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Research article

INVESTIGATION OF SURVIVAL, GROWTH AND BIOCHEMICAL BLOOD PARAMETERS OF COMMON CARP (*CYPRINUS CARPIO* L.) LARVAE FED BY ARTIFICIAL DIETS

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ABSTRACT: Culture of fish larvae is one of the most sensitive stages in production of most fish species. The most important problem is providing suitable and high quality feed which is acceptable, digestible, causes lower mortality and higher growth. In this study three diets (SFK, industrial granules feed), (SFK+ Enzyme (Lipase, Pepsin, Trypsin)) and Gammarus were examined. Each experiment carried out in triplicate with duration of 30 days. The 2400 larvae of common carp with average weight of 5±0.5mg were used for each treatment. Biometry of fishes was conducted every 6 days and data of weight, length, food conversion ratio, specific growth rate and survival were recorded. At the end of the trial, blood sampling was conducted with incision of tail peduncle for analyzing of biochemical blood parameters. Results showed that the larvae fed with SFK+Enzyme had better and significant growth than others ($p<0.05$). Survival of larvae was greater than 70% in all treatment and was not any significant differences between treatments ($p<0.05$). Biochemical blood parameters showed that larvae fed with SFK+Enzyme or gammarus had higher and significant Glucose, Albumin and TP than fish fed only SFK ($p<0.05$). IGM and ALP contents were higher and significant in fish fed Gammarus than others ($p<0.05$). ALT, AST, C3, C4, CPK and BUN did not indicate any significant differences between treatments ($p>0.05$).
Keywords: larvae feeding, growth factors, biochemical blood parameters, Common carp.

INTRODUCTION

The Common carp (*Cyprinuscarpio*) belongs to the family *Cyprinidae* is one of the most important culture fish in the world and especially in Asia. Cyprinidae culture in recent decade has developed noticeably in different countries due to their extensive culture [17]. In fish larviculture, which is one of the most critical and sensitive stages in production cycle of various fish species, the most important things is preparation of easily accepted and adjusted high quality feed by fish larvae (kim,1996). In general the larval feed is the most expensive feed in aquaculture. Culture of fish at larval stage involved high mortality, due to numerous larval enemies in natural pond condition. Taking consideration the physiology of larvae, size of the moth, quality of food and food availability for larvae may reduce the mortality and increase the growth rate and resistant of larvae. Succeed in fish culture, needs to improving culture technique in larval stages, since studies about larval stages characteristics is very basic and important [2]. Undoubtly starter feeding diet is essential factor for successful larvae culture in various fish species and fish larvae such as common carp (*Cyprinuscarpio*) needs to specific diet for providing growth rate and suitable individual development [16]. Live feed almost provide the most necessary feed requirement of larvae. Among live feed, Gammarus belonged to Gamaridae family, contained more than 40% protein [8], and also contained high concentration of carotenoids with important roles in immune stimulation, reproduction and are used in larval stages or starter feeding. Actually the larvae contained sufficient contents of digestive enzymes for live feeds absorption. These contents are inadequate for artificial diets. Artificial diets contained proteins and other feeding elements and anti-nutritional factors which their absorptions are difficult for larvae [13]. One solution for decreasing this problem seems to be using different digestive enzymes. Effect of using enzyme, on absorption, assimilation and growth of *Gilthesdseabream* has been reported by [10]. Therefore using enzyme in intensive fish culture as feed supplementations has been used extensively by fish farmers to increases growth, survival and stimulation of exogenous enzymes for digestion and pre enzyme activity for nutrient absorption in fish.

MATERIAL AND METHODS

In this experiment, 7200 common carp larvae were prepared from local fish propagation center, Sari, Iran. Larvae with mean weight of 5±0.5 mg were cultured with three feeding diets (SFK, industrial granules feed), (SFK+ Enzyme (Lipase, Pepsin, Trypsin)) and Gammarus. Each experiment carried out in triplicate with duration of 30 days. After hatching the larvae were kept in 220 liter fiberglass zoger jar and were adapted to take the feed of milk powder until the yolk sac absorbed. The larvae were transferred to fiberglass tank of 2×2×0.3 m diameter, each contain 800 carp larvae. Mean water temperature during experiment was 22.3±1.4 °C, pH between 8.2±0.7, dissolved oxygen between 8.1±1.7 mg l^{-1}. Larvae fed six times daily and uneaten feeds and fecal removed by siphoning from the tanks once a day. Daily feed adjusted after larvae biometry (every 6 days) considering water temperature and determined 5% of fish body weight for next period. Larvae biometry was conducted every 6 days using measuring larvae length and weight on 20 larvae per treatment.

