

The Intestinal Evacuation and Maximum Daily Consumption of Purified Formulated Diets by Juvenile Grass Carp (*Ctenopharyngodon idella*)

Zhen-Yu Du^{1,2,*}, Li-Xia Tian¹, Gui-Ying Liang¹, Han-Bing Lan³ and Yong-Jian Liu^{1*}

¹Institute of Aquatic Economic Animals, School of Life Sciences, Sun Yat-sen University, 510275 Guangzhou, P.R. China

²National Institute of Nutrition and Seafood Research (NIFES), 5005 Bergen, Norway

³Guangdong Academy of Agriculture Sciences, 510640 Guangzhou, P.R. China

Abstract: The intestinal evacuation and maximum daily consumption of two formulated diets with different lipid content (6 and 10%) was examined in juvenile grass carp at 28°C. Fish were fed formulated diets to satiety and removed at 1 hour interval for intestine weighing. The study showed that both diets were evacuated from intestine in 12h and the process of evacuation was expressed by linear regression. In the later study of maximum daily consumption, fish were fed formulated diets to satiety four times in a single day. The sum of diet consumption in the day was recorded. The experiment was repeated in 4, 7, 10, 13, 16, 19, 22 day by the same fish. The result showed the maximum daily consumption of two formulated diets is 3.64 ± 0.23 % body weight (416.79 ± 28.84 J/g.d⁻¹) and 3.75 ± 0.16 % body weight (481.73 ± 19.44 J/g.d⁻¹).

Key Words: Grass carp, intestinal evacuation, maximum daily consumption, formulated diet.

INTRODUCTION

The rate of gastrointestinal evacuation is one of the major determinants of feeding rate [1-3]. Many factors such as temperature, meal size, fish size, dietary composition and feeding frequency will influence gastrointestinal evacuation [4-8]. Gastrointestinal or gastric evacuation of some fish have been studied [1,4,5,7-14], but these species were carnivorous or omnivorous, also with well-developed stomach. The information on the intestinal evacuation in grass carp (*Ctenopharyngodon idella*), an herbivorous and stomachless finfish, are absent.

The daily food consumption of fish is important in drawing up a feeding schedule in nutrition research and aquaculture productivity, but it is inconstant and affected by water temperature, fish size, food species and photoperiod [15-19]. However, we hypothesized that in a relative constant aquatic environment, such as a good-managed recirculating system in lab, the maximum daily consumption of same fish should be constant.

The object of the present study was to measure the intestinal evacuation and maximum daily consumption of formulated diet in juvenile grass carp. At the same time, in order to know whether diets with different energy level could affect these indexes in grass carp as well as carnivorous species, two diets with different energy level were used.

MATERIALS AND METHODOLOGY

Fish and Diets

The grass carp used in experiments was the strain which is long used in aquaculture and the fish was cultured in our own fish farm before moving in lab. The initial weight of juvenile grass carp was 3.4 ± 0.12 g (mean \pm SD)g. The two experiments were carried out in a recirculating aquatic system consisted of 18 fiberglass tanks (100 L). The fish were acclimated to experimental condition and diet for 2 weeks. During the acclimating period, fish were fed with lipid-free experimental diet (GE 2312 kcal·kg⁻¹) at 1% body weight per day (BW/d). The rate of flow in the system was 5L·min⁻¹. During the two experiments, the water temperature, dissolved oxygen, pH and ammonia were 27.5-28.5°C, 6.75-7.46 mg·L⁻¹, 7.61-7.97 and 0.08-0.26 mg·L⁻¹, respectively. The purified formulated diets were prepared as described by [20], and were stored at -30°C until use. The ingredients of diets were shown in Table 1.

