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Effect of α-tocopherol supplementation on growth performance, antioxidant activity and nutrient digestibility of *Labeo rohita* (Hamilton 1822) fingerlings fed corn gluten meal-based diet

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ABSTRACT

A 70 days feeding trial was conducted to determine the effects of **α**-tocopherol (vitamin E) on the growth performance, antioxidant activity and nutrient digestibility of *Labeo rohita* (Hamilton 1822) fingerlings fed corn gluten meal based diet. Fingerlings (initial average weight: 6.35 g) were fed seven graded levels of **α**-tocopherol *viz.,* 0, 100, 200, 300, 400, 500 and 600 mg kg^{-1}. Triplicate tanks were used and each tank housed 15 fingerlings. Fish were fed at the rate of 5% of live wet weight. Collected data was subjected to one-way analysis of variance (ANOVA). Results showed that fingerlings fed with 200 mg kg^{-1} of **α**-tocopherol showed significantly ($p<0.05$) higher weight gain (32.73 g), weight gain% (261%) and suitable feed conversion ratio (2.49). Among other experimental and control diets, optimum apparent digestibility coefficient (ADC%) of crude protein (CP) (73%), ether extract (EE) (74%) and gross energy (GE) (63%) was noted in the fish fed 200 mg kg^{-1} diet. Minimum oxidation (%) (7.66%) was observed at 600 mg kg^{-1} predicting that the antioxidant activity increased in a dose-dependent manner.

Keywords: α-tocopherol, Antioxidant activity, Aquaculture, Corn gluten meal, Growth

Introduction

Animal protein is an essential part of human food and fish has high protein and low amount of fats which is valuable for human health. Conventional source of protein for fish farming is fish meal (FM), as it has well balanced amino acid and fatty acid profile, has excellent protein content, appetising taste and absorb well in the fish gut (Wang *et al*., 2019). But presently, the FM supply is facing decline and since long its production supports only negligible yield (Bai *et al*., 2019). To combat this imbalance between fish meal production and its demand, scientists are exploring alternative plant protein sources for fish (Teves and Ragaza, 2016). It has become a matter of interest globally for aquaculturists and fish nutritionists over more than two decades (Bai *et al*., 2019).

Corn gluten meal (CGM) is produced as a side-product of corn wet grinding procedure used for the dissociation of protein, starch, gums and fiber components (Wang *et al*., 2016). It is advantageous as compared to other plant meals because of steady and local supply, high protein content within range of 60-70% (dry matter), less amount of anti-nutritional factors, small content of fibres and particularly cost-effective (Glencross, 2016). Because of its high protein value, CGM is commonly used in place of other plants or animal-based proteins such as fish meal and soybean meal (Cha *et al*., 2000). Various studies confirm the successful replacement of FM with CGM at different inclusion levels illustrating more than 50% substitution without decrease in growth performance in carnivorous fishes like seabream, cobia and Japanese seabass (Yigit *et al*., 2012; Luo *et al*., 2013 and Men *et al*., 2014). Molina-Poveda *et al*. (2015) described that 100% replacement of FM with CGM resulted in reduced (30%) growth rate of white shrimp, while only 20-40% inclusion level provided good results.

Labeo rohita is a cyprinid, also called "rohu" or "rui", is the most economical fish among other Indian major carps (IMCs) with its rapid growth rate, nutritional value and high consumer demand (Mohapatra *et al.*, 2012). It is a column feeder and omnivorous in nature and can easily be raised in fish polyculture systems (Goswami *et al*., 2020). It is a highly preferred fish species and according to FAO (2012), over 35% of the total carp production was contributed by this species since previous decade. Vitamin E belongs to the group of fat-soluble molecules; exists in the form of four tocopherols and four tocotrienols; of which highest vitamin E biopotency is present in α-tocopherol (NRC, 2011). It is a valuable antioxidant, benefits the animal by saving cellular membranes, lipids and lipoproteins from free radical based damage (Bender, 2003).

