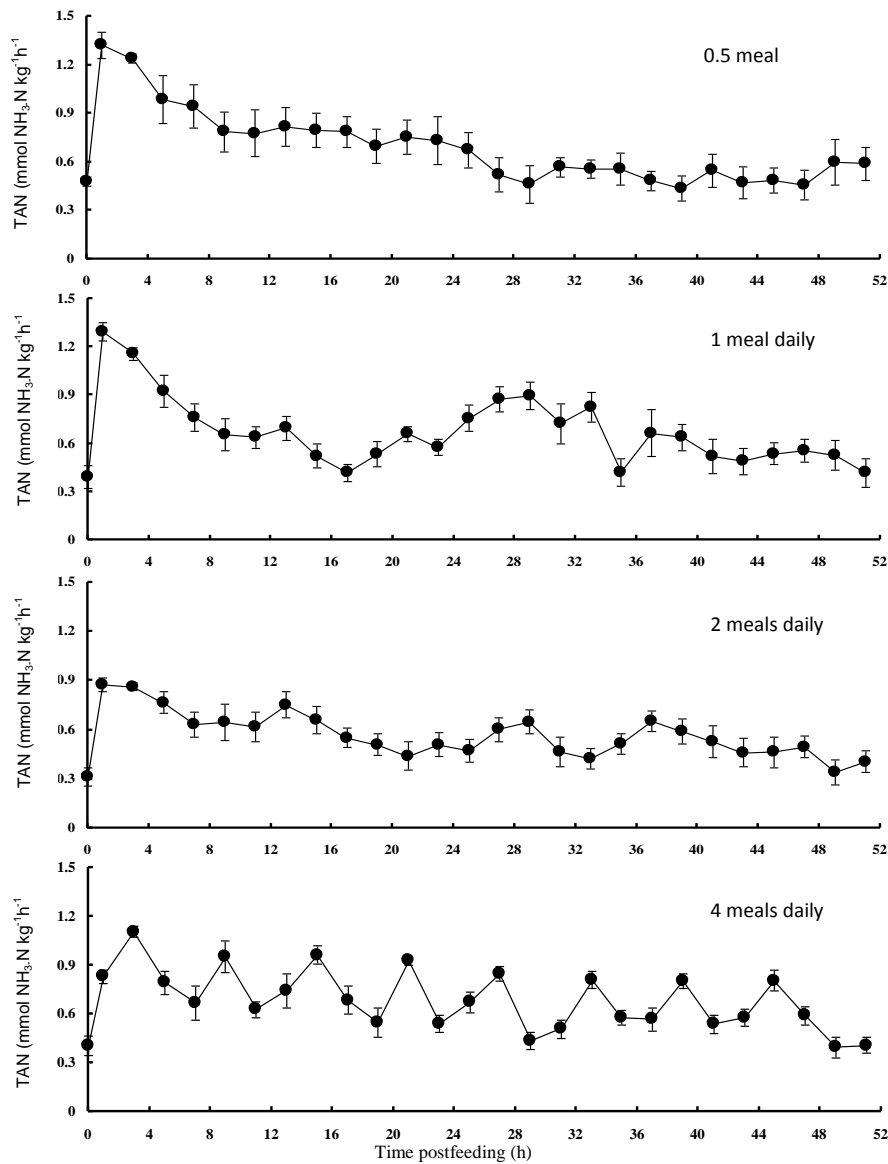
Figure 2. The effect of feeding frequency on the postprandial ammonia excretion rate (TAN, mmol NH_3-N kg^{-1} h^{-1}) of *Channa argus*. The total meal intake for each frequency group was 4% body mass.
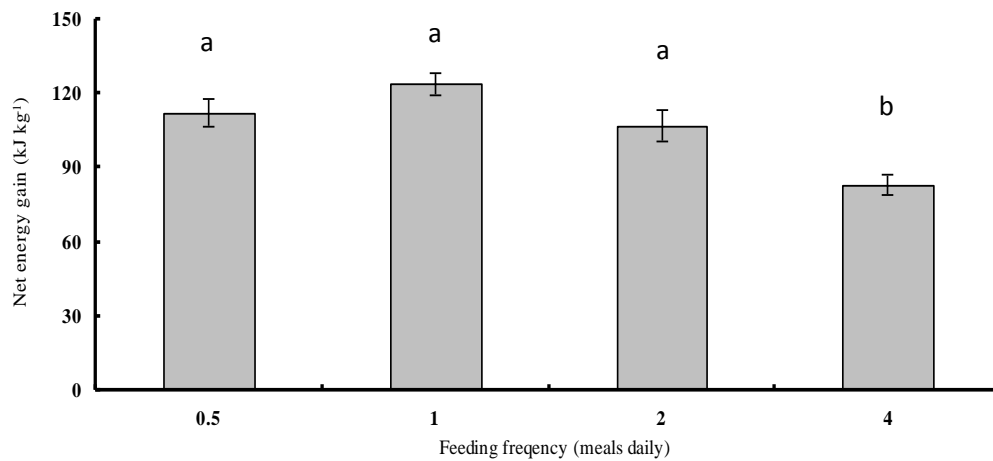

