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Growth, survival and immune responses of juvenile common carp *Cyprinus carpio* fed with diets enriched with α- tocopheryl acetate

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Abstract

Cyprinus carpio is a popular food fish species and is stocked regularly in reservoirs of Sri Lanka. However in semi-intensive aquaculture, growth and survival of this species are very low resulting in high production cost. The present study was carried out to determine the growth, survival and immune enhancement to pathogenic *Aeromonas hydrophyla* of juvenile common carp fed with tocopheryl acetate augmented formulated feed. Experimental set up included four treatments each with three replicates arranged in completely randomized design. Initial total weight of the experimental individuals ranged from 200 to 320 g. Experimental fish, which were stocked in 150 cm x 150 cm x 60 cm cement tanks were fed twice a day. The four treatments were; non-enriched commercial feed (T1), non-enriched formulated feed (T2), formulated feed enriched with Tocopheryl Acetate (TA) 2 mg (T3) and formulated ration enriched with TA 4 mg (T4). After the end of the feed trial, experimental animals were exposed to 5 ppm *A. hydrophila* concentration for 5 minutes duration. The highest Specific Growth Rate (SGR) (1.21) was observed in treatment T4, followed by Control treatment T1 (1.16), which were not significantly different from T3 (1.11). The lowest SGR (1.04) was recorded for T2. The highest (93%) and the lowest (69%) survival were observed in T4 and T3 respectively. The lowest Food Conversion Ratio (FCR) (1.61) was also observed in T4. In T3 and control, they were 1.93 and 1.96 respectively. The highest FCR was also observed in T2 (2.14). No mortality was recorded in T4 after exposing to bacteria concentration while it was 20% in T3. The highest percentage mortality (60%) was recorded from T2 followed by the control (40%). Results indicate that enrichment of prepared feed using Tocopheryl Acetate gives a good growth rate, good survival, hence reducing the production cost. Further, results indicate that Tocopheryl Acetate positively enhances the immunity of the experimental fishes to *Aeromonas* infection.

Keywords: *Cyprinus carpio*, immunity, *Aeromonas hydrophila*, enriched feed, α-tocopheryl acetate

Introduction

Common carp, *Cyprinus carpio* is a popular food fish, which is regularly stocked in inland reservoirs of Sri Lanka (Wijenayake et al. 2005). In the carp rearing ponds, fish are fed mainly on artificial feed. Increased usage of pelleted feeds in carp culture is a necessity to increase the production and fish quality. This is especially important when the human nutritional value of the carp flesh is intended to be increased (István et al. 2013) However, in semi-intensive aquaculture practices, the growth and survival of this species are very low due to bacterial infections resulting in high production cost. This has discouraged the small-scale farmers from culturing this fish species in ponds. As a result, the home gardens in rural areas in the country are not exploited to the maximum potential. Occurrence of bacterial diseases in fish fingerlings is a common problem encountered in hatcheries. *Aeromonas* sp. is one of the important pathogens that is responsible for bacterial diseases. *A. hydrophila* is the causative agent of the disease known as "haemorrhagic septicaemia", "ulcer disease" or "red-sore disease" (Swann and White 1989; Guz and Kozinska 2004). It is considered an opportunistic pathogen (Swann and White 1989) and always capable of producing disease if given the chance (Yildiz et al. 2005). This infection leads to high mortality of these fingerlings. In addition, the operational cost will also increase due to the cost involvement in purchasing drugs, increased labour and prolonged management.

Further, unavailability of high quality feed is a major issue in the industry affecting the small-scale fish farmers. Generally, nearly 60% of the total production cost is for fish feed. Commercial feeds available in the market sometimes do not contain all the required nutrients although the prices are very high. Preparing a low cost feed with essential nutrients using freely available ingredients is an urgent need for the development of the industry. In addition, enrichment of feed is also needed to provide extra nutrients to the target animal. α-Tocopheryl acetate is identified as a very good anti-oxidant that protects the membrane fatty acids of the tissue cells and acts as an immune enhancer. This can enhance the growth by protecting some fatty acids responsible for the well-being of an individual. Therefore the present study was conducted to produce a suitable diet enriched with α-Tocopheryl acetate to facilitate growth and survival of common carp juveniles.

Materials and Methods

Procurement of test animals

Healthy *C. carpio* fingerlings ranging in total weight from 200 to 320 g were obtained from the Freshwater Fish Breeding Centre at Udawalawe of the National Aquaculture Development Authority (NAqDA), Sri Lanka. They were subjected to acclimatization for two weeks at the National Aquatic Resources Research and Development Agency.

Experimental set up

Experimental set up included four treatments each with three replicates arranged in completely randomized design. The test individuals were stocked in 150 cm x 150 cm x 60 cm cement tanks and fed twice a day with experimental feeds listed in Table

1. The composition of the emulsions used for enrichment of formulated feed is given in Table 2. They were fed for four months at a daily ration of 5% body weight. After the end of the feeding trial, the experimental animals were separately exposed to 5 ppm *Aeromonas hydrophila* concentration for 5 minutes.