Generally, vitamin E maintains immunity (Salinthone *et al*., 2013; Zhou *et al*., 2013), saves cell membrane from peroxide damage (Li *et al*., 2014), ameliorates resistance to high stocking density induced stress (Liu *et al*., 2014) and improves tissue composition in fish like black seabream (Peng *et al*., 2009), turbot and gilthead seabream (Tocher *et al*., 2002). Supplemented form of vitamin E in fish diet is α-tocopherol acetate, as it confers higher stability and oxidation resistance even during feed processing events (Peng *et al*., 2009). *α*-tocopherol shows positive effects on antioxidant activity and lipid peroxidation of grass carp and turbot respectively (Li *et al*., 2014; Jia *et al*., 2017). A number of studies reported that *α*-tocopherol is required for fish and terrestrial animals because its function is to improve growth performance, nutrient digestibility, reproductive performance and disease resistance (Lee *et al*., 2003). However, over dosage of *α*-tocopherol in certain fish species may show various abnormal behaviours (Zhang *et al*., 2016). For instance, Wang *et al*. (2015) found that 300 mg kg^{-1} inclusion level of *α*-tocopherol in fish diet worked as a pro-oxidant; in spite of antioxidant. So, proper dietary requirement of the fish should be well known before its supplementation in the diet. Therefore, the main objective of this study was to evaluate the potential of *α*-tocopherol on growth performance, anti-oxidant activity and nutrient digestibility of *L. rohita* fingerlings fed corn gluten meal based diets.

Materials and methods

The experiment was carried out in the Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad, Pakistan.

Fish acclimatisation and culture conditions

L. rohita fingerlings were brought from Govt. Fish Seed Hatchery, Satiana Road, Faisalabad. Fingerlings were stocked in V-shaped tanks having 70 l capacity (especially made for faeces collection) and acclimated to laboratory conditions for 15 days (Rowland and Ingram, 1991). Fish were fed basal diet once a day to apparent satiation (Allan and Rowland, 1992). Prior to the start of the experiment, *L. rohita* fingerlings were bathed in saline solution (NaCl 5 g l^{-1}) so as to free the fish from fungal infection and ectoparasites. On a daily basis, dissolved oxygen, temperature and pH were checked by DO meter (Jenway 970), thermometer and pH meter (Jenway 3510) respectively. Aeration (24 h) was provided by capillary system to all the experimental tanks.

Feed ingredients and experimental diets

All the feed constituents were ground to pass through 0.5 mm sieve and the ground ingredients were mixed in an electric mixer for 5 min, while fish oil was added slowly. To make an appropriate dough, 10-15% water was added (Lovell, 1989). Ingredients composition of the test diets is given in Table 1. The dough was processed in a pelleting machine to make feed pellets. Corn gluten meal-based diet was supplemented with different levels of *α*-tocopherol at 100, 200, 300, 400, 500 and 600 mg kg^{-1}.

Feeding protocol

Rohu fingerlings were fed at 5% of their body weight, two times a day, for a period of 70 days. Tanks in triplicate were set for each experimental diet and 15 fingerlings were kept in each tank. After the feeding period of 2 h, the unconsumed diet was drained out from each tank by opening the valves of the tanks. Faeces were collected through faecal collecting tube of each tank. To minimise leakage of nutrients, care was taken to evade the breaking of thin faecal strings. The material from each treatment was dried in oven, ground and stored for chemical analysis.

Growth study

At the start and end of the experiment, fish in each tank was bulk weighed to determine the growth performance of fingerlings following standard formulae:

$$\text{Weight gain \%} = \frac{\text{(Final weight - Initial weight)}}{\text{(Initial weight)}} \times 100$$

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{SGR \%} = \frac{\text{Final weight (g) - Initial weight (g)}}{\text{No. of experiment days}} \times 100$$

Chemical analysis of feed and feces

With the use of mortar and pestle, feed ingredients, experimental diets and collected faecal samples were

