Aquaculture 364–365 (2012) 13–18

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of different weaning strategies on survival and growth in Chinese longsnout catfish (*Leiocassis longirostris* Günther) larvae

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ARTICLE INFO

Article history:
Received 5 December 2011
Received in revised form 21 April 2012
Accepted 24 April 2012
Available online 29 May 2012

Keywords:
Larvae
Weaning time
Weaning strategy
Digestive enzyme

ABSTRACT

The effects of different weaning strategies during the larval rearing of Chinese longsnout catfish were determined in two trials. In the first trial, the effect of abrupt-weaning from live prey (*Artemia* nauplii) to microdiet at 5, 6, 7, 8, 10 dph, respectively was investigated. The second trial examined the effect of weaning with co-feeding at different ages (6, 8 and 10 dph).

The survival, growth, digestive enzymes, coefficient of variation of final body weight (CV_{FBW}) and body length (CV_{BL}), digestive enzyme activities, fish body lysozyme and fish body glucose were significantly influenced by abrupt-introducing of microdiet ($P<0.05$). When weaning with live prey, only the fish body lysozyme significantly increased in the group introduced to microdiet on 8 and 10 dph ($P<0.05$).

The study showed that abrupt-weaning of Chinese longsnout catfish should be obtained after 10 dph. Co-feeding could reduce the stress to larvae and therefore the weaning could start at 6 dph with co-feeding.

1. Introduction

In fish larviculture, zooplankton and *Artemia* are generally provided at first feeding. But low production and high price have become the bottleneck for large scale culture (Person-Le Ruyet et al., 1993). Formulated microdiets could be the substitute of live food. But the use of formulated microdiets soon after hatching often leads to low survival and poor growth due to improper weaning age or weaning diet (Jones et al., 1993; Person-Le Ruyet et al., 1993; Watanabe and Kiron, 1994).

Low acceptance and low digestibility of the microdiet are also due to inadequate digestive enzyme development in fish larvae. Previous research found that the optimum initiation of feeding was species-specific and was primarily related to the development of the digestive system (Cahu and Zambonino Infante, 2001). Digestive enzyme activities are often used as indicators to judge the maturation of the digestive system and the nutritional status of fish larvae (Gawlicka et al., 2000; Hjelmeland et al., 1996; Oozeki and Bailey, 1995; Ueberschar, 1993). The occurrence time and the development of key enzymes also decide weaning success (Gawlicka et al., 2000; Moyano and Sarasquete, 1993). Improper weaning age or weaning diet could damage the digestive tract and lead to chronic stress with progressive starvation (Pickering, 1998), and delay or impair the development, health and physiological function of fish larvae (Cahu and Zambonino Infante, 1994; Fevolden and Røed, 1993; Fevolden et al., 1999; Hamza et al., 2007; Iwama et al., 2005; Wendelaar Bonga, 1997).

Co-feeding with live prey has been reported to help alleviate these problems and increase the success of early weaning to microdiet (Alves et al., 2006; Curnow et al., 2006a,b; Kestemont et al., 2007). Two reasons have been accepted while explaining this success: (1) unknown nutritional factors carried by live prey stimulate larval pancreatic secretions which then stimulate endocrine responses and are helpful to the maturation of the digestive process (Koven et al., 2001); (2) visual and chemical stimulation of live prey facilitates intake of microdiet subsequently affecting the growth of larvae (Cañavate and Fernández-Díaz, 1999; Kolkovski et al., 1997a,b; Rosenlund et al., 1997).

The ontogeny of digestive enzyme patterns in fish larval has been comprehensively studied to design adequate larval rearing and feeding strategies, and to formulate microdiets (Gawlicka et al., 2000; Lazo et al., 2007; Oozeki et al., 1995). The appearance of functional stomach as a final step of larval development and the acquisition of juvenile-like digestive characteristics were normally considered (Gawlicka et al., 2001). In our previous study, it was observed that Chinese longsnout catfish (*Leiocassis longirostris* Günther) larvae started first feeding at 5 dph and grew well with live prey. From 8 dph, the digestive enzymes such as pepsin, trypsin, amylase and lipase reached a plateau. The increasing values of pepsin and trypsin

Abbreviations: dph, days post hatching; CV_{BL}, coefficient of variation for final body length; CV_{FBW}, coefficient of variation for final body wet weigh; SGR, specific growth rate; FBW, final body weight; NF, not feeding; AT, *Artemia* nauplii; CO, 5% *Artemia* nauplii + 25% microdiet.

