Effects of different levels of vitamin C and prolonged nursing on growth and innate immunity of Nile tilapia, *Oreochromis niloticus*

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Abstract. The study investigated the effects of different levels of dietary vitamin C on growth and lysozyme activity of Nile tilapia (*Oreochromis niloticus*) in relation to nursing duration (short duration, new-season fingerlings (NF) and stunted overwintered fingerlings (OF)) and sexes difference. Semi-purified diets (40% protein) supplemented with four different levels vitamin C (0, 420, 840 and 4,200 mg kg^{-1}) were prepared and fed to triplicate groups of tilapias (mean weight: 7.37 g and 11.31 g for NF and OF, respectively) for four weeks. Kidneys from the sacrificed fishes were sampled to determine the lysozyme activity following turbidimetric assay system. On growth performances, vitamin C had a significant effect ($p<0.05$) and the highest growth had been achieved in fish fed 420 mg kg^{-1} vitamin C, while prolonged nursing had no significant effect ($p>0.05$) and compensatory growth of OF was not achieved which might be due to energy loss associated with breeding activity of OF. Supplementation of vitamin C had no significant effect on kidney lysozyme activity of both NF and OF, although NF showed little increased lysozyme activity with increasing the dietary concentration of vitamin C. Nursing duration negatively affects kidney lysozyme activity ($p<0.05$) and the activity significantly differs with sexes, female individuals showed higher activity compared with male counterparts. Although prolonged nursing of carp fry is a common practice in sub-tropics to achieve faster growth in subsequent culture period but this study revealed that more research needed to make a conclusion on the benefits of overwintering process in terms of growth for *O. niloticus* and over dosages of vitamin C had no clear positive effects considering the growth and kidney lysozyme activity.
Key Words: *O. niloticus*, overwintering, turbidimetric assay, sexes, lysozyme activity.

Introduction. Aquaculture, one of the fastest-growing animal food-producing sectors is being constrained by the availability of good quality desired sized seeds in time, especially for most farmers practice semi-intensive culture system in Asia where seasonal temperature variation is prominent. Meeting the fish seed demand is compelled by its highly seasonal nature in most areas of Asia where farmers want to buy and stock seed at the beginning of the monsoon (Dan & Little 2000). However, unfortunately fish seed production is limited during this time as because of seasonal low temperatures which inhibit spawning of fish prior to the monsoon season (Little et al 2003). Therefore, nursing fries for prolonged periods to produce advanced fingerlings/juveniles or 'overwintering' has now become a common and well established practice in South Asian countries for carps. Typically, the practice involves over-stocking of fries in 'hapas' (small net structure installed in food fish production pond) or earthen ponds and allowing them small amount of food throughout the winter season; resulting a growth 'stunted' populations (Noakes & Balon 1982; Lorenzen 2000; Little et al 2003) of the fingerlings. There are evidences of compensatory growth of these 'stunted' fingerlings when subsequently released in culture pond (Derun & Yakupitiyage 1998; Wangpen 1996; Hossain 1995; Dan & Little 2000). Therefore, prolonged nursing of late-spawned tilapia fries is now getting more attention to the farmers of Asian countries to ensure the availability of fingerlings early in the following grow-out season.

Another potential limiting factor to aquaculture production is outbreak of diseases (Jansen et al 2012) and in fact, infectious diseases are by far the biggest killer of farmed fishes; an outbreak can often wipe out entire stocks, requiring costly decontamination of the associated facilities and equipment (Pillay & Kutty 2005). Rapid increase in aquaculture production has been coupled with intensification of the culture systems i.e., increasing the stocking density, which in turn makes fish more susceptible to stress and disease that finally might cause severe losses of the stock (Schreck 1996). In order to reduce or control fish disease, commonly any of the four basic measures is frequently practiced - treatment with drugs; culling of diseased fish and replacing with new stocks after disinfection; vaccination of stock or genetic selection to improve disease resistance (Sarder et al 2001). Unfortunately, none of the above-mentioned methods are free from negative impacts considering time, cost, mode of administration, availability and environmental concern. Therefore, the approach of reducing disease susceptibility of fish by triggering their own immune system through application of feed additives getting more attention day by days.

