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Growth rate, body composition, digestive enzymes and transaminase activities, and plasma ammonia concentration of different weight Jian carp (*Cyprinus carpio* var. *Jian*)

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ABSTRACT

The present study investigated the effect of body weight on body composition, digestive and absorptive capacity, transaminase activities in hepatopancreas and muscle, and plasma ammonia concentration of Jian carp (*Cyprinus carpio* var. *Jian*). A total of 750 Jian carps (18.0 ± 0.2 g) were randomly distributed into five groups with three replicates and fed the same diet for 56 days. Tissue and plasma samples were collected on days 14, 28, 42, and 56. The results were used to develop a mathematical model for specific growth rate, body moisture and fat content, aspartate transaminase activity and alanine aminotransferase activity in hepatopancreas and muscle, plasma ammonia concentration, and trypsin, chymotrypsin, lipase, and amylase activities in hepatopancreas and intestine, activities of creatine kinase, Na^+/K^+-ATPase, alkaline phosphatase, and γ-glutamyl transpeptidase in intestine in Jian carp. There were linear relationships between natural logarithms of above indexes and body weight. The body moisture and fat content, digestive and absorptive enzymes activities, and transaminase activities showed negative allometry against body weight of Jian carp which were partial reasons to explain fish growth rate decreasing.

1. Introduction

Fish growth is an outcome of feeding, digestion, assimilation, and metabolism of food material. The previous studies indicate that the digestive, absorptive, and metabolic capabilities of nutrients are related to fish size (Beamish, 1972; Fernández et al., 1998; Luo et al., 2013; Usmani and Jafri, 2002). As size increasing, fish growth rate and feed efficiency gradually decrease (Jobling, 1983a; Liu et al., 2016). One reason is associated with a higher body dry matter and lipid contents as fish growing, which leads to a decrease in feed protein and energy deposition rate (Azevedo et al., 2004). However, there are few records of the systematic study of body composition-size-growth relationships for fish. The study in muscle of *Polyodon spathula* shows that when moisture content decreases, fat content increases with size increasing, and protein content increases with size increasing up to a certain weight and remains constant thereafter (Chen et al., 2008). Nutrient deposition is the net result of nutrients anabolic and catabolic metabolism. Piscine amino acid metabolism is a vital metabolic process (Ballantyne, 2001). The alanine aminotransferase (ALT) and aspartate transaminase (AST) are two most important amino acid metabolizing enzymes, which activities reflect the intensity of fish amino acids metabolism (Ballantyne, 2001). The liver is the main organ for amino acid catabolism which results in the production of 50 to 99% ammonia (Ballantyne, 2001). The ammonia is the main end product of nitrogen metabolism in teleost fish (Wilkie, 2002). Up to data, there is

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scarce information concerning the relationship between liver ALT and AST activities and plasma ammonia concentration (PAC) and fish size. Such information is required to be explored.

Another reason is associated with the change of digestive and absorptive capabilities as fish grew. Digestive and absorptive enzymes play a key role in digestion and absorption of nutrients, which activities directly reflect digestive and absorptive capacity and affects fish growth rate (Zhao et al., 2015). The previous study shows that with increasing carp *Cyprinus carpio* size from 25 to 100 g, hepatopancreas proteases activity gradually increases; foregut protease activity firstly increases, and then decreases; midgut protease activity rapidly decreases; and hindgut protease activity rapidly increases (Bai et al., 1999). Intestinal alkaline phosphatase (AKP), Na^+, K^+-ATPase, γ-glutamyl transferase (γ-GT), and creatine kinase (CK) are important brush border enzymes relating to nutrients absorption in fish (Zhao et al., 2015). The AKP activity in intestine during sea bream *Sparus aurata* L. endogenous nutrient is quite weak, and increased slowly in half a month after feeding (Sarasquete et al., 1993). The similar results are observed in the larvae and juvenile stages of turbot *Scophthalmus maximus* (Chen et al., 2005) and *Cynoglossus semilaevis* (Chang et al., 2005). However, to our knowledge, there is still little report concerning the relationship between fish size and digestive and absorptive enzymes activities.

The current study was to examine the growth rate, body composition, activities of digestive enzyme, intestinal brush border enzyme, and ALT and AST, and PAC vary with the size of Jian carp *C. carpio* var. *Jian*, which is one of the main cultured new freshwater species in China. The present results provided some helpful comprehension of nutritional physiology and culture practice in fish.

