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Short communication

Evaluation of the protein requirement of juvenile Chinese soft-shelled turtle (*Pelodiscus sinensis*, Wiegmann) fed with practical diets

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ABSTRACT

A feeding trial was conducted to determine the optimal protein requirement of juvenile Chinese soft-shelled turtle (*Pelodiscus sinensis*). Seven isoenergetic diets containing graded levels of protein (25%, 27%, 29%, 31%, 33%, 35%, and 43%) were fed to turtles initially averaging 4.8 ± 0.7 g for 8 weeks. The specific growth rate (SGR) of turtles increased significantly with dietary protein from 25% to 33% without any further significant increases in SGR at 35% or 43% protein. Feed intake was highest in turtles fed with the diet containing 25% protein, followed by the 27–31% protein groups, and was lowest in turtles fed with the 33–43% protein diets. Feed efficiency (FE) of turtles followed an inverse pattern with feed intake. Protein efficiency ratio (PER) in turtles initially increased with dietary protein levels, with the highest value observed in the 33% protein group, followed by decreases with further increases in dietary protein content. Protein productive value (PPV) of turtles followed the same pattern as PER. Body lipid content decreased with increasing dietary protein level. Analysis of SGR by broken-line regression indicated that the estimated optimal dietary level of protein for juvenile Chinese soft-shelled turtles was 32.8%.

1. Introduction

The Chinese soft-shelled turtle (*Pelodiscus sinensis*), a freshwater reptile, is a high-value aquaculture species that is commercially cultured in Asian countries including China, Japan, Korea, and southern Taiwan (Chen et al., 2014; Ip et al., 2013; Jia et al., 2005; Wu and Huang, 2008). Thus, the development of a nutritionally complete artificial diet is of crucial importance for the successful aquaculture of this species. The level of protein in commercial diets fed to soft-shelled turtles is usually higher than 45%, with more than half of this protein being provided by fish meal. The high cost of formulated feed has restrained further development of turtle aquaculture. Thus, knowledge of soft-shelled turtle protein requirements is important for the formulation of well-balanced and low cost feeds.

The dietary protein requirement of *P. sinensis* estimated by earlier studies was relatively high, ranging from 42.2% to 46.5% (Bau et al., 1992; Nuangsaeng and Boonyaratapalin, 2001; Zhou et al., 2013). However, there is evidence that the minimum protein requirement of the soft-shelled turtle may be lower. For example, Nuangsaeng and Boonyaratapalin (2001) found that turtles fed with a diet containing 55% protein had the highest growth rate, but no statistically significant differences in body weight were observed among turtles fed with diets containing 45–55% protein at the end of the feeding trial. In general, increasing the protein level of diets can lead to improved production, especially for carnivorous animals. However, only a portion of the dietary protein is used to synthesize new tissues for growth, and the surplus is metabolized as a source of energy (Lei, 2007). Diets containing excessive protein not only result in inferior growth performance, but also lead to increased amounts of energy being used for excretion and increased production of nitrogenous waste (Abdel-Tawwab et al., 2010; Guo et al., 2012; Mohanta et al., 2008; Monentcham et al., 2009; Yang et al., 2002). It was previously reported that the soft-shelled turtle experiences higher metabolic losses of nitrogen (nearly 76%; Lei, 2007) than some species of fish (Boujard et al., 2002; Fournier et al., 2004; Yigit et al., 2002). Therefore, it is important to determine the optimal dietary protein level at which the use of protein for growth is maximized, while minimizing the amount of protein metabolized as energy, stored as fat, and used in the production of nitrogenous waste.

The purpose of the present study was to investigate the effects of dietary protein content on growth, feed utilization, and body composition of juvenile soft-shelled turtles to estimate the optimal dietary protein level in order to support industrial scale feed manufacturing.