For protection against pathogen and preservation of internal homoeostasis, like other vertebrates, fish also posses a variety of bio-defence mechanisms. Fish defense mechanism or immune system is usually divided into two parts i.e., innate immunity that recognizes invading microbes by germline-encoded molecules and adaptive immunity where recognition depends on molecules generated by somatic mechanisms during the ontogeny of each individual organism (Medzhitov & Janeway 1997). Due to its earliest evolution, fish mostly depends on non-specific or innate immunity and also its immune system is less well developed as well as studied (Saurabh & Sahoo 2008). Innate immunity essentially serves as the fish's first line of defense against invasion of pathogens where lysozyme plays vital role against pathogenic attacks (Ingram 1980).

Lysozyme (muramidase, EC 3.2.1.17) is a mucolytic enzyme of leucocytic origin which splits the β (1→4) linkages between N-acetylmuramic acid and N-acetylglucosamine in the cell walls (peptidoglycan layers) of gram-positive bacteria (Saurabh & Sahoo 2008). Lysozyme cannot directly attack on gram-negative bacteria, but when the outer cell wall of gram-negative bacteria is disrupted by the action of complement and other enzymes exposing the inner peptidoglycan layer, then lysozyme becomes effective. Besides this function, lysozyme also promotes phagocytosis by directly activating polymorphonuclear leucocytes and macrophages or indirectly by an opsoninic effect (Saurabh & Sahoo 2008). Lysozymes have been isolated and characterized from a number of fish species including salmonids (Lie et al 1989) and presumed to perform substantial antibacterial activity over mammalian lysozyme; not only against gram-positive bacteria but also against gram-negative bacteria in the absence of complement (Saurabh & Sahoo 2008).

Vitamin C (L-ascorbic acid) is a water-soluble, essential nutrient for sound growth of tilapia (Shiau & Lin 2006) and also influences the immune system of animals, including fish (Lim et al 2010). Vitamin C provides cellular defense against reactive oxygen and has also been reported to stimulate immune responses in fish (Li & Lovell 1985; Navarre & Halver 1989; Hardie et al 1993; Verlhac et al 1993; Waagbø et al 1993; Verlhac et al 1996; Ortuno et al 1999).

Although, reports on the effect of vitamin C on disease resistance of fish are not consistent (Lim et al 2010), vitamin C might be a good candidate as feed supplement to increase the innate immune response of finfish including tilapia. 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, *Oreochromis niloticus* and their response as overwintered and new-season fingerlings.

Material and Method

Diet preparation. 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 for the present feeding trial (Table 1). The diets were prepared using a processing machine having 1.5 mm die and dried at room temperature until the moisture level reached below 20%, and were subsequently stored at -20^{o}C (until fed) in airtight polyethylene bags. The proximate composition of diets (Table 1) were determined following the standard procedures for fish feed analysis (AOAC 1995); briefly, analyzing moisture content by drying the sample at 105°C for 24 h, ash by complete combustion of feed sample in muffle furnace at 550°C for 6 h, crude protein (N×6.25) by micro Kjeldahl method, lipid by chloroform-methanol mixture method and carbohydrate was computed by applying the formula [100- (Crude protein + Crude lipid + Crude ash + Moisture)].

Table 1

Common dietary profile of four test diets for juvenile *Oreochromis niloticus*

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

[1]The vitamin mix, diluted in cellulose, provided the following in mg kg^{-1} diet according to the requirement of *O. niloticus* (Shiau & Lin 2006): vitamin A 1.8; vitamin D3 0.025; vitamin E 25; vitamin K 5.2; thiamine 2.5; riboflavin 6; pyridoxine 16; pantothenic acid 10; niacin 121; folic acid 0.82; vitamin B12 0.024; biotin, 0.06; choline chloride, 1000.
[2]Trace mineral premix provided by following minerals (mg kg^{-1} diet): zinc (as $ZnSO_4 \cdot 7H_2O$), 150; iron (as $FeSO_4 \cdot 7H_2O$), 40; manganese (as $MnSO_4 \cdot 7H_2O$), 25; copper (as $CuCl_2$), 3; iodine (as KI), 5; cobalt (as $CoCl_2 \cdot 6H_2O$), 0.05; selenium (as Na_2SeO_3), 0.09.