2. Materials and methods

2.1. Experimental deign and fish feeding

A total of 750 Jian carp (with an average initial weight of 18.0 ± 0.2 g, means ± SD) were randomly distributed into fifteen 100-L glass aquaria, each of 50 fish. Feeding management and all experimental protocols were performed according to our previous study (Jiang et al., 2013). The fish were acclimatized to the experimental conditions for one month prior to the start of the study. A closed system with continuous aeration and daily replacement of water in the aquarium was used. The dissolved oxygen was higher than 5 mg/L. The water temperature was 25 ± 1°C. Other water quality characteristics were monitored and maintained at acceptable levels. A 12:12 h light–dark photoperiod was maintained. The fish were fed the same diet (Table A1), containing 32% crude protein and 5.47% crude fat, four times daily (0700, 1100, 1500, and 1900) for 56 days. Thirty minutes after the feeding, uneaten feed were removed by siphoning.

2.2. Sampling and analysis

The samples were collected on days 14, 28, 42, and 56, respectively. Each sampling was carried out after 12 h of the last feeding. Five fish were selected from each tank to determinate body composition (Horwitz, 2000). Blood samples of five fish from each tank were drawn from the caudal vein using heparinized syringes for PAC determination (Wang et al., 2015). Then another five fish from each tank were anesthetized in 50 mg/L benzocaine bath (Deng et al., 2016) and sampled for determination of protein content, and trypsin, chymotrypsin, lipase and amylase activities in hepatopancreas and intestine, CK, Na^+, K^+-ATPase, AKP, and γ-GT activities in intestine, glutamic oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities in hepatopancreas and muscle (Feng et al., 2013; Tang et al., 2013).

2.3. Calculations and statistics

Intestine and hepatopancreas protein contents (IPC and HPC), specific growth rate (SGR), and geometric mean weight (GMW) were calculated as follows:

$$\text{IPC} = [\text{intestinal protein (g)/wet intestine weight (g)}] \times 100,$$
$$\text{HPC} = [\text{hepatopancreas protein (g)/wet hepatopancreas weight (g)}] \times 100,$$
$$\text{SGR} = 100 \times [(\ln W_2 - \ln W_1)/(t_2 - t_1)],$$
$$\text{GMW} = (W_1 \times W_2)^{1/2}$$ (Árnason et al., 2009).

The relationships between natural logarithm of SGR (Y) and GMW (X), and natural logarithm of digestive enzymes and transaminase activities, and PAC (Y) and Wt (X) were described with the following equations: $Y = A + B X$, where Wt is the weight of the fish measured at time t.

3. Results

The present result showed that there was a linear relationship between ln SGR (Y) and ln GMW (X): $Y = 2.625 - 0.412X$ in Jian carp (Fig. 1). The body moisture decreased with increasing body weight, whereas, body fat content significantly increased with increasing body weight ($P < 0.05$); the protein and ash contents significantly increased with increasing body weight up to 31.4 g and kept a platform thereafter (Table A2). The intestinal and hepatopancreas protein contents significantly increased with increasing body weight (Table A2). The activities of trypsin, chymotrypsin, lipase, and amylase in hepatopancreas and intestine significantly increased with increasing body weight (Table A2). The activities of CK in intestine and AKP, γ-GT, and Na^+/K^+-ATPase in all intestinal segments significantly increased with increasing body weight (Table A3). The PAC, and GOT and GPT activities in hepatopancreas and muscle also significantly increased with increasing body