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doi:10.1016/j.aquaculture.2012.04.051

from 1 dph to a plateau (8–13 dph) seem to indicate that the stomach rather than the pancreas is in rapid development at 5–8 dph (Liu, 2011). In this stage, a progressive enzyme activity shift from alkaline to acid proteases mentioned by Lazo et al. (2007) occurred, and pepsin-like protease finally became the main digestive enzyme in the stomach development (Govoni et al., 1986; Pérez-Casanova et al., 2006; Zambonino Infante and Cahu, 2001).

Chinese longsnout catfish, *L. longirostris* Günther is one of the high value aquaculture species in China. However, high mortality is often encountered during weaning stage. Previous investigations are restricted to juveniles on rearing conditions such as ration level, fish size and water temperature (Han et al., 2004; Liu et al., 2008; Pei et al., 2004; Zhu et al., 2005). The weaning strategy with microdiet is still not confirmed and limits the large scale production.

Therefore, the present study was designed to investigate the effects of abrupt weaning to a microparticulate diet and co-feeding with live prey on survival, growth and how the microdiet would affect the digestive enzyme activities and physiological responses of Chinese longsnout catfish larvae.

2. Materials and methods

2.1. Diet preparation

Commercial powdered feed for juvenile soft shell turtles (Q/WHFL 01-2009, Coland Feed Co., Ltd., Wuhan, China) (crude protein: 50.4%; crude lipid: 6.2%) was used as microparticulate weaning feed. The powder was blended with water and stirred by hand, then sieved to obtain particles of 250–500 μm. The diet was then stored at −4 °C until further use.

2.2. Fish and feeding trial

Chinese longsnout catfish larvae 4 dph (6.2 mg) were obtained from the National Thoroughbred Farm for Chinese longsnout catfish, Shishou, Hubei, PR China and were transported to the laboratory at the Institute of Hydrobiology (Wuhan, China).

The two trials were conducted using the same batch of larvae obtained at 4 dph. The feeding regime is shown in Table 1. During the trials, the larvae were fed microdiet (MD) or *Artemia* nauplii. Newly hatched *Artemia* nauplii (eggs from Tianjin Red Sun Aquaculture Co., Ltd., China) contain about 58% crude protein and 19% crude lipid. The moisture of the microdiet and *Artemia* was determined daily.

During all the experiments, water temperature was measured twice daily (09:00 and 15:00). Total ammonia nitrogen and dissolved oxygen were monitored every 7 days and pH was detected daily. Photoperiod was 13 h:11 h with the light period from 08:00 to 21:00. Light intensity was about 260.25–301.35 lx. Flow-through systems were used in two trials. Tap water was treated by active carbon and zeolite and water flow into each tank was 500 mL/min. Total ammonia nitrogen (TAN) was less than 0.5 mg/L, and pH ranged between 7.0 and 7.5. Water temperature was 24.9 ± 0.15 °C in Trial 1 and 24.3 ± 0.41 °C in Trial 2. Dissolved oxygen (DO) was higher than 5 mg/L.

2.2.1. Trial 1: Abrupt weaning with microdiet

The larvae at 4 dph were randomly transferred into eighteen glass tanks (60 × 40 × 50 cm, water volume: 96 L) at a density of 250 larvae per tank. Six treatments were tested in triplicates. During the experiment, fish larvae were fed at 30% BW/d, three meals per day. The control group was fed with *Artemia* nauplii. Abrupt weaning groups were transitioned from *Artemia* to the artificial microdiet on 5, 6, 7, 8, and 10 dph, respectively. Experimental schedule is shown in Table 1.

2.2.2. Trial 2: Co-feeding with Artemia and artificial microdiet

4 dph larvae were randomly transferred into twelve fiberglass tanks (diameter: 50 cm, water depth: 40 cm, water volume: 80 L). In order to meet the requirement of step sampling, 500 larvae were reared in each tank. Larvae were fed at about 30% BW/d, three meals per day. Four treatments were tested in triplicates. Larvae were fed with *Artemia* nauplii as the control (CT) and co-feeding lasted for four days with 5% *Artemia* nauplii + 25% microdiet beginning on 6, 8, and 10 dph, respectively. Experimental schedule is shown in Table 1.