The weight and length of larvae, feed conversion ratio, specific growth rate and survival were determined according Ronyai et al. (1990) using following formula:

Feed conversion ratio (FCR) = F/(WF-WI) where:

F = consumed feed (mg), WF = final weight (mg), WI = initial weight (mg)

Specific growth rate (SGR) = LnWF-LnWI/(T×100) where:

LnWF = final weight in logarithm (mg), LnWI = initial weight in logarithm (mg), T = experiment periods (days)

Survival Rate (SR) = (larvae at the end of experiment-larvae at the beginning experiment star) ×100

At the end of the experiment, blood sampling was taken using caudal peduncle incision and samples were transformed to laboratory for determining biochemical blood parameters e.g. Glucose, Albomin, total protein (TP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Complement C3, Complement C4, Immunoglobulin (IgM), Alkaline phosphatase (ALP), Creatine phosphokinase (CPK), Blood urea nitrogen (BUN).

For statistical analyses, SPSS,15 software were used. Considering Shapiro-Wilk test and certificating normal distribution of data, one way ANOVA and Duncan were used for comparing means between different treatments at the significance level of 95%. Graphs were drawn using Excel software. Results showed with mean ± standard deviation and when $P<0.05$, differences were significant.

RESULTS

Common carp Growth performance and survival

Common carp weight and length results showed that larvae fed SFK+Enzyme had better and significant growth than larvae fed SFK and Gammarus ($p<0.05$). Weight of larvae in treatment 1 increased from 5.3±0.25 mg at starting to 570.2±0.21mg at final period of experiment. Also the length of fish changed from 5.27±0.21 mm to 30.30±0.25mm in this period. The larvae fed with Gammarus also had pronounced increase weight but lower than the two other treatments (Fig. 1 and 2).

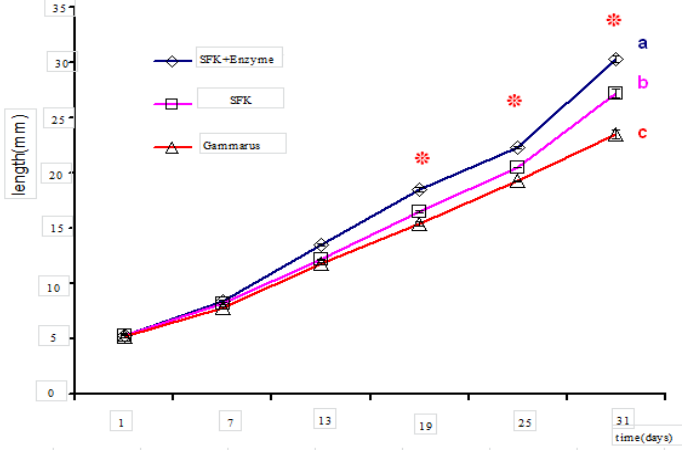

Figure1. Length of common carp larvae fed artificial diets.

*. Shows significant differences between SFK+ enzyme with the two other treatment.

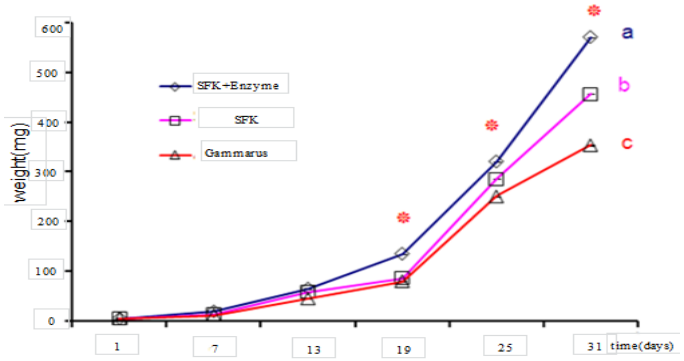

Figure2.weight of common carp larvae fed artificial diets.