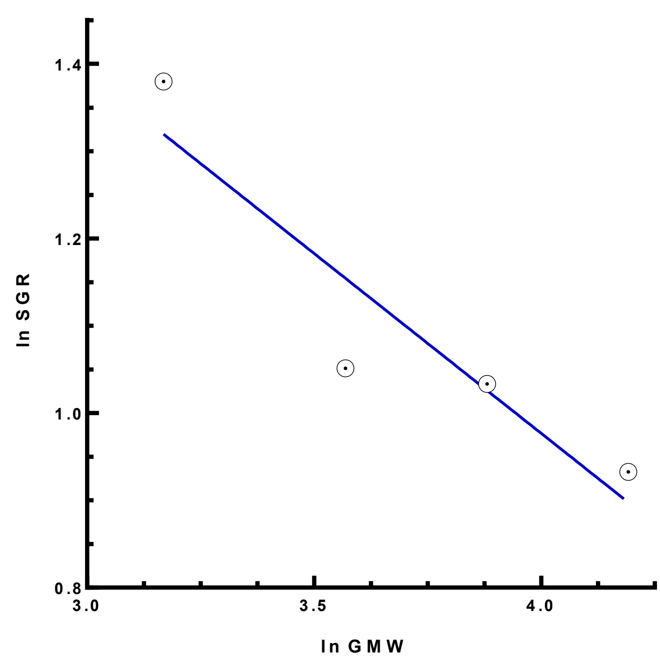

Fig. 1. Linear regression between ln SGR and lnGMW. SGR = specific growth rate; GMW = geometric mean weight.

weight (Table A2). The parameters from the natural logarithm of body moisture and fat contents, digestive enzymes and transaminase activities, and PAC (Y) vs. Wt (X) regressions ($Y = A + BX$) are present in Table 1 and Fig. 2.

4. Discussions

The fish growth is affected by environmental and endogenous factors. The endogenous factors like body weight are known to modify the fish growth rate (Jobling, 1983b). The growth rate of fish usually is determined using SGR in the nutritive study (Brown, 1957). The previous experiments have shown that the relationship between growth rate and fish weight can be described by the equation: $\ln SGR = A + B \ln W$, where W is fish weight in grams (Jobling, 1983a). The intercept (A) represents the ln SGR of a fish of unit weight (Jobling, 1983b). The values of A could be used to investigate the influence of changes in environmental variables, such as temperature and oxygen concentration, and species on growth rates (Jobling, 1983b). The present result firstly showed that the intercept value is 2.625 for Jian carp under the present condition. It is similar to the intercept value (2.367) of turbot *S. maximus* at 22°C (Árnason et al., 2009). These suggest that the ln SGR of two fish species of unit weight are close. The slope value (B) of the regression line relating ln SGR to ln W was −0.412 for Jian carp in the present, which is similar to that of other fish species (Jobling, 1983b). The slope of approximately −0.4 could be generally applicable in all species of fish (Jobling, 1983b). These slope value results indicated that the relative growth of weight is classified as showing negative allometry based on unit weight.

Fish growth is a consequence of proximate composition (such as moisture, lipid, protein, and ash) deposition. The previous study indicates relative water and lipid contents of individual whole fish are in relation to SGR (Holdway and Beamish, 1984). The present study firstly showed that relative water content varied inversely (slope = −0.039), but relative lipid content varied positively (slope = 2.668) with increasing weight in Jian carp. That the rate of fat content increasing is far greater than the rate of water reducing resulted in reduced. A similar trend was found in the Atlantic cod *Gadus morhua* L. (Holdway and Beamish, 1984). Nutrient deposition is the net result of nutrients anabolic and catabolic metabolism. Amino acids, except to playing as the building blocks of proteins, are important energy sources and provide 14 to 85% of the energy requirements of teleost fish (Ballantyne, 2001). Aspartate transaminase and GPT activities can be valuable indicators of the metabolic utilization of amino acids in fish (Jürss and Bastrop, 1995). The end product of nitrogen metabolism mainly is ammonia, which directly excrete into water by gill (Ballantyne, 2001). The present study revealed that AST activities in hepatopancreas and muscle and PAC increased with increasing body weight in the same way (slope = 0.324, 0.321, and 0.308, respectively). These data indicated that the AST in hepatopancreas and muscle maybe play an important role in the amino acid catabolism and ammonia production in Jian carp.

Fish growth is based on protein and fat deposition, which is closely related to the digestion and absorption of nutrients (Zhao et al., 2012). The digestive capacity of fish is governed by the activities of digestive enzymes and brush border enzymes in the intestine (Wu et al., 2011). In the current study, activities of trypsin, chymotrypsin, lipase, and amylase in intestine showed negative allometry against body mass. These results suggested digestive abilities of protein, fat, and starch gradually decreased with increasing unit weight in Jian carp. To our knowledge, exocrine pancreas is the main site for trypsin, chymotrypsin, lipase, and amylase synthesis and secretion in fish (Zambonino and Cahu, 2001). Therefore, the activities of lipase and amylase in hepatopancreas and intestine shared almost the same slopes (B = 0.474 and 0.466, and 0.306 and 0.307, respectively) in the present study. However, the slope of trypsin activity in intestine was six times higher than that in hepatopancreas. At the same time, the slope of trypsin activity in hepatopancreas was two times higher than that in intestine. The reasons may be related to the activation of trypsinogen and chymotrypsinogen in the intestine, but the exactly reasons need to be explored. The negative allometry increase of the digestive enzyme activities may be attributed to the development of hepatopancreas, which is reflected by the HPC (Zhang et al., 2013). In this study, the lnHPC showed negative allometry against body weight, which was similar to the previous result in the hepatopancreas mass of common carp *C. carpio* (Oikawa and Itazawa, 1984).