Table 1. Ingredient composition (%) of test diets

Ingredients	Test Diet-I (Control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI	Test Diet-VII
α-tocopherol (mg kg^{-1})	0	100	200	300	400	500	600
Corn gluten meal	55	55	55	55	55	55	55
Fish meal	16	16	16	16	16	16	16
Wheat flour*	11	11	11	11	11	11	11
Soybean meal	8	8	8	8	8	8	8
Fish oil	6	6	6	6	6	6	6
Vitamin premix	1	1	1	1	1	1	1
Mineral mixture	1	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100

*α-tocopherol was supplemented at the cost of wheat flour

Proximate composition (%) of feed ingredients

Ingredients	Fish meal	Soybean meal	Wheat flour	Corn gluten meal
Dry matter (%)	91.65	93.51	92.49	92.90
Crude protein (%)	48.93	11.17	09.94	57.68
Crude fat (%)	7.23	4.59	2.28	3.75
Crude fibre (%)	0.93	3.82	2.12	1.39
Ash (%)	25.15	10.92	1.97	2.66
Gross energy (kcal g^{-1})	2.63	3.18	3.04	3.93
Carbohydrates	18.67	46.21	82.88	34.16

homogenised and analysed following standard methods (AOAC, 1995). Crude protein (N × 6.25) was assessed by micro-Kjeldahl apparatus, moisture through stove drying at 105°C for 12 h, crude fat by petroleum ether extraction method through Soxtec HT2 1045 system, ash by ignition at 650°C for 12 h in electric furnace (Eyela-TMF 3100) to constant weight and crude fibre as loss on ignition of dried residues that are lipid-free after digestion with 1:1 of NaOH and H_2SO_4. Gross energy was evaluated using oxygen bomb calorimeter.

Digestibility studies

Chromic acid was included in the diet (1%) to determine apparent digestibility coefficient (ADC%) of nutrients. By employing acid digestion method (Divakaran *et al*., 2002) in UV-VIS 2001 spectrophotometer at 350 nm, chromic oxide content in the samples of experimental diets and faeces was oxidised with perchloric reagent. By using the following formula (NRC, 1993), apparent nutrient digestibility coefficient (%) of experimental diets was evaluated.

$$\text{ADC (\%)}=100-100\times\frac{\text{Percent marker in diet}\times\text{Percent nutrient in faeces}}{\text{Percent marker in faeces}\times\text{Percent nutrient in diet}}$$

Determination of antioxidant activity

The effect of α-tocopherol supplemented diets on antioxidant activity, in terms of % inhibition of oxidation in *L. rohita* was checked following methods described by Hussain *et al*. (2011) with some modifications. Collected samples of fish from each group was dried, ground and transferred to different test tubes, then hexane fraction was prepared by mixing 1g of ground sample with 10 ml of n-hexane in each test tube. Following this, test tubes having hexane fraction were heated gently in water bath for about 10 min. To prepare 10 ml solution of 0.2 M, phosphate buffer was added in each test tube. After mixing gently, 200 µl from each test tube was poured into new tubes and equal ratio (200 µl) of 35% ferrous chloride solution and 30% aqueous ammonium thiocyanate solution was added respectively and absorbance was determined in a spectrophotometer at 500 nm by adding 10 ml of 95% ethanol in each test tube. To assess the inhibition of oxidation (%), following formulae were used:

$$\%\ \text{inhibition} = [(A0 - As)/A0] \times 100$$

$$\text{Oxidation (\%)} = 100 - 100 \times (A0 - As/A0)$$

In the above equations, A0 and As are the absorbance of control and sample after 0 to 5 min, respectively.

Statistical analysis

ANOVA was applied on the data of growth performance (Steel *et al*., 1996). The differences between means was compared by Tukey's Honestly Significant Difference Test and considered significant at $p<0.05$

(Snedecor and Cochran, 1991). For statistical analysis, the Co-Stat computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used.