2.3. Sampling

At the beginning of the experiment, three samples (30 larvae/sample) were taken and batch-weighed to calculate average initial body weight. At the end of the experiment, larvae were counted and batch weighed to calculate survival and growth. In the abrupt weaning trial (Trial 1), 60 larvae per tank were sampled for analysis of digestive enzyme activities and whole fish body lysozyme and glucose. In the co-feeding trial (Trial 2), 100 larvae per tank were sampled at 8 and 10 dph while 60 larvae were sampled at the end of

Table 1
Feeding regime of three trials.

Larvae age	Trial 1						Trial 2			
	Control	5 dph	6 dph	7 dph	8 dph	10 dph	Control	T1	T2	T3
4 dph										
5 dph	AT	MD	AT	AT	AT	AT	AT	AT	AT	AT
6 dph	AT	MD	MD	AT	AT	AT	AT	CO	AT	AT
7 dph	AT	MD	MD	MD	AT	AT	AT	CO	AT	AT
8 dph	AT	MD	MD	MD	MD	AT	AT	CO	CO	AT
9 dph	AT	MD	MD	MD	MD	AT	AT	CO	CO	AT
10 dph	AT	MD	MD	MD	MD	MD	AT	MD	CO	CO
11 dph	AT	MD	MD	MD	MD	MD	AT	MD	CO	CO
12 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	CO
13 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	CO
14 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	MD
15 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	MD
16 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	MD
17 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	MD
18 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	MD
19 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	MD
20 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	MD
21 dph	AT	MD	MD	MD	MD	MD	AT	MD	MD	MD
22 dph	End	End	End	End	End	End	End	End	End	End

NF: no feeding; AT: *Artemia* nauplii; CO: 5% *Artemia* nauplii + 25% microdiet; MD: microdiet.

Table 2
Growth, CV of final body length and weight, and survival of Chinese longsnout catfish larvae at different sudden weaning times (mean ± S.E.).

Weaning time	FBW[1] (mg)	SGR[2] (%/d)	Survival[3] (%)	CV_{BL}[4]	CV_{FBW}[5]
Control	112.3 ± 2.32^{a}	18.0 ± 0.13^{a}	95.5 ± 1.19^{a}	0.07 ± 0.01^{a}	0.19 ± 0.02^{a}
5 dph	81.5 ± 0.44^{b}	16.0 ± 0.03^{b}	66.1 ± 2.15^{c}	0.15 ± 0.02^{b}	0.56 ± 0.12^{b}
6 dph	79.9 ± 3.70^{b}	15.9 ± 0.28^{b}	73.3 ± 8.42^{cb}	0.16 ± 0.05^{b}	0.38 ± 0.05^{ab}
7 dph	77.8 ± 2.16^{b}	15.8 ± 0.18^{b}	80.2 ± 4.11^{abc}	0.12 ± 0.02^{ab}	0.41 ± 0.07^{ab}
8 dph	78.2 ± 3.21^{b}	15.8 ± 0.26^{b}	79.5 ± 6.60^{cb}	0.12 ± 0.02^{ab}	0.38 ± 0.07^{ab}
10 dph	61.2 ± 4.62^{c}	14.2 ± 0.48^{c}	88.0 ± 1.22^{ab}	0.10 ± 0.01^{ab}	0.36 ± 0.04^{ab}

Values are mean ± S.E. Different superscript letters in the same row show significant differences between treatments ($n = 3$, $P<0.05$).
[1] FBW: final body weight (mg).
[2] SGR: specific growth rate (%/d) = 100 × [ln (final wet body weight) − ln (initial wet body weight)]/days.
[3] Survival rate (%) = 100 × live larvae left in the tank/initial larvae number.
[4] CV_{BL}: coefficient of variation for final body length.
[5] CV_{FBW}: coefficient of variation for final body wet weight.

the trial for analyzing digestive enzymes activities and whole fish body lysozyme and glucose. For calculating CV_{FBW} (coefficient of variation of final body weight) and CV_{BL} (coefficient of variation of final body length), 10 larvae per tank were sampled for measuring body wet weight and body length. Sampling was carried out in the morning (07:00–08:30) before feeding. The samples were frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.4. Analytical methods