Experimental Progress

After 2 weeks of acclimation, fish were distributed randomly into 12 tanks (30 fish/tank, 6 tanks for each experiment). In order to study intestinal evacuation, 48 h-fasted fish were fed the formulated diets to visual satiety at 9:00AM. Six fish (two fish per tank) were then gently removed at 1h intervals for intestinal content weighing until the intestinal content was emptied entirely in all the samples. At the same time of removing fish, the remaining diet or faeces were cleaned up. The percentage of intestinal content weight (% body weight) was used for statistical analysis. The experiment was repeated twice.

*Address correspondence to these authors at the National Institute of Nutrition and Seafood Research (NIFES), Postboks 2029 Nordnes, 5817 Bergen, Norway; Tel: +47 91132279; Fax: +47 5590 5298; E-mail: zdu@nifes.no; Institute of Aquatic Economic Animals, Sun Yat-sen University, Xin'gang Xi, Road, 510275 Guangzhou, P.R. China; Tel: +86 84110789; E-mail: eds@mail.sysu.edu.cn

Table 1. Formulation and Composition of Experiment Diets (% Dry Matter)

Formulation		
Casein	32	32
Gelatin	8	8
Corn starch	25	25
Fish oil	3	5
Corn oil	3	5
Cellulose	18.50	14.50
Vitamin mix ¹	1	1
Mineral mix ¹	8	8
Ascorbic phosphate ester	1	1
Choline chloride	0.5	0.5
Composition		
Moisture	9.5	9.7
Crude protein	35.85	35.24
Crude lipid	5.6	9.7
Ash	5.64	5.55
Crude energy (kcal/g) ²	3	3.3

¹ Vitamin mix and mineral mix was prepared as previous described by Du *et al.* [31].

² The crude energy was calculated using physiological fuel value of 4.0, 4.0 and 9.0 kcal·g⁻¹ for protein, carbohydrate and lipid respectively.

Maximum daily consumption was determined after the intestinal evacuation experiment. Two control tanks without fish were used to measure the dissolving rate of the diet. The diets were oven-dried at 105°C for constant weight (W_a) and then put in eight tanks at 9:00AM, 12:00AM, 3:00PM and 6:00PM to let fish eat to satiety. The remaining diet in each tank was collected 90min after feeding and then oven-dried to constant weight (W_e for experimental tanks and W_c for the controls). The dissolving rate (DR) of diet was calculated as $(W_a - W_c)/W_a$. The diet consumption of fish during each feeding period in a single tank was calculated as $W_a - W_e/(1 - DR)$. The maximum daily consumption of diet (W_m) in a single tank was calculated by summing the consumption of four time-points in a single tank. The maximum daily consumption of grass carp towards the two diets was calculated as $100 \times W_m/\text{fish weight}$ in a single tank. The experiment was repeated in a time course as 1, 4, 7, 10, 13, 16, 19, 22 day with the same fish. Except experimental day, fish were food-restricted. When all experimental repeats were completed, fish were weighed again.

Statistical Analysis

The data were subjected to one-way ANOVA to test the maximum daily consumption ratio in eight experimental repeats. When significant ($P < 0.05$) difference was found, a Duncan's multiple range test was used to rank the repeats. All figures were made by Excel 2000 (Office 2000, Microsoft).

RESULTS AND DISCUSSION

The variation of intestinal content of grass carp after feeding is shown in Fig. (1). The intestinal content tended to decrease gradually until the 12th h and no significant difference was found between two diets with different energy level. However, in most of other fish species studied, gastrointestinal evacuation time normally exceed 24h [7,8,10,11,13,21,22]. The intestinal evacuation time of grass carp (12h) is much shorter than other fish. But it should be noticed that most fish studied are carnivorous or omnivorous, and also have well-developed stomach. In natural environment, the prey of these carnivorous or omnivorous fish consists of small fishes, shrimps and other aquatic animals [23]. However, grass carp is herbivorous and stomachless. Compared to the prey of other carnivorous or omnivorous fish, the natural food of grass carp is low-energy and low-protein content, so it should be very necessary to improve the efficiency of digestion and absorption to shorten the passing time of food in intestine, for obtaining enough energy and protein. This hypothesis is supported by several evidences: 1) Grass carp have much longer intestine, 2.29-2.54 times to its body length, than carnivorous fish [24]. The long intestine could enlarge the surface area of digestion and absorption and it also could lead to shorten the evacuation time. 2) Li *et al.* [25] found in a single daytime, the time used of grass carp for eating is about 20h and it only stops eating in midnight for 4 h in natural environment. It proves that the ability of digestion in grass carp was strong. 3) Li *et al.* [25] also found when grass carp was fed phytoplankton and vege-

