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Effects of ration level and feeding frequency on digestibility in juvenile soft-shelled turtle, *Pelodiscus sinensis*[*]

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Abstract: The effects of ration level and feeding frequency on digestibility in juvenile soft-shelled turtle, *Pelodiscus sinensis*, were investigated. Four ration levels 1.5%, 2.5%, 4.0% and satiation (6.0% BW/d) were used. Apparent digestibility (AD) of dry matter (DMAD), protein (PAD) and protein real digestibility (PRD) were significantly affected by ration level, but not by feeding frequency when the ration level was similar. However, the feeding frequency affected the AD, DMAD, PAD and PRD significantly when the turtles were fed to satiation. The relationship between fecal protein content (Y) and protein intake (X) can be expressed as a quadric equation: $Y=-0.1742+0.1476X-0.0003X^2$ ($r^2=0.876$, $n=27$, $F=93.92$, $P<0.01$).

Key words: Digestibility, Ration level, Feeding frequency, Juvenile soft-shelled turtle, *Pelodiscus sinensis*
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INTRODUCTION

Chinese soft-shelled turtle *Pelodiscus sinensis* (Reptilia, Testudines, Trionychidae) is an important and high-valued commercial species in China and Southeast Asia where it is commonly cultivated in ponds and canals (Cox *et al*., 1998). The native range of the species includes China, Korea, Japan, and northern Vietnam (Ernst and Barhour, 1989; Iverson, 1992; Zhao and Adler, 1993), but it has been introduced to Hawaii (Iverson, 1992), Guam (Zhao and Adler, 1993) and much of southeastern Asia (Cox *et al*., 1998). The Chinese soft-shelled turtle is an aquatic, mostly carnivorous species, feeding on insects, crustaceans, mollusks, fish and the seeds of marsh plants (Ernst and Barhour, 1989; Cox *et al*., 1998). It is highly prized as food and medicinal tonic.

Digestibility of feeds for cultured animals is a major concern for reasons of economic efficiency and pollution control (Cho *et al*., 1994; Lawrence and Lee, 1997; Azevedo *et al*., 1998). Intensive production of turtles may not be feasible in the future unless careful consideration is given to the environmental effects of feeding regimes. Digestibility data will be important for the formulation of low pollution feeds by reducing the amount of empirical environmental chemistry required for certifying the feed (Cho *et al*., 1994).

Digestibility of a feedstuff depends not only on the animal's digestive physiology and the feedstuff's physical and nutrient characteristics but also on the environmental conditions. Reports about effects of many factors, such as temperature (Olsen and Ringoe, 1998; Lei *et al*., 2000; Usmani and Jafri, 2002; Bendiksen *et al*., 2003; Ng *et al*., 2004), salinity (Storebakken and Austreng, 1987; Lei and Li, 2000; Cabanillas-Beltrán *et al*., 2001), ration size (Henken *et al*., 1985; Fernández *et al*., 1998; Windell, 1978), feeding frequency (Hudon and de La Noue, 1984), and feed composition (Giri *et al*., 2000; Patra, 2001; Fagbenro and Davies, 2001; Mundheim *et al*., 2004; Hevroy *et al*., 2005) on digestibility in aquatic animals such as fish, shrimp, crab and abalone have been

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studied extensively, but few studies were focused on soft-shelled turtles.

MATERIALS AND METHODS

Experimental animals and design

The study was carried out at Department of Biological Engineering, Shenzhen Polytechnic, Shenzhen, China. Juvenile turtles were purchased from the Fishery Extension Service, Nanshan, Shenzhen and stocked in fiberglass water tubs (150 cm×65 cm×45 cm) where they were acclimated for over a month before the experiments, during which time the water temperature was gradually (2 °C/d) increased from 24 °C to 30 °C and they were fed a pellet diet with pellet feed designed specifically for juvenile turtles (Xinguang Feed Limited, Shenzhen). One week before the beginning of the study, turtles weighing 28.7~53.0 g were randomly distributed to 37.5 L glass aquaria (50 cm×25 cm×30 cm). For temperature control the aquaria were submerged in a large water tub (150 cm×65 cm×45 cm) equipped with heaters. The aquaria were supplied with freshwater ((30±0.5) °C) which was changed every other day. Dissolved oxygen was maintained at 5 mg/L or higher and photoperiod of 12 h light:12 h darkness.