Results

Graded levels of α-tocopherol significantly affected growth performance of *L. rohita* fingerlings (Table 2). The weight gain (WG), weight gain percentage (WG%) and suitable feed conversion ratio (FCR) values of *L. rohita* fingerlings fed α-tocopherol at 200 mg kg^{-1} was significantly ($p<0.05$) different than the other experimental and control diets. Initial weight of all the fingerlings was more or less similar but final weight was significantly different from each other. Maximum weight gain and weight gain% of 23.67 g and 261%, respectively were recorded in *L. rohita* fingerlings fed at 200 mg kg^{-1} of α-tocopherol followed by the fish fed at 300 mg kg^{-1} (21.09 g and 232%). Weight gain increased with an increase in the levels of vitamin E up to 200 mg kg^{-1}, while further increase of vitamin E from 300-600 mg kg^{-1} decreased WG significantly ($p<0.05$). The best FCR value was detected at 300 mg kg^{-1} of α-tocopherol (2.49) while the second optimum value was at 200 mg kg^{-1} (2.39).

Nutritional composition of all the test diets fed to *L. rohita* fingerlings was similar (Table 3). The analysed composition of nutrients like crude protein, ether extract and gross energy in the faeces is given in Table 4. It was observed from the results that α-tocopherol supplementation in corn gluten meal based diet played a significant ($p<0.05$) role in improving apparent digestibility of CP (73%), EE (74%) and GE (63%) at 200 mg kg^{-1} level (Table 5), so less nutrients were excreted out from the fish body. An increasing trend was observed in nutrients digestibility up to diet III having 200 mg kg^{-1} of α-tocopherol in diet, where it reached its maximum. However, further increase in α-tocopherol supplementation resulted in reduced nutrient digestibility. The second higher values of nutrients absorption were met by applying 300 mg kg^{-1} of α-tocopherol (CP 68%, EE 73%, GE 62%). Alternatively, control diet (*i.e.*, having no α-tocopherol) resulted in highest excretion of nutrients in faeces as; CP 55%, EE 61% and GE 52%, which were significantly different from each test diet.

The antioxidant activity of α-tocopherol supplemented corn gluten meal based diet at various levels is presented in Table 6. Results were obtained by using percentage of oxidation as a parameter to know the effect of α-tocopherol in each diet. Experimental diet VII having 600 mg kg^{-1} of α-tocopherol was found to be

Table 2. Growth performance of *L. rohita* fingerlings fed α-tocopherol supplemented corn gluten meal based diets

Growth parameters	α-tocopherol levels (mg kg^{-1})						
	Diet I (Control diet)	Diet II	Diet III	Diet IV	Diet V	Diet VI	Diet VII
	0	100	200	300	400	500	600
IW (g)	9.09±0.02^{a}	9.08±0.13^{a}	9.06±0.03^{a}	9.08±0.02^{a}	9.10±0.10^{a}	9.06±0.03^{a}	9.10±0.03^{a}
FW (g)	23.71±0.13^{g}	29.11±0.05^{c}	32.73±0.05^{a}	30.17±0.06^{b}	27.44±0.19^{d}	27.44±0.19^{e}	26.85±0.05^{f}
WG (g)	14.62±0.15^{e}	20.03±0.04^{de}	23.67±0.15^{c}	21.09±0.08^{bc}	19.11±0.08^{a}	18.39±0.16^{b}	17.75±0.04^{b}
WG (%)	160.84±0.82^{a}	220.71±0.83^{c}	261.32±0.68^{a}	232.19±0.87^{b}	209.97±0.78^{d}	203.02±1.11^{e}	195.16±0.29^{f}
WG ($fish^{-1}$ day^{-1}) g	0.21±0.00^{d}	0.29± 0.00^{d}	0.34±0.00^{cd}	0.30±0.00^{b}	0.27±0.00^{a}	0.26±0.00^{bc}	0.25± 0.00^{d}
FI	0.41±0.03^{a}	0.42±0.01^{d}	0.43±0.03^{cd}	0.42±0.01^{ab}	0.43±0.02^{e}	0.44±0.02^{ab}	0.45±0.02^{a}
FCR	2.77±0.14^{a}	2.62±0.07^{cd}	2.49±0.10^{d}	2.39±0.03^{bc}	2.49±0.10^{ab}	2.52±0.10^{a}	2.55±0.08^{e}