Figure 3. The net energy gain during the specific dynamic action of *Channa argus* fed at different frequencies. a, b Different superscripts in each row indicate significant differences between treatments (P<0.05).

Table 2. Metabolic parameters post the first feeding of fish fed at different frequencies (Mean±SE)

Feeding Frequency (meals daily)	0.5	1	2	4
Meal size of body mass (%)	4	2	1	0.5
Number	7	11	11	9
Body mass (g)	1.59±0.13	1.86±0.13	1.78±0.14	1.69±0.15
meal size (g)	0.07±0.01[a]	0.04±0.00[b]	0.02±0.00[c]	0.01±0.00[d]
meal energy (kJ)	0.29±0.02[a]	0.19±0.01[b]	0.09±0.01[c]	0.04±0.00[d]
Oxygen				
Resting M_{O_2} (mmol O_2 kg^{-1} h^{-1})	4.50±0.22	4.02±0.37	3.78±0.36	4.22±0.30
Peak M_{O_2} (mmol O_2 kg^{-1} h^{-1})	8.44±0.36[a]	6.72±0.50[ab]	6.06±0.41[b]	6.66±0.31[ab]
Factorial scope	1.89±0.08	1.73±0.11	1.71±0.17	1.61±0.08
Peak time (h)	1.57±0.37	1.55±0.28	1.18±0.18	1.00±0.00
Duration (h)	26.18±2.53[a]	16.49±0.87[b]	6.99±0.57[c]	4.42±0.21[c]
SDA (mmol O_2 kg^{-1})	50.50±5.26[a]	25.06±3.90[b]	8.14±1.89[c]	5.82±0.60[c]
SDA (kJ kg^{-1})	22.15±2.18[a]	11.10±1.73[b]	3.61±0.84[c]	2.58±0.27[c]
SDA coefficient (%)	11.98±1.20	10.96±1.58	7.20±1.65	10.61±1.12
Total energy consumption (kJ)	0.11±0.01[a]	0.07±0.01[b]	0.03±0.00[c]	0.02±0.00[c]
Total energy consumption (kJ kg^{-1})	73.06±4.84[a]	39.78±3.06[b]	14.79±1.23[c]	10.81±0.66[c]
Ammonia				
Resting TAN (mmol NH$_3$-N kg^{-1} h^{-1})	0.48±0.03	0.40±0.04	0.31±0.02	0.40±0.33
Peak TAN (mmol NH$_3$-N kg^{-1} h^{-1})	1.49±0.11[a]	1.40±0.09[a]	1.01±0.08[b]	1.11±0.10[ab]
Factorial scope	3.17±0.26	4.01±0.55	3.28±0.23	2.89±0.35
Peak time (h)	2.43±0.84	1.91±0.50	2.09±0.42	3.22±0.22
Duration (h)	26.66±2.93[a]	16.23±1.75[b]	11.51±0.36[b]	6.00±0.00[c]
SDA TAN (mmol NH$_3$-N kg^{-1})	9.87±1.46[a]	6.66±0.80[b]	4.20±0.59[c]	2.03±0.37[c]
Ex$_{NH_3-N}$/In$_{-N}$ (%)	29.94±3.28[ab]	28.58±1.68[ab]	23.28±2.33[a]	35.28±3.88[b]

reflected by gradually increasing peak values of M_{O_2}, in gilthead sea bream (Guinea and Fernandez, 1997) but not in walleye (Beamish and MacMahon, 1988) and southern catfish (Fu *et al.*, 2005b). Our results showed that there was no cumulative effect in snakehead after multiple feeding times daily (Figure 1), which may be because of the relatively longer interval between feedings and smaller meal size compared to the study of Guinea and Fernandez (1997).

