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Original article

Effects of different levels of vitamin C and prolonged nursing on growth and innate immunity of Nile tilapia

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Introduction

Aquaculture largely depends on the availability of good quality desired sized seeds in time. Again, seed production is compelled by its highly seasonal nature in Asia where farmers want to stock seed at the beginning of the monsoon [1]. Therefore, nursing fries for prolonged periods or 'overwintering' to produce advanced fingerlings or juveniles for culture in the subsequent pre-monsoon has now become a common practice for carps. Typically, the practice involves over-stocking of fries with minimum amount of feed to pass the winter season and produce a 'stunted' population [2]. There are evidences of compensatory growth of 'stunted' fingerlings when subsequently released in culture pond [1].

Outbreak of disease is a another major potential limiting factor to aquaculture production and rapid increase in aquaculture has been coupled with intensification of the culture systems, which in turn makes fish more susceptible to stress and disease. Unfortunately, none of the methods presently practiced to control fish diseases is free from negative impacts considering time, cost, availability and environmental impacts. Thus the approach of reducing disease susceptibility by triggering the immune system through the application of feed additives getting more attention day by days.

Therefore, this study was conducted aiming to evaluate the effects of different levels of dietary vitamins C on growth performance and lysozyme activity of Nile tilapia and their response as overwintered and new-season fingerlings.

Materials and methods

Four semi-purified experimental diets each of which containing 40% dietary protein and supplemented with four different levels of vitamin C (0, 420, 840 and 4,200 mg kg^{-1} feed) were prepared (Table 1). The proximate compositions of diets (Table 1) were determined following the standard procedures for fish feed analysis [3].

Table 1. Common dietary profile of test diets for juvenile Nile tilapia

Ingredients (%)	
Soybean meal	40.0
Rice powder	6.0
Rice bran	15.0
Brown Fish meal	25.0
Feed oil	6.0
Vitamin C free vitamin mixture[1]	2.5
Mineral mixture[2]	2.5
Binder (Carboxymethyl cellulose)	3.0
Proximate composition (%)	
Crude Protein	40.4
Crude Lipid	10.2
Carbohydrate	26.5
Crude Ash	10.4
Moisture	12.5

Prolonged stunted Nile tilapia juveniles (PJ) were prepared by collecting from the Nikko River in Aichi prefecture, Japan in November, 2015; kept in 100 L tanks and fed at 2% fish biomass day^{-1}. New-season juveniles (NJ) were collected from the same place in May, 2016 and acclimatized for one month.

Four (04) PJ (marked by fin clipping) and three (03) NJ were randomly distributed into each of twelve aquaria (45 L) at three replicates per treatment and reared for four weeks. At the end of the feeding trial, kidney lysozyme activity was determined using the turbidimetric assay system [4]. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 per min. Data were analyzed by two-way analysis of variance (ANOVA) using the software SPSS vers. 16.

Results and discussion

Fish Growth

During the experimental period, no mortality had occurred. The PJ and NJ showed similar trend in growth in effects of vitamin C (Fig. 1) and significantly higher weight gain ($p<0.05$) had been achieved in fish group fed 420 mg kg^{-1} vitamin C which is also the ideal

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requirement for Nile tilapia [5].

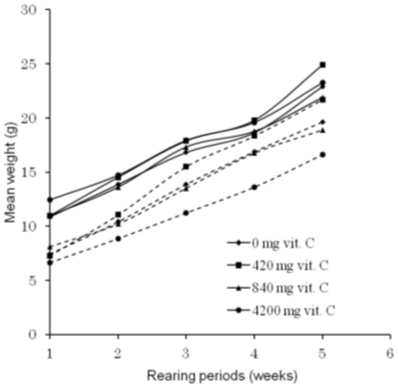

Fig.1. Growth response of Nile tilapia supplemented with different dietary levels of vitamin C; solid lines and fragmented lines represent prolonged nursed and new-season juveniles, respectively.

Compensatory growth of overwintered fingerlings (PJ) was not observed and nursing duration did not show any significant effects (p>0.05) on growth performances; might be due to the energy loss associated with courtship behavior observed during the study. Similar findings had also been reported by others [2]. Although no newly hatched fry was found in the aquaria but well developed ovary with advanced staged eggs were observed only in overwintering females (PJ).

Lysozyme activity

Vitamin C supplementation had no significant effect (p>0.05) on kidney lysozyme activity of both the prolonged stunted juvenile (PJ) and new-season juvenile (NJ) (Table 2). Although vitamin C is a well known antioxidant but published articles on the benefits of excess supplementation of this vitamin at levels higher than the requirement on innate immunity are inconsistent. Many authors reported the stimulating effects of vitamin C [6] while some reported no significant effects [7].

Lysozyme activity differs significantly and higher activity (p<0.05) was found in new-season fish (Table 2). In case of PJ, female individuals always showed higher lysozyme activity than Male counterparts (Fig. 2) and the difference is significant (p<0.05). The immune response of animals differs with sex and published reports are also available suggesting the sexual difference of immunity in fish, but the results are inconsistent and varied with fish species.

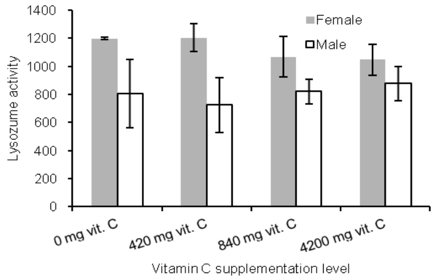

Fig. 2. Kidney lysozyme activity (unitsmin^{-1}mL^{-1}) of male and female Prolonged nursed juveniles (Mean ± SD).

Table 2. Final mean weight (FMW), weight gain (WG), specific growth rate (SGR) and kidney lysozyme activity (unitsmin^{-1}ml^{-1}) of prolonged nursed (PJ) and new-season juveniles (NJ) fed different levels of vitamin C diets for a period of four weeks (Mean ± sd)

Nursing duration	Vitamin C (mg kg^{-1})	FMW (g)	WG (g)	SGR (%)*	Lysozyme activity
PJ	0	22.90±1.77	12.04±1.66	2.66	1002±264.4
	420	24.90±2.89	13.93±2.68	2.91	964±297.20
	840	21.90±1.29	10.95±0.87	2.47	944±172.70
	4200	23.26±0.23	10.82±0.11	2.24	962±139.60
NJ	0	19.64±1.95	12.20±0.96	3.48	1055±138.28
	420	21.70±2.86	14.42±1.87	3.97	1116±75.28
	840	18.80±1.30	10.76±2.34	3.03	1142±320.58
	4200	16.62±2.25	10.00±2.50	3.28	1232±216.60
Vitamin C effect (*p* level)			0.012	0.063	0.897
	0		12.12^{ab}	3.07	1028.5
	420		14.16^{a}	3.44	1040
	840		10.86^{b}	2.75	1043
	4200		10.41^{b}	2.76	1097
Prolonged nursing effect (*P* level)			0.90	<0.05	0.026
PJ			11.94	2.57^{b}	968^{b}
NJ			11.86	3.44^{a}	1136.25^{a}
Vitamin C X Prolonged nursing			0.93	0.77	0.725

*SGR was calculated using the formula: SGR = {ln (final weight) – ln (initial weight)} X 100/ Rearing periods (in days).
**Values with different superscripts are significantly different (*p < 0.05*).

Conclusions

The present study demonstrated that Prolonged nursing practice might be suitable to get larger sized fingerlings during high demand (pre-monsoon) and supplementation of excess vitamin C more than requirement had no positive effects considering the growth and kidney lysozyme activity of Nile tilapia.

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