Means within rows having different superscripts are significantly different at $p<0.05$
Data are means of three replicates
IW= Initial weight, FW= Final weight, WG= Weight gain, FI= Feed intake, FCR= Feed conversion ratio

Table 3. Nutrient compositions (%) in feed of *L. rohita* fingerlings fed on corn gluten meal based diet with supplementation of α-tocopherol

Experimental diets	α-tocopherol levels	CP	EE	GE (kcal g^{-1})
Diet I	0	30.87±0.02^{a}	7.70±0.14^{bc}	4.12±0.01^{a}
Diet II	100	30.87±0.02^{a}	7.73±0.08^{b}	4.26±0.04^{a}
Diet III	200	30.89±0.02^{a}	7.81±0.06^{a}	4.30±0.03^{a}
Diet IV	300	30.88±0.03^{a}	7.90±0.06^{b}	4.23±0.02^{a}
Diet V	400	30.86±0.02^{a}	7.82±0.06^{bc}	4.26±0.03^{a}
Diet VI	500	30.88±0.02^{a}	7.81±0.07^{bc}	4.23±0.02a
Diet VII	600	30.87±0.02^{a}	7.80±0.06^{c}	4.26±0.03^{ab}

Means within rows having different superscripts are significantly different at $p<0.05$
Data are means of three replicates

Table 4. CP, EE and GE (kcal g^{-1}) in faeces of *L. rohita* fingerlings fed on α-tocopherol supplemented corn gluten meal based diet

Experimental diets	α-tocopherol levels (mg kg^{-1})	CP	EE	GE (kcal g^{-1})
Diet I	0	14.55±0.78^{a}	2.84±0.10^{a}	2.13±0.15^{b}
Diet II	100	11.35±0.25^{e}	1.78±0.16^{b}	1.76±0.04^{ab}
Diet III	200	9.02±0.33^{e}	2.23±0.09^{d}	1.73±0.08^{ab}
Diet IV	300	10.94±0.03^{f}	2.30±0.02^{f}	1.56±0.13^{b}
Diet V	400	11.49±0.33^{d}	2.39±0.03^{e}	1.86±0.01^{b}
Diet VI	500	12.47±0.43^{c}	2.48±0.03^{d}	1.94±0.01^{ab}
Diet VII	600	13.93±0.06^{b}	2.65±0.04^{c}	2.06±0.02^{ab}

Means within rows having different superscripts are significantly different at $p<0.05$
Data are means of three replicates

Table 5. Apparent digestibility coefficient (%) of corn gluten meal based diet with α-tocopherol supplementation in *L. rohita* fingerlings

Experimental diets	α-tocopherol levels (mg kg^{-1})	CP	EE	GE
Diet I	0	55.05±0.55^{g}	61.18±0.20^{d}	52.37±0.84^{b}
Diet II	100	65.97±0.77^{c}	66.19±0.93^{c}	60.09±0.72^{ab}
Diet III	200	73.57±0.34^{b}	74.48±0.76^{a}	63.61±0.94^{a}
Diet IV	300	68.72±0.81^{a}	72.67±0.41^{ab}	61.43±0.26^{a}
Diet V	400	67.06±0.33^{d}	73.72±0.04^{b}	59.74±0.47^{ab}
Diet VI	500	63.95±0.45^{e}	71.70±0.82^{b}	58.00±0.86^{ab}
Diet VII	600	59.69±0.16^{f}	70.26±0.26^{b}	54.55±0.35^{ab}

Means within rows having different superscripts are significantly different at $p<0.05$
Data are means of three replicates

Table 6. Antioxidant activity of α-tocopherol supplemented corn gluten meal diets in *L. rohita* fingerlings

Experimental diets	α-tocopherol levels (mg kg^{-1})	Absorbance	Oxidation (%)
Diet I	0	0.0274±0.00012	100.00±0.00
Diet II	100	0.0258±0.00010	95.89±0.35
Diet III	200	0.0239±0.00019	78.34±0.84
Diet IV	300	0.088±0.00013	45.28±0.62
Diet V	400	0.0072±0.00022	31.15±0.34
Diet VI	500	0.0064±0.00015	17.23±0.38
Diet VII	600	0.0017±0.00011	7.66±0.44

the best because oxidation (%) was minimum (7.66%) as compared to other diets. Decreasing trend of oxidation was observed with increasing level of α-tocopherol in all the groups.