Whole fish body homogenate was used for all analysis. Trypsin activity was measured using N-benzoyl-L-arginine ethyl ester (BAEE) as the substrate (Bergmeyer et al., 1974). Trypsin activity was expressed as the equivalent content per milligram of protein that made the absorbency change to 0.001 at pH 8.0. Amylase activity was measured using starch as the substrate (Métais and Bieth, 1968). Amylase-specific activity was expressed as the equivalent enzyme activity that was required to hydrolyze 1 mg of starch in 30 min at 37 °C. Lipase activity was determined photometrically (OD 420 nm) with p-nitrophenylpalmitate as substrate (Jaeger et al., 1992). One unit of activity was defined as the amount of enzymes necessary to liberate 1 μmol of p-nitrophenol per minute from p-nitrophenylpalmitate. Pepsin activity was determined at pH 2.0 using bovine hemoglobin as a substrate (Anson, 1938). Pepsin activity was expressed as specific activity with 1 U representing 1 μg tyrosine liberated per minute, per milligram of protein at 37 °C. Lysozyme was measured using *Micrococcus lysodeikticus* according to the modified method of Hultmark et al. (1980). Glucose content was determined using the glucose oxidase–peroxidase method (Barham and Trinder, 1972). Protein concentration was determined using the Bradford (1976) method with bovine serum albumin as the standard.

2.5. Statistical analysis

Statistica 6.0 for Windows was used for data analysis. Results are expressed as means ± SE (standard error). Homogeneity was tested (Levene's test) before ANOVA. After one-way analysis of variance (ANOVA), Duncan's multiple range test was used in Trial 1 and unequal LSD was used in Trial 2 to detect the differences of means between groups. Discrimination of values was identified at a significant level of $P<0.05$.

3. Results

3.1. Survival and growth performance

In the abrupt weaning trial, survival significantly increased with increasing weaning age ($P<0.05$, Table 2). Abrupt weaning on 7 and 10 dph resulted in no significant difference between the group and the control ($P>0.05$) (Table 2). Fish final body weight (FBW) and specific growth rate (SGR) significantly decreased while CV_{BL} and CV_{FBW} decreased with the increased weaning time ($P<0.05$) (Table 2).

In the co-feeding trial, no significant differences of survival were observed between groups ($P>0.05$) though the values increased with increasing weaning age (Table 3). No significant differences, but a slightly decreased SGR, CV_{BL} and CV_{FBW}, were observed with increasing weaning age ($P>0.05$) (Table 3).

3.2. Digestive enzymes, lysozyme and glucose

In the abrupt weaning trial, fish body pepsin activity decreased when weaned at 5–7 dph (Fig. 1A, $P<0.05$), while it had a close value to that of the control when weaned at 10 dph ($P>0.05$). Fish body trypsin activity increased by abrupt weaning ($P<0.05$) though

Table 3
Growth, CV of final body weight and length, and survival of Chinese longsnout catfish larvae with different weaning methods (mean ± S.E.).

Diet	FBW[1](mg)	SGR[2]	Survival (%)[3]	CV_{BL}[4]	CV_{FBW}[5]
CT (*Artemia*)	93.27 ± 16.36	16.80 ± 1.11	86.42 ± 0.58	0.36 ± 0.02	0.11 ± 0.01
T1 (4 days co-feeding from 6 dph)	83.26 ± 2.58	16.18 ± 0.19	80.82 ± 3.73	0.39 ± 0.04	0.13 ± 0.00
T2 (4 days co-feeding from 8 dph)	84.14 ± 2.36	16.25 ± 0.18	86.67 ± 0.00	0.33 ± 0.02	0.10 ± 0.01
T3 (4 days co-feeding from 10 dph)	73.78 ± 0.18	15.43 ± 0.02	89.50 ± 0.50	0.29 ± 0.03	0.08 ± 0.02

Values are mean ± S.E. ($n = 3$). Different superscript letters in the same row show significant differences between treatments ($n = 3$, $P<0.05$).
[1] FBW: final body weight (mg).
[2] SGR: specific growth rate (%/d) = 100 × [ln (final wet body weight) − ln (initial wet body weight)]/days.
[3] Survival (%) = 100 × live larvae left in the tank/initial larvae number.
[4] CV_{BL}: coefficient of variation of final body length.
[5] CV_{FBW}: coefficient of variation of final body wet weight.