2. Materials and methods

2.1. Experimental diets

A feeding trial was conducted using a completely randomized design. Seven isoenergetic diets containing graded levels of protein (25%,

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27%, 29%, 31%, 33%, 35%, and 43%) were prepared (Table 1). Russian steam-treated fish meal and soybean meal were used as the main protein sources. The diets were kept isoenergetic by adjusting the α-starch and cellulose content of the diet. The ingredients were first ground into a fine powder through a 320 μm mesh. All the ingredients were then thoroughly mixed with soybean oil, and water was added to produce a stiff dough. The dough was then pelletized using an experimental feed mill (F-26 (II), South China University of Technology, Guangzhou, China) with a long single-screw and a die size of 2 mm diameter. The noodle-like strands were broken up and sieved into pellets with about 2 mm and then stored in at −20 °C until used.

2.2. Experimental procedure

Seventy rectangular glass tanks ($L \times W \times H = 60 \times 30 \times 40$ cm) equipped with separate electric thermostat heaters (MX300 IC, WEIPRO Aquarium Equipment Co. Ltd, Zhongshan, Guangdong, China) were used in the experiment. During the trial, mean water temperature was kept at 30 ± 0.5 °C. Juvenile *Pelodiscus sinensis* were obtained from a local commercial hatchery 3 days after hatching. Upon arrival, turtles were randomly stocked in tanks with about 18 L of filtered groundwater, and acclimated to laboratory conditions for 2 weeks. Turtles were fed with a commercial diet (Haitai company, Shijiazhuang, Hebei, China) to satiation during this period. The proximate composition (%) of the commercial diet was: crude protein 41.0; lipid, 7.5; ash 12.0. At the end of the acclimation, turtles were fasted for 24 h and weighed. Twenty turtles were weighted and sampled after being anesthetized with eugenol (1: 10, 000) (Shanghai Reagent, China) for measurement of initial body composition. Turtles of similar size (4.8 ± 0.7 g) were randomly distributed into 70 tanks (n = 5 turtles per tank). The tanks were then randomly divided into seven dietary treatments (n = 10 tanks per treatment). Turtles were fed 3% of their body weight per day. This amount was close to the maximal daily ration for turtles according to feed consumption during the acclimation period and was the amount that the turtles could consume in 0.5 h. The daily ration was subdivided into three equal meals fed at 0800 h, 1400 h, and 2000 h. Any uneaten food was removed 0.5 h after feeding, then dried at 65 °C and weighed to get the actual amount of the feed consumed daily. Potential loss of uneaten food was determined by placing food in water for 0.5 h, followed by drying and weighing. This value was used to adjust the calculated feed intake. Mean feed intake of turtles was calculated at the end of the feeding trial. Turtles were weighed once every 2 weeks, and the daily ration was adjusted accordingly. Any dead turtles were removed and were not replaced during the experiment. The tanks were cleaned twice per week, and approximately half of the culture water was replaced. Turtles were fed with the experimental diets for an 8-week period.

Water column characteristics were monitored weekly in the morning, using an YSI model 556 (Yellow Spring Instrument Co., Inc., Yellow Spring, Ohio). Moderate aeration was provided continuously with the use of air stones to maintain dissolved oxygen above 6 mg L^{-1}, except that aeration was stopped 0.5 h before uneaten food collection, and was resumed after collection. A photoperiod of 12 h light and 12 h dark was used.

Table 1
Formulation and proximate composition of the experimental diets.

Ingredients (%)	Dietary protein level (%)						
	25	27	29	31	33	35	43
Russian white fish meal	16	20	24	28	32	37	45
Soybean meal	15	15	15	15	15	15	10
Yeast meal	9	9	9	9	8	7	8
Liver meal	3	3	3	3	3	3	9
Cellulose	14.5	13	10.5	8.5	6.5	4	0
Soybean oil	2.2	2.2	2.2	2.2	2.1	2.1	1.5
Wheat meal	11	10	10	9	9	8	4
α-starch	25	24	23	22	21	20	19
Vitamin premix [a]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix [b]	3	3	3	3	3	3	3
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (%)							
Moisture	7.31	7.67	7.2	7.04	7.3	7.56	7.26
Ash	14.88	14.99	15.03	15.40	15.35	15.88	16.62
Crude protein	25.25	27.15	29.12	31.10	32.98	34.96	43.16
Crude lipid	7.12	6.95	7.09	7.31	7.11	7.13	6.51
Gross energy (KJ g^{-1} diet)	16.5	16.6	16.8	17.1	17.1	17.3	17.2
CP/GE (mg kJ^{-1})	15.2	16.3	17.3	18.1	19.3	20.2	25.0