The nutrient absorption in fish intestine is related to brush border enzyme activities, such as CK, Na^+/K^+-ATPase, AKP, and γ-GT, which activities may reflect the fish intestinal absorption capacity (Chen et al., 2012). The activities of CK, Na^+/K^+-ATPase, AKP, and γ-GT in intestine and IPC showed negative allometry against body weight in the present study. The CK, Na^+/K^+-ATPase, AKP, and γ-GT are synthesized and secreted by cells of the intestine. The IPC can be sensitive to indicate the growth and development of intestine (Feng et al., 2013). The similar result has been reported in the intestine mass of common carp *C. carpio* (Oikawa and Itazawa, 1984).

5. Conclusions

The present study indicated digestive and absorptive functions of young Jian carp showed negative allometry against body weight. The activities of transaminase in hepatopancreas and muscle, PAC showed negative allometry against body weight, which indicated negative allometry of amino acid metabolism capacity. In conclusion, the body composition, digestive and absorptive enzymes activities, transaminase activities and PAC showed negative allometry

Table 1
Parameters from the natural logarithm of digestive enzymes and transaminase activities, and plasma ammonia concentration (Y) vs. Wt (X) regressions ($Y = A + BX$) in Fig. 2.

Parameters	n	A	B	R^2	P-value
Body moisture content	5	4.474	−0.039	0.735	0.040
Body fat content	5	−3.805	2.668	0.712	0.046
IPC	5	0.863	0.276	0.834	0.019
HPC	5	1.338	0.194	0.973	0.018
Hepatopancreas trypsin	5	0.319	0.145	0.932	0.005
Intestine trypsin	5	0.654	0.870	0.831	0.020
Hepatopancreas chymotrypsin	5	−2.606	1.208	0.993	0.000
Intestine chymotrypsin	5	−0.869	0.522	0.926	0.006
Hepatopancreas lipase	5	4.598	0.473	0.984	0.001
Intestine lipase	5	3.995	0.466	0.888	0.011
Hepatopancreas amylase	5	6.034	0.306	0.806	0.025
Intestine amylase	5	6.014	0.307	0.765	0.033
Intestine creatine kinase	5	2.341	0.358	0.884	0.011
Proximal intestine Na^+, K^+-ATPase	5	2.650	0.622	0.855	0.016
Mid intestine Na^+, K^+-ATPase	5	3.556	0.290	0.785	0.029
Distal intestine Na^+, K^+-ATPase	5	2.364	0.546	0.831	0.020
Proximal intestine AKP	5	−1.501	0.985	0.917	0.007
Mid intestine AKP	5	−1.021	0.822	1.000	0.000
Distal intestine AKP	5	−1.064	0.497	0.968	0.002
Proximal intestine γ-GT	5	1.279	0.417	0.949	0.003
Mid intestine γ-GT	5	1.340	0.509	0.986	0.000
Distal intestine γ-GT	5	3.186	0.119	0.979	0.001
Hepatopancreas AST	5	6.956	0.324	0.896	0.009
Hepatopancreas ALT	5	6.389	0.321	0.916	0.007
Muscle AST	5	5.134	0.704	0.756	0.035
Muscle ALT	5	5.256	0.538	0.706	0.047
Plasma ammonia content	5	6.371	0.308	0.938	0.004

IPC = intestine protein content; HPC = hepatopancreas protein content; AKP = alkaline phosphatase; γ-GT = γ-glutamyl-transpeptidase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