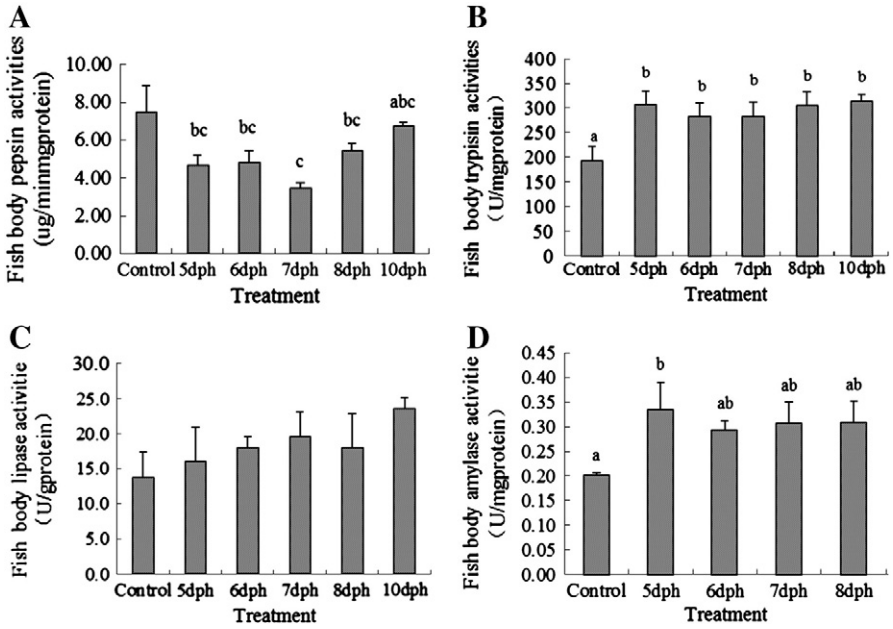

Fig. 1. Effects of different weaning age with abrupt-weaning to microdiet on fish body digestive enzyme activities in Chinese longsnout catfish larvae (mean ± S.E.). Different superscripts show significant differences between treatments ($P<0.05$). (A) Pepsin; (B) trypsin; (C) lipase; (D) amylase.

there was no difference among different weaning ages ($P>0.05$) (Fig. 1B). No significant differences were found in lipase activities of different treatments ($P>0.05$) (Fig. 1C). Abrupt-weaning caused increased amylase activities and the highest value was observed at 5 dph ($P<0.05$) (Fig. 1D). Abrupt weaning resulted in increased fish body lysozyme ($P<0.05$) which was however not significantly different among different weaning ages ($P>0.05$) (Fig. 3A). Fish body glucose showed slightly lower values after abrupt weaning, with significantly lower values ($P<0.05$) (Fig. 3C) obtained in the group weaned at 8 dph.

In the co-feeding trial, no significant differences in fish body pepsin, trypsin, lipase or amylase activities and glucose content were observed between groups ($P>0.05$) (Fig. 2A–D; Fig. 3D). Fish body lysozyme at 8 and 10 dph significantly increased in the group introduced to microdiet (Fig. 3B, $P<0.05$).

4. Discussion

Larvae survival is the most important parameter to justify the success of weaning. Increased survival with weaning age of Chinese longsnout catfish larvae as observed in Trial 1 of the present study might be related to a gradually progressive functioning of the stomach. The depressed activities of pepsin (Trial 1) indicated malnutrition in weaned groups. Those results are consistent with the studies of