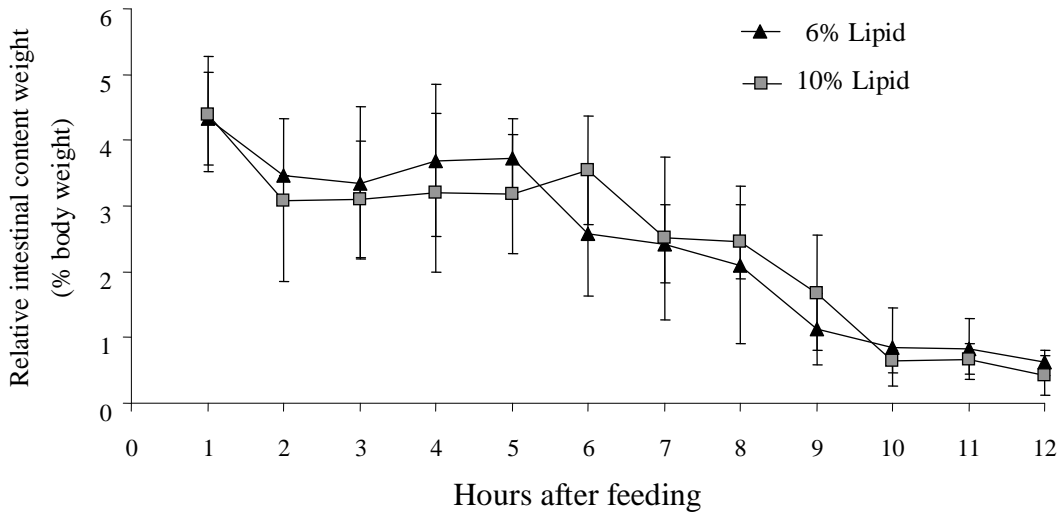


Fig. (1). Intestinal evacuation rate of grass carp towards two purified formulated diets with different lipids content.

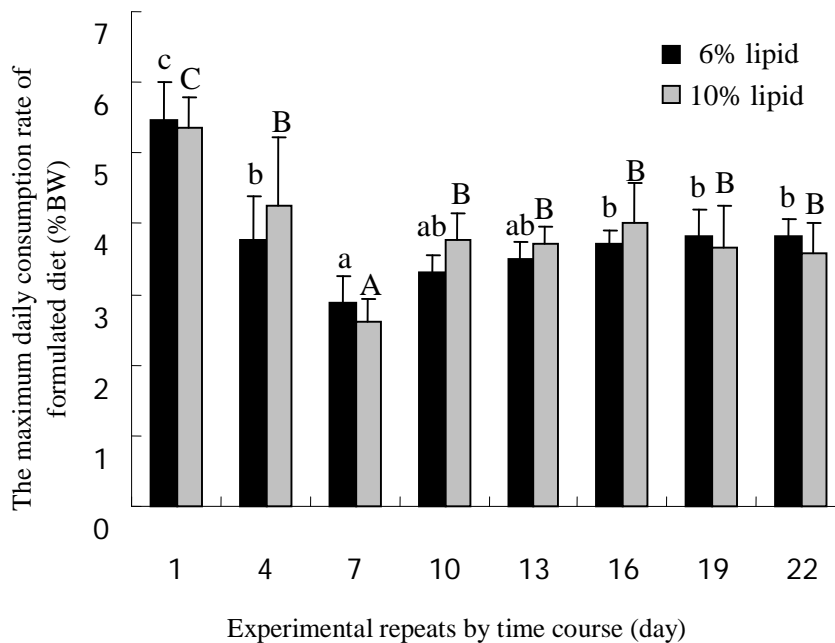


Fig. (2). The maximum daily consumption of two purified formulated diet with different lipids content in grass carp. Within the same diet, the values with different superscripts are significantly different ($P < 0.05$).