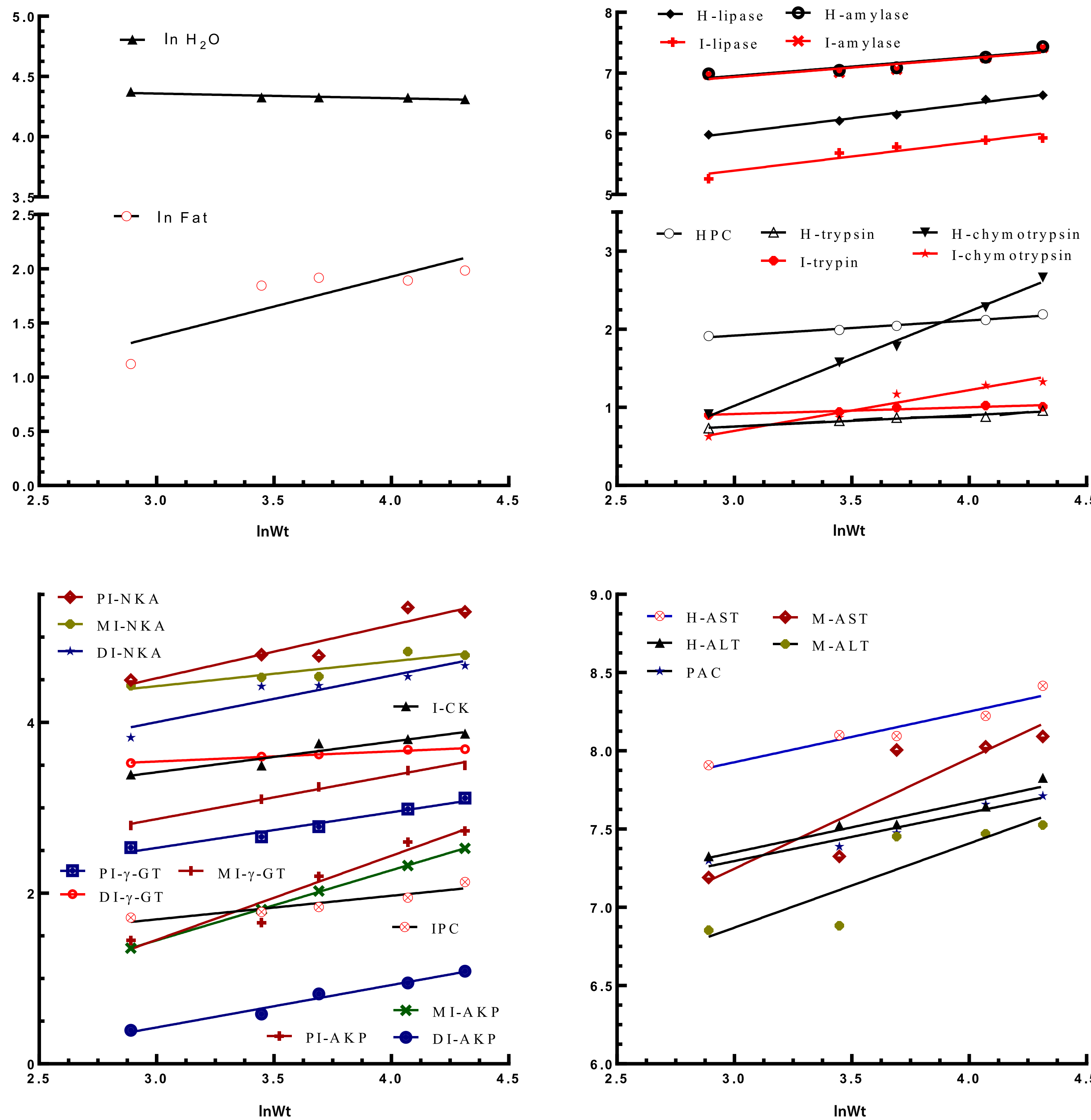

Fig. 2. Linear regressions between the natural logarithm of body moisture and fat contents, digestive enzymes and transaminase activities, and plasma ammonia concentration vs. weight (Wt). H = hepatopancreas; I = intestine; HPC = hepatopancreas protein content; PI = proximal intestine; NKA = Na^+, K^+-ATPase; MI = mid intestine; DI = distal intestine; CK = creatine kinase; γ-GT = γ-glutamyl-transpeptidase; IPC = intestine protein content; AKP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; PAC = plasma ammonia concentration.

against body weight of Jian carp, which were partial reasons to explain fish growth rate decreasing.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aninu.2015.12.006.

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