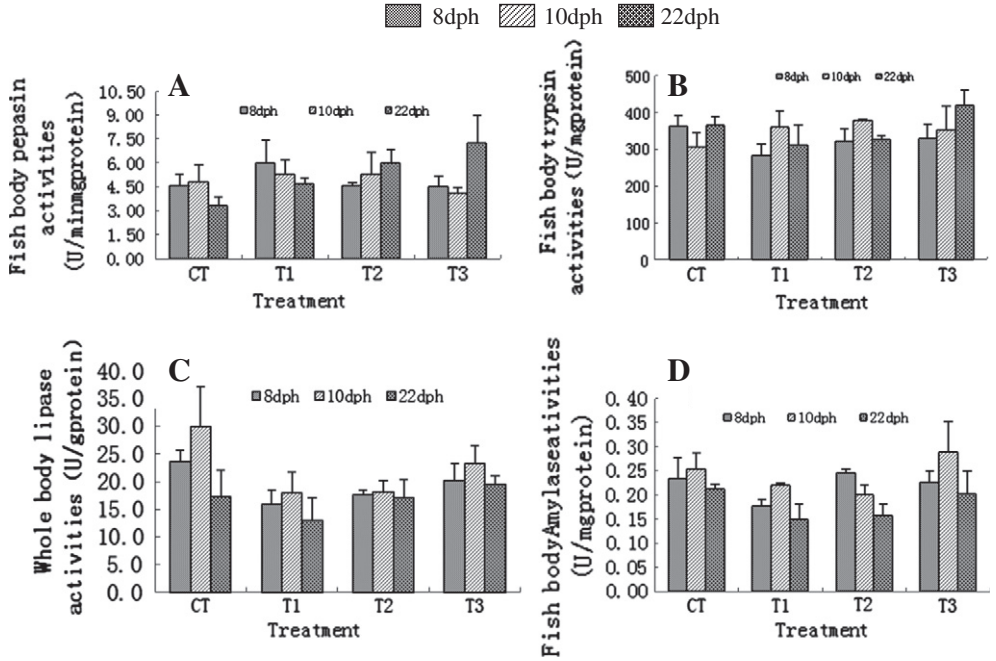

Fig. 2. Effect of different weaning age with co-feeding on fish body digestive enzyme activities in Chinese longsnout catfish larvae (mean ± S.E.). (A) Pepsin; (B) trypsin; (C) lipase; (D) amylase.

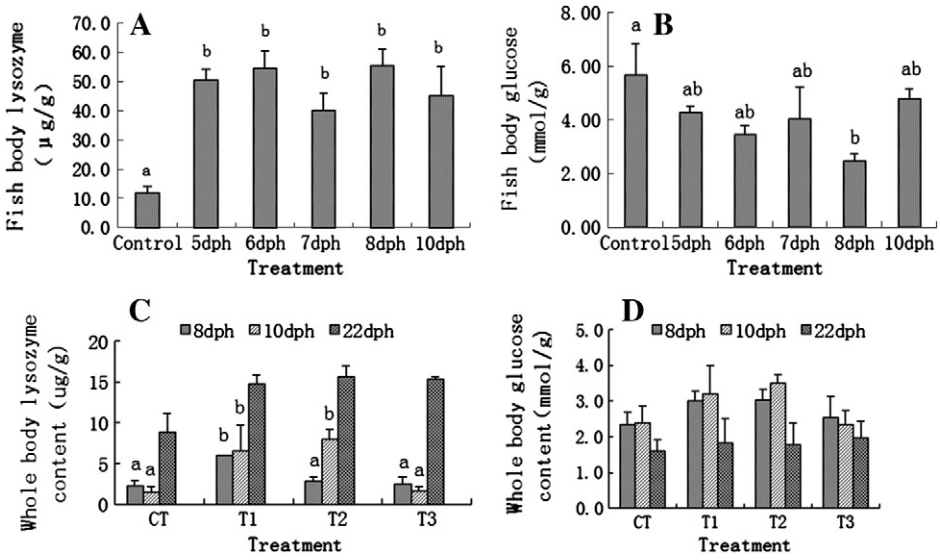

Fig. 3. Fish body lysozyme and glucose in Chinese longsnout catfish larvae subjected to different weaning strategies (mean ± S.E.). Different superscripts show significant differences between treatments ($P<0.05$). (A) Fish body lysozyme in abrupt-weaning; (B) fish body lysozyme in co-weaning; (C) fish body glucose in abrupt-weaning; (D) fish body glucose in co-weaning.

morphology and digestive enzyme activities during Chinese longsnout catfish larvae development in our previous study (Liu, 2011). During 5–8 dph, the stomach is not functional enough for artificial diets, and the digestive enzyme activities are in rapid increase (Liu, 2011). At this stage, an improper abrupt-weaning time and microdiet diets might delay the stomach development or impair epithelial cell as observed by Hamza et al. (2007). Formulated feed was reported to delay or even prevent the maturation process of fish larvae (Cahu and Zambonino Infante, 2001). Engrola et al. (2007) also found that fish larvae had an adaptation period to inert diets with perturbation of enzymatic secretion processes and this adaptation period is inversely proportional to post-larvae age. This may explain the facts that survival was lower until 7 dph and pepsin activity significantly decreased except for that in groups of 8 and 10 dph in Trial 2. The delayed development is prevented or repaired only until 8 dph.