Experimental fish. Overwintered *O. niloticus* fingerlings were prepared by collecting local stock of *O. niloticus* fries from a small tributary of the Nikko River in Aichi prefecture, Japan in early November, 2015. Fries were transported using plastic jar equipped with mobile aerator. After two weeks of acclimatization, the fries (initial weight 3.12±0.11 g) were kept in rectangular plastic tanks (capacity 100 L) at a stocking density of 2,000 m^{-3} and fed a commercial fish feed (40% protein) at a rate of 2% fish biomass day^{-1} daily when water temperature exceeded 14°C. Survival rate of fry after overwintering was 82% and the fingerlings were size graded before the onset of rearing experiment. New-season *O. niloticus* fingerlings were collected from the same place in May, 2016 and kept in plastic tank for a period of one month for acclimatization and fed the same commercial feed as for overwintered fish but at a rate of 10% fish biomass day^{-1}.

Rearing of overwintered and new-season fingerlings. Before starting the rearing experiment, the fingerlings were size graded to obtain similar sized fish, and then seven fingerlings (four overwintered + three new-season) were randomly distributed into each of twelve rectangular polycarbonate aquaria (each equipped with aerator, pump and filter; capacity: 45 L) at three replicates per treatment. The stocking density was determined after Richter et al (2003). The mean initial weights of overwintered and new-season fingerlings were 11.31 and 7.37 g, respectively. In each tank, overwintered fingerlings were marked by clipping a small portion of their pelvic fins. Fish were hand-fed to apparent satiation twice daily (between 09:30-10:30 and 16:00-17:00 h) and reared for a period of four weeks. Photoperiod was maintained as 12 h light and 12 h dark by fluorescent lights.

Individual fish weight (accuracy: ±0.01 g, using SP-401 balance) and total length (with digital slide calipers, accuracy 0.01 mm) were taken weekly after anaesthetizing them in 450 ppm 2-phenoxyethanol water. Everyday excreta and other wastes were siphoned and approximately one third of the aquaria's water was exchanged and once a week the aquaria were scrubbed and the filters were replaced by the new ones. Fish were fed only in the afternoon on cleaning days. Water quality was monitored every week for total phosphorus (TP), ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) using the HACH portable data logging colorimeter DR-850 (HACH Co. Ltd, USA) and dissolved oxygen level (DO) and pH were measured by Lutron DO-5509 (Lutron Electronic Enterprise Co.; Ltd., Taiwan) meter and HACH HQ40d model (HACH Co. Ltd, USA), respectively.

Lysozyme assay. At the end of the feeding trial, kidney was taken from the sacrificed fish to determine the lysozyme activity using the turbidimetric assay system according to Parry et al (1965) with little modifications. Briefly, after taking the weight of the kidney, acetate buffer (0.5 M acetic acid + 0.5 M sodium acetate; pH = 5) was added five times of the tissue's weight and were homogenated. After homogenization, the tissue suspension was centrifuged at 4000 rpm for 15 min and the supernatant was collected. 140 μ*l* Micrococcus solution [1 mg mL^{-1} lyophilized *Micrococcus lysodeikticus* in 0.05 M sodium phosphate buffer (pH = 6.2)] was mixed with 10 μL tissue homogenates. The reduction in absorbance (delta A) was recorded at 450 nm (0 min.) and again after 30 min. incubation at room temperature (20^{o}C). One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 per min. and the formula used to calculate the activity: Δ A X 1000 $min^{-1}mL^{-1}$ (Lie et al 1989).

Statistical analysis*.* Data were analyzed by two-way analysis of variance (ANOVA) using the general linear model (GLM) to test the effects of dietary levels of vitamin C, nursing duration and their interactions. If there was a significant F-test, subsequent comparisons of treatment means were performed using the Duncan's Multiple Range test. Differences were considered significant at the 0.05 probability level. All analyses were performed using the software SPSS vers. 16 (IBM®, New York, USA).