Similar size turtles were used for each feeding trial to test the effects of ration level and feeding frequency. The turtles were placed randomly into each aquarium, one turtle per aquarium because of their truculent nature. Five turtles were assigned randomly to each ration level in the ration trial and three to five turtle for the feeding frequency trial (Table 1 and Table 2). The turtles were fed with pellet feed produced by Xinguang Feed Limited, Shenzhen. The major ingredients of the feed were wheat, ground fish and shrimp, ground pig liver, and pea, with crude protein, crude lipid and energy contents of 46%, 5% and 15.5 kJ/g dry weight respectively. Chromic oxide (0.5%), which could not be digested by the animals, was well mixed into all the pellet feed as an indicator for digestibility determination. In the ration level trial, the turtles were fed twice a day (10 a.m. and 5 p.m.). Ration levels were designed in four groups, which were 1.5%, 2.5%, 4.0% and satiation. In satiation group, the leftover was collected two hours after the turtles were fed. Effects of feeding frequency were tested by feeding the turtles either once daily at 5 p.m. or twice daily at 10 a.m. and 5 p.m. The experiment lasted four weeks.

Upon completion of the experiment, the turtles were starved for two days before being weighed in order to empty their stomachs and intestines. Turtle faeces were siphoned out and dried at 105 °C to obtain dry weight. Faeces were collected at least 10 times/d. All the faeces used to measure digestibility were encased in fecal strands that protect the feces from leaching.

Chemical analyses

Total nitrogen of diet and feces was determined by analyzing triplicate samples by the Kjeldahl method (AOAC, 1990); crude protein was calculated as %N×6.25. Total lipid content of the fish and pellet diets were determined on triplicate samples (each 5 g) using a column procedure with methanol-chloroform/water as the eluting solvent (Bligh and Dyer, 1959). Chromic oxide content was determined by analyzing triplicate samples by the procedure described by Tejada (1992).

Data analysis

The apparent digestibility coefficient (ADC) was calculated from the equation (Laining *et al*., 2003):

$$ADC(\%) = 100 \times \left[1 - \left(\frac{A' \times B}{A \times B'}\right)\right],$$

$$\text{Real protein digestion rate } (\%) = \frac{C - (F - e)}{C} \times 100,$$

where A is the concentration (% dry matter (%DM)) of nutrient in the ingested diet, A' is the concentration (%DM) in the feces (%), B is the concentration of Cr_2O_3 in the pellet feed (%), B' is the concentration of Cr_2O_3 in the feces (%), C is protein intake (g), F is protein content in the feces (g), e is nitrogen loss of basic metabolism (g) (protein content in the feces with zero protein intake, using the regression equation between protein intake and fecal protein (see results)).

Dry mater intake rate (*FIR*) (%)=100×average daily dry food intake/[(initial+final wet weight of turtles)/2]; Protein intake rate (*PIR*) (%)=100×average daily protein intake/[(initial+final wet weight of turtles)/2].

One-way analysis of variance (ANOVA) was

used to compare the various digestion parameters among the different treatments, and the Duncan method was used to determine significant differences among treatment groups. Curve estimation (quadratic and linear models) was used in the regression analyses. The results are presented as means±standard error. SPSS software, version 11.0 was used for statistical calculations, and $P<0.05$ was considered to be significant.