In the current study, the SDA and SDA coefficient were independent of the feeding frequency, indicating unchanged energy consumption for the processes associated with digestion (Table 1). Similar results were also reported in carp (Hamanda and Maeda, 1983), walleye (Beamish and MacMahon, 1988), gilthead sea bream (Guinea and Fernandez, 1997), southern catfish (Fu *et al.*, 2005b), and eastern water skink *Eulamprus quoyii* (Iglesias *et al.*, 2003). Natural selection allows animals to adopt a specific strategy to maximize the ratio of energy income and energy expenditure (Priede, 1985). Our results suggest that the feeding frequency has little effect on the energy consumed for the SDA response, which may allow for stable energy consumed in the process from ingestion to assimilation for different foraging strategies, either frequently on small meals or infrequently on a large meal. However, we determined that the SDA duration significantly increased as feeding frequency increased. This is not surprising as when meals are given throughout the day, the SDA response continues through the day. Thus, it may limit the snakehead spend more time to complete digestion after multiple meals. Similarly, the prolonged effect of feeding frequency on postprandial oxygen consumption was also observed in walleye

(Beamish and MacMahon, 1988). A longer SDA process may be related to a longer time for digestion, which limits the fish to start next foraging later (Priede, 1985). Furthermore, the prolonged SDA duration also increased total energy consumed (including SDA and resting metabolism) during the SDA process. Thus, the net energy gain, calculated as meal energy minus total energy consumed during SDA process was lower when feeding with high frequency (Figure 3). Because the digestion energy gain per digestive turnover time may be of importance to the foraging strategies of the animal (Verlinden and Wiley, 1989), the results suggest that very high frequent feeding with a small meal may be less beneficial for food energy utilization in the sedentary ambush foraging snakehead fish, in according with our hypothesis.

Ammonia is the primary end product of protein catabolism in freshwater fish, and ammonia excretion indicates protein turnover (Mommsen and Walsh, 1991; Wood, 1993). The AQ equals 0.33 when aerobic metabolism is fueled completely by protein, and the percentage of oxygen consumed during protein metabolism is estimated as AQ/0.33×100 (Kutty, 1978). Based on the pre-feeding AQ (0.09-0.11), we estimated that 27-33% of the oxygen consumed was used for oxidizing protein in the pre-feeding snakehead (Table 1). This is similar to the value for southern catfish in a comparable warm water region (Luo *et al.*, 2009), but higher than those of many other cold water species, including rainbow trout, Atlantic salmon (*Salmo salar*), Arctic charr (*Salvelinus alpinus*), and Eurasian perch (*Perca fluviatilis*) (Lauff and Wood, 1996; Forsberg, 1997; Lyytikäinen and Jobling, 1998; Zakęs *et al.*, 2003). Snakehead showed increasing TAN and AQ values

post-feeding compared to the pre-feeding values (Figure 1, Table 1), indicating enhanced protein turnover during the SDA process. Similarly, increases in TAN and AQ post-feeding have been observed in many other fish species (Clarke and Thomas, 1997; Owen *et al.*, 1998; Peck *et al.*, 2003; Also and Wood, 1997; Lyytikäinen and Jobling, 1998; Luo and Xie, 2009). Our results showed that eight fold differences in feeding frequency reduced only very limited changes (around 1/4) in peak rate of ammonia excretion (Table 2). It suggests that adjusting feeding frequency has only limited effect for the water quality controlling in the culture water environment. Our results also indicate that the percentage of oxygen consumed during protein metabolism in the SDA process is independent of the feeding frequency. However, the highest Ex_{NH3-N}/In_{-N} occurs in fish fed a high frequency of meals (4 meals daily), suggesting reduced dietary protein efficiency.

The metabolic parameters of the fish after the first feeding in the present study can be used to compare the effect of meal size (Table 2). The SDA, peak M_{O2}, and SDA duration of the snakehead post-first feeding increased with decreasing feeding frequency (increasing meal size). Our results are consistent with the majority of previous fish studies (Jobling and Davies, 1980; Soofiani and Hawkins, 1982; Fu *et al.*, 2005a; Wang *et al.*, 2012).

In conclusion, our results demonstrate that the SDA, SDA coefficient, and AQ are independent of feeding frequency, indicating stable energy efficiency and protein turnover; however, frequent feedings result in a prolonged digestion process and reduce the net energy gain and dietary protein efficiency. Consistent with our hypotheses, the results suggest that very frequently (4 meals a day) may be less beneficial for food energy utilization in the sedentary ambush foraging snakehead.

Acknowledgements

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