Results

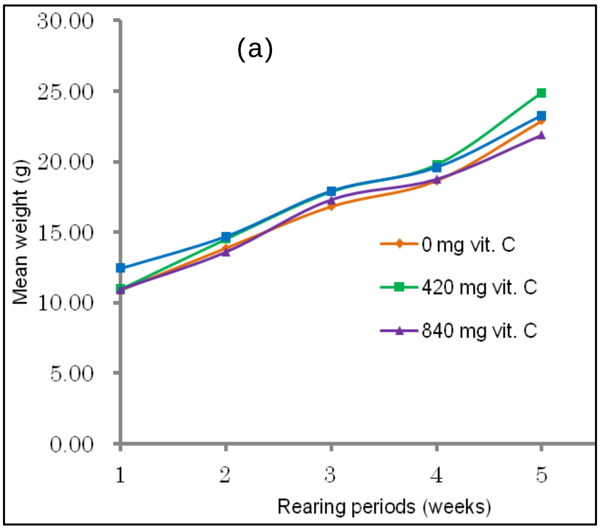
Water quality parameters. The physico-chemical parameters of the rearing tank water (Table 2) during the experimental period were always kept within the suitable range. DO values in the rearing system were always within the range of DO necessary for fish culture and never went below 6.00 ppm. pH value was also within the suitable range. Other important water quality parameters which are the results of fish's excretion and left-over feed, such as NH_3-N, NO_2-N and NO_3-N values were also within the range (Table 2). With the progress of the experiment, the total phosphorus (TP) level in aquaria water increased slightly might be due to gradual increment of feed delivered.

Table 2

Water quality parameters of aquaria in which *Oreochromis niloticus* were reared during the experimental period

Parameters	*Number of samples taken*	*Mean of parameter*	*Range of parameter*	*Suitable range for O. niloticus culture (DeLong et al 2009)*
Temperature (^{0}C)	35	29.5	24.5–33.0	25-32
DO (mg L^{-1})	48	7.30	7.2–7.4	>3.50
pH (mg L^{-1})	48	7.33	7.2–7.5	6.0–9.0
NH_3-N (mg L^{-1})	48	0.090	0.08–0.10	<1.00
NO_2-N (mg L^{-1})	48	0.012	0.007–0.018	<5.00
NO_3-N (mg L^{-1})	48	2.26	1.20–2.90	<300
Phosphorous (mg L^{-1})	48	0.453	0.34–0.58	
Turbidity (FAU)	48	4.25	2.00–7.00	

Growth of overwintered and new–season fingerlings. During the experimental period, no mortality had occurred. Both the overwintered and new-season fingerlings of Nile tilapia showed an exponential growth. The overwintered and new-season fingerlings showed similar trend in growth in effects of vitamin C and the highest weight gain had been achieved in vitamin C level 420 mg kg^{-1} (in both types of fingerlings) which were significantly different ($p<0.05$) (Figure 1).



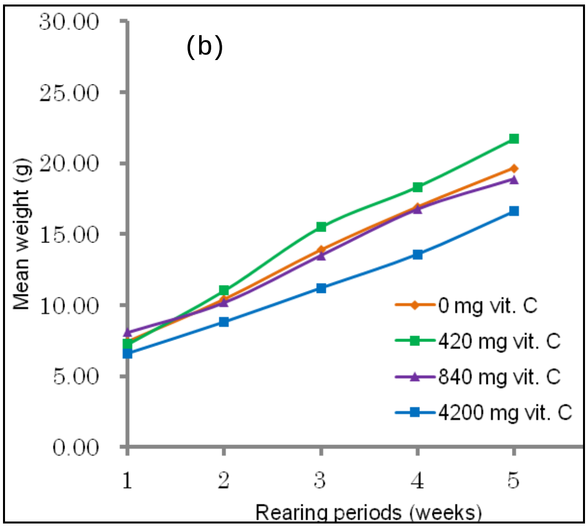


Figure 1. Growth response of *Oreochromis niloticus* supplemented with different dietary levels of vitamin C; (a) - overwintered fingerlings; (b) -new-season fingerlings.