RESULTS

Effect of ration on digestibility

Protein intake rate, apparent digestibility (AD) of protein (PAD) and dry mater (DMAD), protein real digestibility (PRD), fecal protein content (FPC) and amount of fecal protein (AFP) are presented in Table 1.

There were no significant differences in DMAD among different ration levels ($P>0.05$). Significant differences existed in PAD, PRD, FPC and AFP. PAD and PRD were significantly lower in the ration level 4 than those in any other levels. Moreover, the FPC at this level 37.6% is the highest of all ration levels.

Regression analysis showed that the relationship between total protein intake (g) (X) and AFP (g) (Y) could be expressed as follow:

$$Y=0.2025+0.1417X+0.0021X^2,\quad (r^2=0.808,\ n=23,\ P<0.01). \tag{1}$$

Effect of feeding frequency on digestibility

Feeding frequency did not affect DMAD, PAD, PRD, FPC and AFP when the FIR was similar, whereas, when the FIR was different in turtles fed to satiation, significant differences existed in DMAD, PAD, PRD, FPC and AFP (Table 2).

Because a linear relationship between the feed frequency and FIR existed ($r=0.9272$, $P<0.001$), partial correlation analyses were used (Table 3). Partial correlation analyses showed that the significant linear relationship between feeding frequencies and DMAD, PAD and PRD were not caused by the FIR difference

Table 1 Effects of ration on digestibility, fecal protein content and fecal protein excretion in juvenile *Pelodiscus sinensis* (N=5)

	Level 1	Level 2	Level 3	Level 4	P
Initial weight (g)	40.5±3.3	39.5±3.8	44.2±1.9	40.3±1.5[a]	0.635
Real ration rate (%)	1.64±0.102[a]	2.54±0.108[b]	4.19±0.124[c]	5.96±0.118[d]	<0.001
Ration level	27.5% satiation	42.6% satiation	70.3% satiation	Satiation	<0.001
DMAD (%)	66.7±3.0	72.6±4.3	73.6±1.6	65.7±2.4	0.109
PAD (%)	80.8±1.7[b]	80.4±0.9[b]	83.1±2.1[b]	71.7±2.8[a]	0.007
PRD (%)	86.1±1.0[b]	85.4±1.4[b]	85.3±1.9[b]	75.0±2.4[a]	0.001
FPC (%)	28.4±1.2[a]	30.4±1.9[a]	29.1±2.0[a]	37.6±1.5[b]	0.003
AFP (g)	0.03±0.001[a]	0.05±0.002[a]	0.10±0.01[b]	0.2±0.02[c]	<0.001

Note: Means in the same row that do not share a common superscript letter differ significant ($P<0.05$)

Table 2 Effect of feeding frequency on digestibility in juvenile *Pelodiscus sinensis*

	Satiation group		Same ration level	
	Once/day	Twice/day	Once/day	Twice/day
	[**]N=3	N=5	[**]N=3	[*]N=5
Initial weight (g)	41.8±2.1	40.3±1.5	41.8±2.1	43.2±1.7
FIR (%)	1.65±0.09	2.73±0.12[#]	1.65±0.09	1.46±0.24
PIR (%)	0.77±0.01	1.26±0.06[#]	0.77±0.01	0.67±0.08
DMAD (%)	75.3±0.1	65.7±2.4[#]	75.3±0.1	74.4±2.1
PAD (%)	84.3±0.6	71.7±2.8[#]	84.3±0.6	84.0±1.7
PRD (%)	85.1±0.5	75.0±2.4[#]	85.1±0.5	87.5±1.4
FPC (%)	29.4±1.1	37.6±1.5[#]	29.4±1.1	26.7±0.9
AFP (g)	0.08±0.01	0.21±0.02[#]	0.08±0.01	0.07±0.01

[*] Not fed to satiation but fed the same ration level as once per day group, which was fed to satiation; [**] Indicates the same turtles;
[#] Indicates significant different from turtles fed once per day ($P<0.05$)