Table 1. The experimental feeds.

Feed	Description
T1	Commercial feed (control diet)
T2	Formulated feed non-enriched
T3	Formulated feed enriched with 2 mg α-Tocopheryl Acetate (TA) per 1 kg feed
T4	Formulated feed enriched with 4 mg α-Tocopheryl Acetate (TA) per 1 kg feed

Table 2. Composition of the emulsions used for the enrichment of formulated feed.

Composition	Emulsion for Feed 1	Emulsion for feed 2
Water	40 ml	40 ml
Egg yolk	50 ml	50 ml
Gelatin	5 g	5 g
α-TocopherylAcetate (TA)	2 mg	4 mg

Assessment of growth performance

Specific Growth Rate

Specific growth rate (SGR) of fish in each treatment was calculated by the following formula.

$$\mathrm{SGR}(\%) = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Time period}} \mathrm{x}100$$

Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) was calculated for each treatment by the following formula.

$$\mathrm{FCR} = \frac{\text{Weight of feed given during the experimental period)}}{\text{Wet body weight gain in the same period}}$$

Statistical Analysis

Statistical analysis were carried out using the SPSS statistical package (SPSS inc., Chicago, IL, USA). Results were presented as mean and standard error of mean. Difference among the control and treatment means were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's new multiple range tests. Differences were considered statistically significant when $p < 0.05$.

Results

The highest Specific Growth Rate (SGR) (1.21) was observed in treatment T4, followed by Control treatment T1 (1.16), which was not significantly different from the treatment T3 (1.11). The lowest SGR (1.04) was obtained for the treatment T2. The highest percentage survival (92.6) was in T4 while the lowest (68.5) was recorded in treatment T3. The lowest Food Conversion Ratio (FCR) (1.61) was also observed in treatment T4. In T3, it was 1.93 while in the control treatment it was 1.96. The highest FCR was observed in the treatment T2 (2.14) (Table 3).

No mortality was recorded in treatment T4 when they were exposed to bacteria concentration while it was 20% in treatment T3. The highest percentage mortality (60%) was recorded in treatment T2 followed by the control treatment (40%) (Table 4).

Table 3. Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Percentage Survival of the experimental animals fed with different feeds. In each column, values indicated with different superscripts are significantly different from each other ($p < 0.05$).

Treatment	SGR	FCR	% survival
T1 (control)	1.16^{b}	1.96^{b}	85.36
T2	1.04^{c}	2.14^{c}	87.69
T3	1.11^{b}	1.93^{b}	68.52
T4	1.21^{a}	1.61^{a}	92.59

Table 4. Percentage mortality of the experimental animals after exposing to *A. hydrophila* for 5 minutes.

Treatment	Number of animals used	Number of deaths after immunity test	% mortality
T1 (control)	10	04	40
T2	10	06	60
T3	10	02	20
T4	10	00	00

Discussion

Culture of *C. carpio* would be a useful means to upgrade the small-scale farmers' livelihood and to increase the per capita consumption of fish in Sri Lanka. Being an omnivorous fish, it is easy to use these species in extensive culture (i.e., culture-based fisheries) in the reservoirs of Sri Lanka especially in dry zone (Wijenayake et al. 2005). Aquaculture in home gardens and cage and pen–culture in shallow reservoirs can be popularized because captive breeding technology is well established in the

country at present. Fast growth, high survival and resistance to bacterial diseases make this species popular among fish farmers (FAO 2004-2015).

Formulated feed is an attractive alternative because it eliminates the need for costly and labor-intensive fresh feeds. In addition, formulated feeds are available throughout the year, conveniently stored, easy to use and their nutrient composition could be easily modified. In some cases, commercial products are not very rich in all nutritional qualities. Nutritive quality of fish feed is highly important, as growth is significantly correlated with it.

Results indicated that ingestion of 4 mg TA per kg feed enriched feed enhances the growth and survival than feeding with 2 mg per kg feed enriched feed. It is well known that TA acts as an anti-oxidant and also serves as a potent free radical scavenger (Lovell 1989). It helps preventing auto oxidation or peroxidation of poly-unsaturated fatty acids and proteins in the cell membranes (Dutta-Roy 1999). Further, TA is responsible to the development of immunity in some fish species (Montero et al. 1998). The lipid and fatty acid composition have been identified as major dietary factors that determine successful growth and survival of fingerlings (Coutteau et al. 1997). It is evident that in some fish varieties, highly unsaturated fatty acids (HUFA) in fish diets increase the early growth rates (Coutteau et al. 1997).