Discussion

Vitamin E is considered as one of the important vitamins due to its vital role in improving physiological processes of life. For commonly cultured fish species, optimum dietary range of α-tocopherol is 6.25-200 mg kg^{-1} (NRC, 2011). The quantitative dietary *α*-tocopherol requirement of *L. rohita* based on corn gluten meal is 200 mg kg^{-1}, which is relatively lower than that of *Piaractus mesopotamicus* (Pacu) which is 250 mg kg^{-1} (Garcia *et al*., 2007), while relatively higher than that of *Cirrhinus mrigala* (mrigal) - 99 mg kg^{-1} (Paul *et al*., 2004), *Rachycentron canadum* (Cobia) - 78 or 111 mg kg^{-1} (Zhou *et al*., 2013) and *Ctenopharyngodon idella* (Grass carp) - 100.36 mg kg^{-1} (Li *et al*., 2014). The difference in dietary requirement of each species is attributed to rearing conditions, synergistic effect of vitamin E with other antioxidants present in the diet, fish species, different vitamin E storage capacity of each organ and size and life stage of fish (Lozano *et al*., 2017).

In the present study, parameters related to growth performance significantly enhanced in *α*-tocopherol supplemented groups when compared with the control group. In terms of WG, WG% and FCR, *L. rohita* fingerlings fed 200 mg kg^{-1} of α-tocopherol showed improved results. Our results co-relate with Kim *et al*. (2015) who evidenced that 200 mg kg^{-1} of vitamin E to *Panaeolus olivaceus* enhanced the growth of fish significantly. Muchlisin *et al*. (2016) reported in their study that 150 mg kg^{-1} of *α*-tocopherol in feed supplemented to keureling (*Tor tambra*) is an optimum dosage for better growth. Significant increase in WG, SGR and FCR was observed when Gao *et al*. (2012) fed red sea bream juveniles at 100 and 200 mg kg^{-1} vitamin E supplemented

diet, as compared to the control fish fed without vitamin E supplemented diets.

Sau *et al*. (2004) also gave positive results of vitamin E supplementation for 12 weeks in *L. rohita* in lerms of SGR, WG and FCR. Wang *et al*. (2019) found growth promoting effects of dietary vitamin E at 68.75 mg kg^{-1} in *Nibea albiflora* (Yellow drum) juveniles and stated results by drawing broken line model of WG%. Pan *et al*. (2017) described that vitamin E deficient diet depressed the SGR and WG% in *C. idella*, while optimal vitamin E supplemented diet reversed the negative growth parameters. Supplementation of vitamin E at 480 mg kg^{-1} in *S. maximus* (turbot) improved growth performance and provided suitable FCR value (Jia *et al*., 2017). Improved digestibility of crude protein, total lipids and dry matter of *Notemigonus crysoleucas* (golden shiner) was observed by Chen *et al*. (2004) by feeding 98 mg AA kg^{-1} α-T supplemented diets to the fish.