Table 3 Partial correlation coefficients

			DMAD	*PAD*	*PRD*
Zero-order partials	Feed frequency	*r*	0.7812	0.8096	0.7810
		P	0.022	0.015	0.022
	FIR	*r*	0.5614	0.6064	0.5741
		P	0.148	0.111	0.137
Controlling for FIR	Feed frequency	*r*	0.8411	0.8305	0.8107
		P	0.018	0.021	0.027
Controlling for feed frequency	FIR	*r*	0.6971	0.6562	0.6411
		P	0.082	0.109	0.121

Note: Feed frequency trial: satiation group; n=6~8

(P<0.05 when controlling FIR and P>0.05 when controlling feed frequency).

DISCUSSION

Ration

Ration level is very important to the growth, nutrient absorption, energy budget, and body composition of cultured aquatic animals. Moreover, ration level may affect the rates of nutrient loading in the environment. It is necessary to optimize feeding ration to improve the economical and environmental sustainability of aquaculture.

There are some studies on the effect of ration on digestibility in fish. But the results are conflicting. Some studies showed a decrease in digestibility of protein, other dietary components or energy with increase in ration level (Windell, 1978; Bergot and Breque, 1983; Henken *et al*., 1985; Xie *et al*., 1997), whereas other studies have not found this decreased digestibility (Bondi *et al*., 1957; Beamish, 1972; Staples and Nomura, 1976; Storebakken and Austreng, 1987). On the other hand, others have found elevated ADs at high ration levels in grass carp, sturgeon and gibel carp (Cui *et al*., 1994; 1996; Zhu *et al*., 2000). Henken *et al*.(1985) found that AD of protein (and dry matter) by African catfish (*Clarias gariepinus*) was negatively correlated with feeding level. Fernández *et al*.(1998) found that the effect of ration level was correlated with diet type in gilthead sea bream (*Sparus aurata*). Increase daily rations produced a decrease in the ADs in all intestinal samples in the case of diets made with brown whole fish and trash fish meal, mainly in those with higher C/N ratios; but in the case of diet composed of capelin meal, such a decrease was restricted to the anterior region of the intestine and did not affect AD values for posterior intestine or faces even in the case of capelin based diets with high C/N ratios and high content of gelatinized corn meal.

In the present study, ration had no significant effect on the AD of dry matter; although the PAD and PRD were significantly affected by the ration. The PAD and PRD do not change much with the increase in the ration from level 1 to 3 (1.5%~4.0% BW/d), whereas they decreased rapidly when the ration increased to level 4 (satiation, 6.0% BW/d). The stability of PAD and PRD with increased amounts of food from level one to level three may be due two factors: (1) increased enzyme secretion with increased food intake into the stomach and intestine (von Liebich and Mann, 1950; Windell, 1967), and (2) longer retention of food in the stomach and intestine (Elliott, 1972; Kitchell and Windell, 1968; Garber, 1983). However, excessive food intake may obviate the effects of increased enzyme secretion and retention time (Garbar, 1983), resulting in lower digestibility as seen in the present study.

Feeding frequency

Feeding frequency does not seem to affect digestibility in fish greatly. Hudon and de La Noue (1984) did not find any difference in apparent digestibility of dry matter, protein and energy when feeding frequency was increased from two to six times per day.

In the present study, feeding frequency had no significant effect on digestibility of either diet when the ration level was similar; however, digestibility did change when the ration level was different. Partial correlation analyses showed that feeding frequency has significant effects on the DMAD, PAD and PRD after eliminating the effect of ration level.

CONCLUSION

To optimize the digestibility and minimize nutrient outputs from soft-shelled turtle culture, the turtles should be fed twice a day to 4.0% BW/d. Feeding turtles based on maximum digestibility will give the most cost-effective utilization of the feed used, and minimizing nutrient outputs will help in decreasing the environmental impacts of soft-shelled turtle culture.

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