Effect of \(\alpha\)-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 \(\alpha\)-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 \(\alpha\)-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 \(\alpha\)-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: \(\alpha\)-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.
Materials and methods

*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 induced stress (Li 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:

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

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

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

**Chemical analysis of feed and feces**

With the use of mortar and pestle, feed ingredients, experimental diets and collected faecal samples were
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 H₂SO₄. 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:

\[
\text{% inhibition} = \left(\frac{A_0 - A_s}{A_0}\right) \times 100
\]

\[
\text{Oxidation} (%) = 100 - 100 \times \left(\frac{A_0 - A_s}{A_0}\right)
\]

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.
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(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 | Diet I (Control diet) | Diet II | Diet III | Diet IV | Diet V | Diet VI | Diet VII |
|-------------------|-----------------------|---------|----------|---------|--------|---------|---------|
| IW (g)            | 9.09±0.02a            | 9.08±0.13a | 9.06±0.03a | 9.08±0.02a | 9.10±0.10a | 9.06±0.03a | 9.10±0.03a |
| FW (g)            | 23.71±0.13a           | 29.11±0.05c | 32.73±0.05a | 30.17±0.06b | 27.44±0.19a | 27.44±0.19a | 26.85±0.05b |
| WG (g)            | 14.62±0.15a           | 20.03±0.04a | 23.67±0.15a | 21.09±0.08a | 19.11±0.08a | 18.39±0.16a | 17.75±0.04a |
| WG (%)            | 160.84±0.82           | 220.71±0.83a | 261.32±0.68a | 232.19±0.87a | 209.97±0.78a | 203.02±1.11a | 195.16±0.29a |
| WG (mg fish⁻¹ day⁻¹) | 0.21±0.00b        | 0.29±0.00d | 0.34±0.00e | 0.30±0.00c | 0.27±0.00d | 0.26±0.00e | 0.25±0.00c |
| FI                | 0.41±0.03a            | 0.42±0.01d | 0.43±0.03a | 0.42±0.01e | 0.43±0.02a | 0.44±0.02b | 0.45±0.02b |
| FCR               | 2.77±0.14a            | 2.62±0.07d | 2.49±0.10a | 2.39±0.03b | 2.49±0.10a | 2.52±0.10a | 2.55±0.08b |

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 (mg kg⁻¹) | CP (g 100 g⁻¹) | EE (g 100 g⁻¹) | GE (kcal 100 g⁻¹) |
|--------------------|-------------------------------|----------------|----------------|------------------|
| Diet I             | 0                             | 30.87±0.02a    | 7.70±0.14b     | 4.12±0.01a       |
| Diet II            | 100                            | 30.87±0.02a    | 7.73±0.08b     | 4.26±0.04a       |
| Diet III           | 200                            | 30.89±0.02a    | 7.81±0.06c     | 4.30±0.03c       |
| Diet IV            | 300                            | 30.88±0.03a    | 7.90±0.06c     | 4.23±0.02c       |
| Diet V             | 400                            | 30.86±0.02a    | 7.82±0.06bc    | 4.26±0.03c       |
| Diet VI            | 500                            | 30.88±0.02a    | 7.81±0.07bc    | 4.23±0.02a       |
| Diet VII           | 600                            | 30.87±0.02a    | 7.80±0.06c     | 4.26±0.03bc      |

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±0.78ᵃ | 2.84±0.10ᵇ | 2.13±0.15ᵇ   |
| Diet II            | 100                           | 11.35±0.25ᵇ | 1.78±0.16ᵇ | 1.76±0.04ᵇ   |
| Diet III           | 200                           | 9.02±0.33ᵇ  | 2.23±0.09ᵇ  | 1.73±0.08ᵇ   |
| Diet IV            | 300                           | 10.94±0.03ᵇ | 2.30±0.02ᵇ  | 1.56±0.13ᵇ   |
| Diet V             | 400                           | 11.49±0.33ᵇ | 2.39±0.03ᵇ  | 1.86±0.01ᵇ   |
| Diet VI            | 500                           | 12.47±0.43ᵇ | 2.48±0.03ᵇ  | 1.94±0.01ᵇ   |
| Diet VII           | 600                           | 13.93±0.06ᵇ | 2.65±0.04ᵇ  | 2.06±0.02ᵇ   |

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±0.55ᵍ | 61.18±0.20ᵈ | 52.37±0.84ᵈ |
| Diet II            | 100                           | 65.97±0.77ᶜ | 66.19±0.93ᵗ | 60.09±0.72ᵃ |
| Diet III           | 200                           | 73.57±0.34ᵇ | 74.48±0.76ᵃ | 63.61±0.94ᵗ |
| Diet IV            | 300                           | 68.72±0.81ᵃ | 72.67±0.41ᵇ | 61.43±0.26ᵗ |
| Diet V             | 400                           | 67.06±0.33ᵈ | 73.72±0.04ᵇ | 59.74±0.47ᵃ |
| Diet VI            | 500                           | 63.95±0.45ᵃ | 71.70±0.82ᵇ | 58.00±0.86ᵃ |
| Diet VII           | 600                           | 59.69±0.16ᶜ | 70.26±0.26ᵇ | 54.55±0.35ᵃ |

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                           | 0.0088±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     |

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

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 Cirrhus 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 Panaceolus 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 keurling (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 terms 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). Lim 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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