Compensatory growth of overwintered fingerlings was not observed in this study and nursing duration did not show any significant effects on growth performances ($p>0.05$). New-season fingerlings performed better than overwintered fingerlings in 420 mg kg^{-1} and the condition is reversed in case of over dosages of vitamin C (Table 3).

Table 3

Final mean weight (FMW), weight gain (WG), specific growth rate (SGR) and kidney lysozyme activity (units min^{-1} mL^{-1}) of overwintered (OF) and new-season (NF) fingerlings fed different levels of vitamin C diets over a period of four weeks (Mean ± SD)

Nursing duration	*Vitamin C levels (mg kg^{-1} diet)*	*FMW (g)*	*WG (g)*	*SGR (%)**	*Lysozyme activity*
OF	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±297.2
	840	21.90±1.29	10.95±0.87	2.47	944±172.7
	4200	23.26±0.23	10.82±0.11	2.24	962±139.6
NF	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
Overwintering effect (*P* level)			0.90	<0.05	0.026
OF			11.94	2.57^{b}	968^{b}
NF			11.86	3.44^{a}	1136.25^{a}
Vitamin C X Overwintering			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 ($p<0.05$).

Sexual maturation of fish. No newly hatched fry was found in the experimental aquaria, however after dissected, well developed ovary with advanced staged eggs were observed in overwintering females whereas in case of new-season fish the ovary were very thin with nearly no detectable eggs when observed with unaided eyes.

Lysozyme activity. Kidney lysozyme activity were measured and compared among the treatments. Vitamin C supplementation had no significant effect ($p>0.05$) on kidney lysozyme activity of both the overwintered and new-season fish; although lysozyme activity of new-season fish increased linearly with the supplementation of vitamin C (Table 3). Lysozyme activity differs significantly ($p<0.05$) between the overwintered and new-season fish where higher activity was found in new-season fish. In case of overwintered fish, female individuals always showed higher lysozyme activity than Male counterparts (Figure 2) and the difference is significant ($p<0.05$).

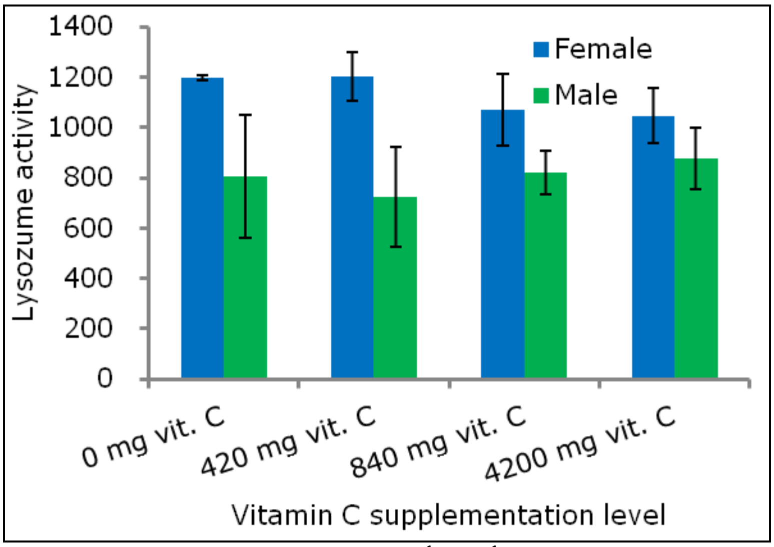


Figure 2. Kidney lysozyme activity (units min^{-1} mL^{-1}) of male and female overwintered fish (Mean ± SD).

Discussion. Availability of quality seeds in-time is the first priority to flourish aquaculture production and in any change in technology has to consider impacts on both the juvenile and adult stages of cultured food fish. Obviously, any change or adjustment that benefits the seed producer through improved survival and/or growth also influences the production during culture period (Little et al 2003). In subtropical regions growth of many aquatic organisms including different fish species is retarded due to seasonal dropping of temperature to below critical limits. Such a period of retarded growth or stunting condition is mainly due to physiology of fish (poikilotherm) along with a shortage of availability of food organisms; and this phenomenon has been reported for many freshwater fish species under both natural and cultured condition (Hossain et al 2003). It has been reported that growth stunting is a phenotypic change generating from unfavorable environmental conditions, such as over-crowding and limited food availability (Noakes & Balon 1982; Bjornsson et al 1988). Many authors reported that overwintering or juveniles of fish whose growth has been retarded under controlled conditions can subsequently compensate growth when they released in suitable environmental conditions with adequate food supply, especially for carps (Hossain 1995; Hossain et al 2003).