*. Shows significant differences between SFK+ enzyme with the two other treatment.

Feed conversion ratio decreased by spending the time and feeding of the larvae. During experiment, the best feed conversion ratio observed in SFK+Enzyme had significant differences than treatments 2 and 3 ($p<0.05$). The highest feed conversion ratio was observed in larvae fed Gammarus which decreased significantly as time spent ($p<0.05$; figure3).

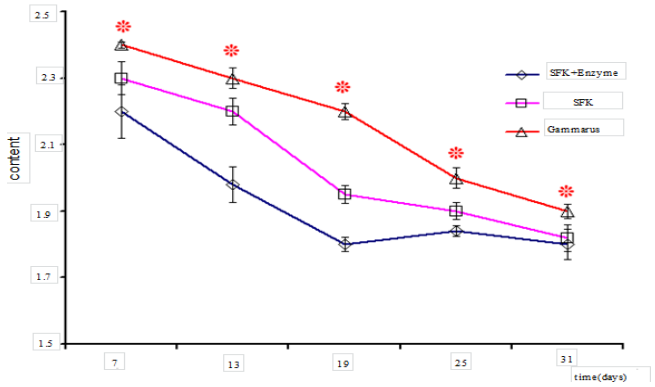

Figure3. FCR of common carp larvae fed artificial diets.

***. Shows significant differences between Gamarous feed with the two other treatment.**

Specific growth rate of larvae had significant decrease with spending time ($p<0.05$). The highest SGR was observed in larvae fed SFK+Enzyme while the lowest SGR was observed in larvae fed Gammarus (Fig. 4). Common carp larvae survival at the end of experiment showed that larvae fed SFK+Enzyme had better survival rate (more than 70%) than larvae fed SFK or Gammarus, however the difference was not significant ($P>0.05$).

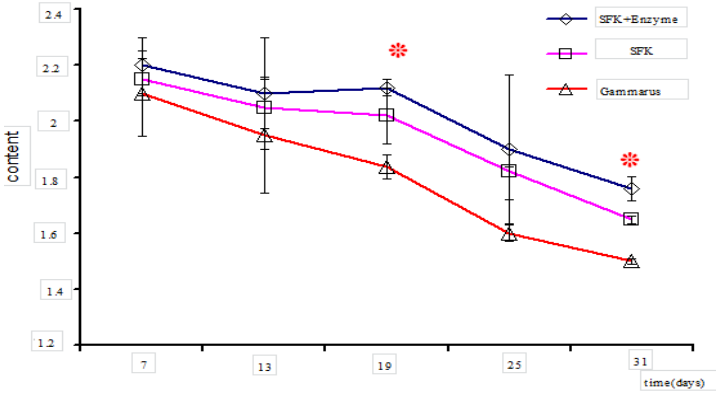

Figure4. SGR of common carp larvae fed artificial diets.

*. Shows significant differences between SFK+ enzyme with the two other treatment.

Analysis of biochemical blood parameters

Results showed that larvae fed SFK+Enzyme or Gammarus had higher and significant glucose, albumin and total protein than larvae fed SFK ($p<0.05$). Blood IGM and ALP contents in larvae fed Gammarus were higher and significant than other treatments ($p<0.05$). ALT, AST, C3, C4, CPK and BUN had not significant differences among treatments ($p>0.05$; Table 1).

Table1.biochemical blood parameters of common carp larvae fed artificial diets.

Blood parameters	SFK+Enzyme	SFK	Gammarus
Glucose(mg/dl)	5.2±0.7[a]	3.5±0.6[b]	6±0.8 [a]
Albumin(mg/dl)	0.5±0.4[a]	0.4±0.2 [b]	0.6±0.2[a]
TP(g/dl)	6.5±1.2[a]	4.8±2.3[b]	7.3±2.1[a]
ALT(IU/L)	17.5±3.8[a]	20±6.1[a]	20.2±4.3[a]
AST(IU/L)	44.7±7.3[a]	28.6±8.8[a]	35.5±9.2[a]
C3(mg/dl)	23.4±3.2[a]	19.7±2.7[a]	24.3±1.9[a]
C4(mg/dl)	14.2±1.9[a]	13.3±4.3[a]	18.9±3.7[a]
IgM(mg/dl)	106±9.1[b]	102±7.2[b]	144±6.3[a]
ALP(IU/ml)	89±4.3[b]	68±7.6[b]	110±6.4[a]
CPK(IU/L)	30.2±7.6[a]	28.1±8.4[a]	25.3±4.7[a]
BUN(mg/dl)	1.6±0.4[a]	1.8±0.3[a]	1.5±0.6[a]