[a] Vitamin mixture (IU or mg kg^{-1} of diet): retinal, 5000 IU; calciferol, 5000 IU; tocopherol, 700 mg; menadione, 50 mg; thiamin, 70 mg; riboflavin, 200 mg; cyanocobalamin, 50 mg; Ca pantothenate, 320 mg; nicotinic acid, 400 mg; folic acid, 20 mg; inositol, 800 mg; ascorbic acid, 3000 mg; biotin, 20 mg; choline chloride, 4000 mg.

[b] Mineral mixture (g or mg kg^{-1} diet): $CaHPO_4 \cdot 2H_2O$, 250 g; $MgSO_4$, 5 g; KCl, 5 g; NaCl, 2 g; $CuSO_4 \cdot 5H_2O$, 50 mg; $ZnSO_4 \cdot 7H_2O$, 280 mg; $FeSO_4 \cdot 7H_2O$, 300 mg; $MnSO_4 \cdot 5H_2O$, 1 mg; KI, 0.5 mg; $NaSeO_3 \cdot 5H_2O$, 0.4; CoCl, 0.10 mg.

2.3. Analysis and measurement

At the end of the feeding trial, the total number of turtles in each tank was recorded, and turtles were individually weighed after fasting for 24 h. Growth and feed efficiency data were then calculated. Three turtles were randomly sampled from each tank and pooled for body composition analysis (AOAC, Association of Official Analytical Chemists, 1995). Gross energy in feed and feces was determined using a bomb calorimeter (CA-4P, Shimadzu, Kiyamachi-Nijo, Kyoto, Japan). All analyses were carried out in triplicate for each sample.

2.4. Calculations and statistical analysis

The following variables were calculated:

Survival (%) = $100 \times N_t/N_0$

Specific growth rate (SGR: % day^{-1}) = $100 \times (\text{Ln } W_t - \text{Ln } W_0)/t$

Feed intake (FI, g 100 g^{-1} BW day^{-1}) = $100 \times D_f/((W_t + W_0)/2 \times t)$

Feed efficiency (FE) = $(W_t - W_0)/D_f$

Protein efficiency ratio (PER) = $(W_t - W_0)$/protein intake

Protein productive value (PPV) = 100 × protein gain in g/protein fed in dry basis in g.

Where BW is the wet body weight, W_t is the mean final body weight (g), W_0 is the mean initial body weight (g), t is experimental duration in day. N_0 and N_t represent the initial and final numbers of turtles in each tank, respectively. D_f is the dry diet intake (g).

Results were analyzed by one-way analysis of variance (ANOVA) with six protein levels as main effect, using the Statistic 6.0 statistical software (StatSoft, Tulsa, USA), and significance was set at $P < 0.05$. Multiple comparisons among means were made with Duncan's new multiple range test, and the results are presented as means ± SEM (standard error of the mean). Dietary protein requirement for juvenile turtle was estimated by the broken-line model (Robbins et al., 1979) based on growth rate of the turtle.

3. Results

3.1. Growth performance and feed utilization

Survival during the feeding trial was generally high and not related to dietary protein level (Table 2). Specific growth rate (SGR) of turtles initially showed a gradual increase with increasing dietary protein, reaching a plateau in turtles fed with the 33–43% protein diets. Feed intake (FI) was highest ($P < 0.05$) in turtles fed with the 25% protein diet, followed by the 27–31% protein groups, and was lowest in turtles fed with the 33–43% protein diets. Feed efficiency (FE) of turtles followed an inverse pattern with feed intake. Protein efficiency ratio (PER) of