Vitamin E is a potent antioxidant that protects fish tissues from oxidative damage (Rainis *et al*., 2007). Function of antioxidant system is to balance dynamically the production and removal of free radicals at an equal rate. In case of high number of free radicals, peroxidation of lipid membranes takes place. Important antioxidant enzymes in the fish body are superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) which have the potential to balance free radicals' concentration in the body (Jia *et al*., 2017). Linn *et al*. (2014) treated the red seabream with vitamin E supplemented diets and evidenced that 200 mg kg^{-1} of α-tocopherol reduced lipid peroxidation in fish muscles and improved health status of the fish as well. Sahoo and Mukherjee (2002) explained that the use of vitamin E (1000 mg kg^{-1}) in *L. rohita* which is even greater than recommended level leads to improved immunity and enhances protection of cellular membranes from oxidative damage. Tocher *et al*. (2002) found decreased levels of α-tocopherol in tissues of fish fed with low dietary α-tocopherol level and generally decreased activities of the liver antioxidant enzymes and higher levels of lipid peroxides in juvenile turbot (*S. maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and seabream (*Sparus aurata* L.). Vitamin E at the level of 150 mg kg^{-1} in the feed led to an increase in blood antioxidant activity in *Coturnix coturnix japonica* (Shah *et al*., 2016).

Muchlisin *et al*. (2016) concluded that the optimum dose for keureling (*T. tambra*) was 150 mg kg^{-1} of vitamin E in feed. Addition of more than 100 mg kg^{-1} vitamin E could stop tissues from lipid oxidation as well as better growth and health of juvenile red seabream (Gao *et al*., 2012). Higher SOD activity at 36.2 mg kg^{-1} of vitamin E in *N. albiflora* was noted down by Wang *et al*. (2019); in addition to it, low level of serum malondialdehyde (MAD) was also present at the same level and hence resulted in improved antioxidant activity. Huang and Huang (2004) reported that vitamin E deficient diet in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) resulted in elevated levels of MAD in muscular and liver tissues, hence oxidatively damaged the fish. Pan *et al*. (2017) stated vitamin E deficiency related reduced antioxidant activity in grass carp because radical scavenging ability in head, kidney and spleen of fish was depressed and MAD level started to elevate. Hong *et al*. (2004) explained that α-tocopherol as an antioxidant inhibits superoxide radical accumulation in the brain of streptozotocin-induced diabetic rats. Reduction of antioxidant enzyme activity is attributed to decrease in mRNA levels of fish immune system (Xu *et al*., 2016). Jia *et al*. (2017) also noted renewed antioxidant enzyme activity at 480 mg kg^{-1} of vitamin E in turbot and so lipid peroxidation was prevented.

A contradictory result was found by Chen *et al*. (2004) regarding WG, FCR and feed intake (FI) in *N. crysoleucas* fed on vitamin C and vitamin E supplemented diets even after 14 weeks. This is because vitamin C leads to the sparing effect on vitamin E, in which oxidised vitamin E can be reduced again by ascorbate (Tappel, 1972). It affects growth performance, fillet composition or immunological parameters and has been noted in some fish species (Yildirim-Aksoy *et al*., 2008; Hamre, 2011; Betancor *et al*., 2012). Duration of feeding trial also exerts impact on vitamin E depletion or deposition. Similarly, Gao *et al*. (2013) and Sahoo and Mukherjee (2002) noticed insignificant change on growth performance of *Apostichopus japonicus* and *L. rohita*, respectively after using vitamin E supplemented diets. They used 2-5 folds higher vitamin level than that required for fishes. This difference may be due to excessive levels of vitamins in the diet. Similarly, SGR (1.4 to 1.5%), FI (1.9 to 2.1 g $fish^{-1}$ day^{-1}) and FCR (0.73 to 0.95) was detected in *A. regius* when fed on vitamin E supplemented diets (Lozano *et al*., 2017). This divergence can be explained on the basis of different feeding trial period (72 days) and high concentration of vitamin C (5000 mg kg^{-1}) in the diet.

The results of the present study revealed that α-tocopherol supplementation in corn gluten meal based diet has significant effect on growth performance, nutrient digestibility and antioxidant activity of *L. rohita* fingerlings at dietary level of 200 mg kg^{-1}. Such inclusion level of α-tocopherol resulted in improved antioxidant activity by lowering oxidation of lipids in *L. rohita.* Therefore, corn gluten meal along with supplementation of α-tocopherol proved to be a cheap and highly productive fish feed which is expected to produce nutritionally healthy fish.

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