The highest SGR observed in treatment T4 in the present study indicates that there is a positive relationship between the growth of experimental fish and TA. The second better SGR was observed in the control treatment (T1), which is not significantly different from that of treatment T3. These results suggest that feed enrichment with TA is effective for the growth of juvenile common carps. In general, growth of animals vary with several factors such as the food availability, environmental factors, social hierarchy of the population and nutritional qualities of the food etc. In addition, the high level antioxidants in enriched diets support the lipid and/or fatty acid protection in fish body tissue (Cowey et al. 1983). Several researchers have observed that supplementation of TA affect the growth of fish (Cowey et al. 1983; Tocher et al. 2002; Dube and Trung 1993; He and Lawrence 1993). Supplementation of vitamin E to the diet increased survival and growth of sea bream (Tocher et al. 2002). Dube and Trung (1993) showed that supplementation of vitamin E in the diet of goldfish *Carassius auratus* influenced its growth at 50 mg vitamin E/100 g of diet. Positive effect of vitamin E on growth of *Renibacterium* sp. infected Chinook salmon (*Oncorhynchus tshawytscha*) was observed by Thorarinsson et al. (1994). Significant increase in the weight gain of *Penaeus vannamei* fed with vitamin E was reported by He and Lawrence (1993). Present results are in agreement with the findings of those workers. In some cases, it was evident that there was no any increase in the growth parameters when the animals fed with TA supplemented feed. For example, PUFA and vitamin E have not shown any significant effect on growth of Turbot (Stephan et al. 1995). Agradi et al. (1993) reported similar results in Sturgeon. Our results are not in agreement with their results. This may be due to difference in the composition and quality of the emulsion, enrichment time, location environmental factors, experimental design and duration of the experiment, age, digestibility and metabolic activity of the experimental animals and feed preparation method etc.

As the best percentage survival was also observed in treatment T4, the use of 4mg/1kg TA may be positively effective for them. However, the lowest survival was in animals fed with 2mg/1kg TA enriched feed. Kanazawa (1985) and He et al. (1992) reported that addition of vitamin E to diets resulted in improved survival of *Marsupenaeus japonicus* and *Lithopenaeus vannamei*. Dube and Trung (1993) reported positive influences on the survival of goldfish *Carassius auratus* fed with 50 mg vitamin E/100g of diet. Results of the present study are in agreement with them.

Best FCR was also observed in treatment T4 indicating that good enrichment practice would positively affect the juvenile common carps. According to Solan et al. (2011), incorporation of low concentration of vitamin E in to fish feed has positively improved the FCR in *Tilapia*. Parakrama et al. (2011) have shown that vitamin E enriched feed has increased FCR in the post larvae of freshwater prawn. Present results are in agreement with the above findings.

Present study showed that the immune response of juvenile common carp to *A. hydrophila*, the most disease causing agent to freshwater fishes in Sri Lanka, was high in treatment T4. It should be due to the proper concentration of the enrichment media. The second best immune response was found in treatment T3, where 2mg/1kg TA enriched feed was used. These results clearly indicate the immunity enhancement ability of the TA. Several authors have indicated that the use of TA has increased the immunity (Montero et al. 1998). Significant differences of phagocytic and lysozyme activities have been observed by Ardo *et al.* (2010) between the challenge resistant and sensitive individuals of common carp. According to Falco et al. (2012), a diet supplemented with β-glucan has reduced the gene expression levels of some inflammation-related cytokines in common carp against *A. salmonicida.* Mohamad and Abasali (2010) observed an enhanced immunity to *A. hydrophila* in common carp fed with plant extracts. In animals, the primary role of vitamin E is likely to be providing antioxidant protection for membrane-bound PUFAs (Montero et al. 1998). Vitamin E is the only major antioxidant located in the cell membrane. It provides additional health protection through its immune-stimulant property (Montero *et al*. 1998). Jeney and Jeney (2012) observed that parameters of non-specific immune response, level of specific and natural antibodies against *A. hydrophila*, phagocyte activity of leukocytes, plasma lysozyme activity and specific antibodies are higher in the resistant varieties of common carps than in the sensitive ones. The results of the present study are in accordance with their findings.

The fish fed with commercial feed showed a lower percentage of mortality than those fed with non-enriched formulated feed. This result further supports the immune enhancement ability of TA. It is well known that tocopheryl acetate is a good antioxidant and it has the ability of protecting the membrane fatty acids of the tissue cells (Dutta-Roy 1999). Also, it is a good free radical scavenger and is able to prevent the auto oxidation of fatty acids (Dutta-Roy 1999). It is also a fact that the HUFAs are responsible to the early growth and also the reproductive ability of freshwater fish species (Parakrama et al. 2011)

According to the present results, 4 mg /1kg TA dry feed concentration is more effective for protecting essential unsaturated fatty acids in the body tissue, while help to increase the growth, survival and the immune response to target bacteria.

Conclusion

It is evident that enrichment can be a good solution for enhancing the nutritive quality of feeds of juvenile common carp. Enrichment of prepared feed using Tocopheryl Acetate has resulted in high growth rate and survival of the juvenile common carp. This will reduce the culture period reducing the production cost. Further, as the experimental animals showed zero or low mortality, it appear that TA has enhanced development of immunity in fishes to *Aeromonas* infection.

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