On the other hand, trypsin and amylase activities in fish larvae fed with microdiet (Trial 1) significantly increased. The enhanced digestive enzymes after weaning are often considered as the result of compensatory adaptation for malnutrition (Hamza et al., 2007; Zambonino Infante and Cahu, 1994). In Trial 1, fish with higher digestive enzymes did not have good survival and growth. One possible reason could be that the absorption and assimilation capacities of fish larvae did not meet the growth requirement and caused chronic starvation (Ragyanszki, 1980). Fish larvae modulate their digestive capacity to compensate lower digestion caused by lower pepsin activity. Higher fish body lysozyme with lower fish body glucose (Trial 1) also supported the fact that the fish larvae were in sub-healthy state. Based on the improved fish body lysozymes in Trial 1 and Trial 2, it could be concluded that the introduction of microdiet was a clear stress, both in the abrupt-weaning and in the co-weaning, even though no apparent difference was observed at the end of Trial 2. This phenomenon was ameliorated when the larvae were weaned with live prey. The survival and growth in Trial 2 showed that Chinese longsnout catfish larvae could be weaned at 6 dph with 4 days of co-feeding. The data of four selected digestive enzymes (Trial 2) showed that co-weaning alleviated the malnutrition status that appeared during abrupt-weaning. These results are consistent with previous observations that co-feeding strategies can improve survival and growth performance of fish larvae even in early stages (Alves, et al., 2006; Baskerville-Bridges and Kling, 2000; Engrola et al., 2007). According to the present results, the activity of pepsin rather than other three digestive enzymes could be one of the key indicators to evaluate the weaning time and the nutritional status of Chinese longsnout catfish larvae. The fact that adequate timing of weaning was determined by stomach differentiation and pepsin secretion was also advocated by many authors (Person-Le Ruyet et al., 1993; Segner et al., 1993; Walford and Lam, 1993).

A lower survival rate was observed in the co-feeding experiment (Trial 2) compared with the control group of the sudden weaning trial (Trial 1), while the growth performance in both the two trials decreased with increasing survival rate. Similar results were observed in summer flounder *Paralichthys dentatus* (King et al., 2000) and matrinxã, *Brycon cephalus* (Characidae) (Gomes et al., 2000), and have been attributed to the differences in space shared by the larvae (Jobling and Wandsvik, 1983). Lower survival in Trial 2 could be due to the stress of step sampling on 8 and 10 dph as the stocking density was not a stress to longsnout catfish larvae culture. Our previous study showed that stocking density of 26.4 ind/L could be used with no detrimental effect on the survival of Chinese longsnout catfish (Liu, 2011).

CV_{BW} and CV_{BL} are also two important parameters used to evaluate the success of larviculture (Engrola et al., 2007; Rosenlund et al., 1997). In the present study, CV_{BW} and CV_{BL} were significantly improved by the abrupt change from *Artemia* to microdiet and the variation was reduced with weaning age. This could be due to the social hierarchy caused by differential ontogenesis and individual adaptability to the microdiet in the same batch of larvae (Jobling and Wandsvik, 1983; McCarthy et al., 1992). Co-feeding could thus improve the nutritional status of larvae to avoid this big variation. Similar results were reported in barramundi (*Lates calcarifer* Bloch) (Curnow et al., 2006a), fat snook (*Centropomus parallelus* Poey1864) (Alves et al., 2006), Senegalese sole (Engrola et al., 2007) and Dover sole (*Solea solea* L.) (Rueda-Jasso et al., 2005).

In conclusion, considering the values of pepsin activity, abrupt-weaning of Chinese longsnout catfish should be obtained after 10 dph. Co-feeding could reduce the stress to larvae and therefore the weaning could start at 6 dph with co-feeding.

Acknowledgments

Thanks are due to Guanghan Nie for the technical support. This study was funded by the National Key Basic Research Program (NKBRP) (2009CB118702) and partly by the innovation project of the Institute of Hydrobiology (IHB), Chinese Academy of Sciences

(CAS). Thanks should also be given to those anonymous reviewers for their helpful suggestions.

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