DISCUSSION

Larval stages are very sensitive and important because at these stages, fish is passing from endogenous feeding to exogenous feeding and this issue is accompanied to development of organs related to digestive activity. This passing stage is a critical phase in larvae life [20].

At the end of the experiment at the present study, the best Length and weight, FCR and SGR were observed in larvae fed SFK+Enzyme which had significant differences with other treatments ($p<0.05$). Larvae fed Gammarus had lowest growth comparing to the others. Generally, fish in natural conditions, receive some of their essential elements such as carotenoids from their consumed food like algae, crustaceans and mollusks enriched with this substances however in cultivated environments this substances should added to diets as supplementation (Wozniak,1996). Gammarus contained carotenoid compounds and essential fatty acids such as EPA and DHA in their bodies. Carotenoids are not confined to muscle and skin pigmentations but also their presence in diet, increased absorption and assimilation of feed and resulted in growth improvement [19, 18, 5], however higher contents of Gammarus powder in present study caused lower growth. Actually Gammarus powder contained higher EFA and carotenoids than SFK but it does not fulfill the requirement of fish in protein, carbohydrate and fat of larvae, considering needs of Cyprinidae to more than 50 % proteins for optimum growth [15]. Using crustaceans like Gammarus in fish diets due to high level of Chitin, had adverse impact on digestibility and assimilation of macronutrients which resulted in decreasing fish growth [12]. Using pure Gammarus, due to increasing in fiber, ash and chitin in fish diet have adverse effect on digestibility of Macronutrients and other elements which are essential for optimum growth of rainbow trout growth [1]. In the other hand, in spite of high carotenoids and EFA content, Gammarous have relatively low protein content and other elements needed for optimum growth.

The SGR index clearly did not indicate the best feed for larvae. By the time the SGR decreased in all 3 treatments. This result is in agreement of those reported in Salmonids by [3]. The survival rate did not showed any significant differences between treatment however the larvae fed SFK+Enzyme had better survival rate (more than 70%) comparing the two other treatments. In artificial propagation and rearing of larvae the techniques is preparation of pond for natural live feed production. This technique is fully depending on environmental condition and sometimes there is failure of live feed production. In this case high mortality is expected in rearing of larvae. The present work showed we may use SFK diet as a starter feed for Cyprinid larvae cultivation. In using artificial feed some author recommended using commercial diet accompanied to live feeds, improved assimilation and digestion of artificial diets [11]. Using enzyme as supplemented in commercial feed showed the best effective diet in improving growth rate and survival rate as well. This result is agreement with [4]. They stated that using enzymes cause better assimilation in fish larvae.

At larval stage the common carp passing the carnivorous stage of their life, and eat only zooplankton, later common carp change to omnivorous stage of their life. Improved growth performance and feed efficiencies in enzyme supplemented feeds could somewhat due to decreasing adverse impact of plant ingredients of SFK. This statement also is in agreement with findings of [6] on Salmon and [7] on Rainbow trout.

Considering obtained results, it seems that because common carp is a resistant fish, immune parameters such as C3, C4 and IGM have changed at smallest amount with feed changes. Results of blood factors indicate positive effects of enzyme and Gammarus on blood biochemical parameters.

Larvae and fingerling production with high survival and economically benefits is one of the most important stages in cyprinid culture. Larvae feeding issue with artificial diets and achieving to optimum feed in the first stage of growth resulted in aquaculture development. The development and certainly resulted in better development in life cycle of cyprinid in aquaculture systems, technological development of cyprinid larvae and their culture until fingerling is essential for fattening culture. Considering high cost in production of live feeds, manufacturers of formulated diets should makes feeds which meetentire nutritional requirements of larvae. High survival of larvae fed artificial diets, it is a potential rearing of larvae in causes when earthen pond are not prepared or natural feed is not available.

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