table to satiety, it only needed 4h to evacuate all the content in intestine. Jobling [23] believed the evacuation of high-energy density artificial diet was slower than prey in natural environment, which was also confirmed by the present study which demonstrated that the intestinal evacuation time of formulated diet (12h) is 3 times longer than natural food of grass carp.

Linear, square root and exponential regression were normally used for description of gastrointestinal evacuation progress [26]. In the present study, linear, square root and exponential regression models were built and compared respectively, and the linear model was better than the other regressions. For 6 and 10% diet, the linear regression was $Y=4.6988-0.3498X$ ($R^2=0.9206$) and $Y=4.5321-0.3268X$ ($R^2=0.8471$) respectively. Here X is the time (h) and Y is the

intestinal content. This result is similar to many mammals [27], but contrary to carnivorous/omnivorous fish [6,11,12]. It may be explained by the large surface-to-volume ratio in intestine of grass carp and it makes the progress of digestion and absorption turn evener and smoother than other fish which have stomach and shorter intestine.

The maximum daily diet consumptions of eight experimental repeats are shown in Fig. (2). The trend of two diets was similar. In the first three experimental repeats, the maximum diet consumption decreased quickly ($P < 0.05$). From the 10th day, the maximum daily diet consumption increased again and stayed constant ($P > 0.05$). The average of the data from 10th day to 22th day was $3.64 \pm 0.23\%$ BW ($456.46 \pm 28.84 \text{ J/g}\cdot\text{d}^{-1}$) for 6% dietary lipid level and $3.75 \pm 0.16\%$ BW ($481.73 \pm 19.44 \text{ J/g}\cdot\text{d}^{-1}$) for 10% dietary lipid

level, and there was no significant difference. It could be explained that after three repeats, fish had acclimated the feeding quantity and frequency. Li *et al.* [25] found when grass carp (4.9-650g) were fed phytoplankton and vegetable, the maximum daily consumption ratio was 41.5-49.8% BW in July and August at south China, which is much higher than 3.6% and 3.8% BW in present study. But considering the high content of moisture and fiber in water plant, high food consumption is necessary for grass carp to intake enough energy for survive in nature.

Many mathematical models were set up to estimate the relationship between daily food consumption and fish size or water temperature [2,16,28]. In an appropriate range of temperature, daily food consumption increased with the increase of fish size and temperature, but daily food consumption decreased with the increase of fish size. The maximum daily food consumption of some fish in different conditions was monitored, such as 2.99-3.46% BW in juvenile Florida pompano [19], 436.2 J/g.d⁻¹ in southern catfish [29], and 810.84 J/g.d⁻¹ in Crescent Sweetlips (*Plectorhynchus cinctus*) [30]. In the present study, the maximum daily diet consumption, $3.64 \pm 0.23\%$ BW (456.46 ± 28.84 J/g.d⁻¹), gained at relative high water temperature, could be regarded as the maximum daily consumption of grass carp towards these formulated diets in this experimental condition. However, in an 8 weeks trial performed in winter [31], the proper feeding rate of a similar formulated diet in grass carp was determined as 2% BW and excess feed intake would decrease the feed efficiency and digestibility efficiency. So the maximum daily consumption which is got from a short term trial probably needs to regulate to fit longer aquaculture term. In other longer feeding trials [32,33], the differences in growth, body composition and biochemical alteration induced by different dietary energy level have been observed, and these studies also indicated that compared to other carnivorous and omnivorous fish, grass carp is a low-energy requirement fish species. However, in this short term trial, no significant differences were found between two diets with different energy content.

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