In comparison, very few studies have been conducted using overwintered tilapia and results were not consistent (Dan & Little 2000; Little et al 2003). However, the range of observed survival rate (82%) and growth during overwintering were within the expected range which is similar to the findings of others (Sodsook 1989; Wangpen 1996). No mortality was observed in grow-out aquaria, which was achieved due to larger size of fingerlings at stocking and completely controlled environment of rearing. Water quality was always maintained within the suitable range of *O. niloticus*, as reported by several authors for tank culture of *O. niloticus* (DeLong et al 2009; Caldini et al 2011). The most critical chemical parameter in tank culture is dissolved oxygen (DO) and pH and these values were always kept within range.

During grow-out phase, compensatory growth of overwintered fingerlings was not achieved; rather new-season fingerlings performed better than overwintered ones although the difference was not significant ($p>0.05$). Little et al (2003) also reported comparatively lower growth of overwintered fingerlings (six months nursing) than new-season fingerlings (two months nursing) in a 168-day grow-out period which was mainly attributed due to the fact of breeding i.e., new progeny were found in the mixed-sex populations and the overwintered fingerlings spend a significant amount of energy for courtship and breeding. Although no progeny was found in this trial but a conspicuous amount of courtship behavior observed among the overwintered fish and all the female individuals found with ovaries full of well developed matured oocytes. Therefore, overwintered fish had to spend some portion of its energy for their gonadal development and subsequent courtship behavior in comparison with new-season fish. Prolonged nursing of seeds to get larger sized fingerlings present both opportunities and constraints and survival rate of seeds is the key criteria for successful seed production and thus in aquaculture. The present study showed that *O. niloticus* fries could be nursed for prolonged periods (up to seven months) without any negative effects on survival and the major advantage of overwintered fingerlings is the availability of comparatively larger sized fingerlings in the early monsoon when most farmers wish to stock their ponds and which ultimately benefits polyculture of fish.

Growth performance against different levels of vitamin C showed no significant effect on both overwintered and new-season fingerlings ($p>0.05$). The highest growth had been achieved in fingerlings (both overwintered and new-season) fed with 420 mg vitamin C kg^{-1} feed which was the ideal level of vitamin C requirement for the sound growth of *O. niloticus* (Shiau & Lin 2006). Over dosages of vitamin C (840 mg kg^{-1} and 4,200 mg kg^{-1}) affects more in new-season fish than overwintered fish although the difference is not significant. This might be due to the fact that overwintered fingerlings were much more strong and resistant to environmental fluctuations than new-season ones.

Singly or in combinations with other antioxidant, vitamin C perhaps is the most extensively studied vitamin for its influence on immunity and disease resistance of different fish species (Hardie et al 1993; Waagbø et al 1993; Verlhac & Gabaudan 1994; Wahli et al 1998; Ortuno et al 1999; Ortuno et al 2001; Sealey & Gatlin 2002). Many authors reported gross deficiency syndrome (Lim & Webster 2006) as well as the immunosuppressive effects of *O. niloticus* (lower red blood cell count and hemoglobin; higher mean corpuscular volume) fed unsupplemented vitamin C (Lim et al 2010) and reports are also available on the stimulating effects of vitamin C on lysozyme activity of various fish species (Waagbø et al 1993; Roberts et al 1995; Verlhac et al 1996; Sahoo & Mukherjee 2003); but unfortunately, published articles on the benefits of excess supplementation of this vitamin at levels higher than the requirement on innate immunity are inconsistent and often varied depending on species of fish. In this study, no gross deficiency syndrome (phenotypic abnormality) was observed in fish fed unsupplemented vitamin C diet might be due to the fact of presence of little amount of vitamin C coming from the ingredients (not determined) coupled with the short duration of the experimental periods.

Kidney lysozyme activity was measured in this study to evaluate the innate immunity as because levels of lysozyme are much higher in fish kidney than in the blood (Lindsay 1986). Regarding kidney lysozyme activity, Vitamin C supplementation had no significant effect ($p>0.05$) in both the overwintered and new-season fish. The result of the current study is comparable to the findings of Lim et al (2010), who reported significantly lower lysozyme activity and higher total immunoglobulin of *O. niloticus* fed diets supplemented with 2,000 mg kg^{-1} vitamin C. Lysozyme activity of new-season fish increased linearly with the supplementation of vitamin C indicating the quick response new-season fish. Roberts et al (1995) observed increased serum lysozyme activity in turbot with increasing concentrations of dietary vitamin C on day 71 but on day 127, however, there were no significant differences in activity among different treatments. No effect on phagocytosis was observed in channel catfish fed megadose levels (3,000 mg kg^{-1}) of vitamin C but significantly enhanced antibody production and serum complement activity had been achieved (Li & Lovell 1985). In case of fish, lysozyme has been identified in monocytes and neutrophils (Murray & Fletcher 1976) with activity related to some degree with the fluctuations in the number of these cells (Muona & Soivio 1992). Therefore, it is possible that variation in the proportion of various leukocytes might exist in different groups of tilapias. In the present study, since no significant difference was found in lysozyme activity among the different groups of fish fed different levels of vitamin C; therefore, blood leukocyte count or other innate immune parameters could have been checked in future study to get a concise conclusion on innate immunity at high supplemental levels of vitamin C.

Lysozyme activity differs significantly ($p<0.05$) between the overwintered and new-season fish where higher activity was found in new-season fish. In carps, the highest level of lysozyme activity occurred in spawners (Studnicka et al 1986) but in case of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*), the activity markedly decreased during smoltification (Muona & Soivio 1992). It has been reported that sexually matured salmonid fish appears to favor parasitic infestation and infection than immature fish (Pickering & Christie 1980; Richards & Pickering 1978) and might be associated with the production of mucus which contain non-specific molecules like lysozyme (Fletcher & Grant 1968; Fletcher & White 1973); these results indirectly proved the low level of lysozyme in matured fish compared with young ones as observed in the present study. Although requiring further research for an explanation of the results of the present study, it could be inferred that the lysozyme production in overwintered fish was in some way impaired compared with new-season fish.

However, in case of overwintered fish, female individuals always showed significantly higher lysozyme activity than male counterparts ($p<0.05$). The immune response of animals differs with sex and which is more prominent in higher vertebrates. Although published reports are also available suggesting the sexual difference of immunity in fish, but the results are not consistent and varied with fish species and the types of pathogen. Serum lysozyme activity of lumpfish (*Cyclopterus lumpus*) was found to be higher in male than in female (Fletcher et al 1977) and again, Grinde et al (1988) reported that there were no significant differences in kidney lysozyme activity between males and females of rainbow trout (*Oncorhynchus mykiss*), and no significant correlation exists with weight of the fish. On the other hand, some authors reported that sexually mature male fish (brown trout) were more prone to infestation (Pickering & Christie 1980) or infection than were mature female fish and the patterns of infection differ between the two sexes (White 1975; Richards & Pickering 1978) indicating comparatively weak resistance of male than female; which also supports the findings of the present study. Although the reason behind the difference of innate immunity (with sexes) is not fully understood but it seems possible that steroid-induced immunosuppression may be important (Pickering & Christie 1980) which in turn associated with sex-chromosome of fish. If the amount of corticosteroids is related to immunosuppression of fish then it is interesting that available reports suggesting the female fish have higher levels of circulating corticosteroids than have the males (Schmidt & Idler 1962; Donaldson & Fagerlund 1970; Fuller et al 1974); whereas from the present study it would appear that the male fish have lower lysozyme. Therefore, more future work is needed to measure the levels of circulating hormones with particular attention being paid to the distinction between innate immunity changes.

Conclusions. The present study demonstrated that overwintering practice might be a suitable solution to get larger sized fingerlings early in the culture period (monsoon) and supplementation of ovcerdosages of vitamin C had no clear positive effects considering the growth and kidney lysozyme activity of *O. niloticus*.

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