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Full Length Article

Effects of α-Tocopherol Supplemented Corn Gluten Meal Based Diet on Growth, Nutrient Digestibility and Antioxidant Activity of *Catla catla* Fingerlings

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Abstract

A feeding trial of 70 days was conducted to assess the effects of *α*-tocopherol on growth performance, antioxidant activity and nutrient digestibility of *C. catla* fingerlings (7.05 ± 0.02 g body weight and 6.05 ± 1.07 cm body length) were fed on corn gluten meal-based diets. The *α*-tocopherol was supplemented at various concentrations *viz*., 0, 100, 200, 300, 400, 500 and 600 mg/kg of body weight. Triplicate tanks were used for feeding trial and each tank housed 15 fingerlings. During trial, fingerlings were fed at 5% of their body weight. Completely randomized design (CRD) was followed to allocate test diets to the fish groups. Our results showed that 300 mg/kg of *α*-tocopherol was the optimal level that had a significant role in improving growth parameters such as weight gain (230%) and feed conversion ratio (1.80). Nutrient digestibility parameters *i.e*., crude protein (75.79%), crude fat (76.54%) and gross energy (61.03%) were best observed at the same level compared with the control and other test diets. Furthermore, antioxidant status of the fish was improved with the increase in concentration of α-tocopherol, so, only 5.46% oxidation was observed at 600 mg/kg. It was concluded from present project that corn gluten meal-based diet supplemented with 300 mg/kg level of α-tocopherol proved to be effective in developing low cost, nutritionally balanced and environment friendly feed for *C. catla*.

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

Introduction

Nowadays, fish farming is becoming the top growing industry for producing good quality food. However, we are facing challenges in improving aquaculture sector like high demand of fish products and limited supply of fish feed ingredients especially fish meal (Zarantoniello *et al*. 2020). Along with nutritional values, this sector also provides employment for millions of the people especially belonging to poor and rural areas (Salim 2006). The main target for most of the fish nutritionists is to prepare good quality feed for fish at minimum cost. It is estimated that cost of feed is 50 to 60% of the whole expenditure of aquaculture (Shahzad *et al*. 2018). Requirement of fish meal due to increase in its cost and shortage made it essential to look for the other sources of protein for fish feed (Pham *et al*. 2008). To replace fish meal, appropriate plant feed ingredients such as grains and other plant by-products are being used as the most favorable source of protein for feed formulation (Manuel *et al.* 2019). Corn gluten meal is produced during processing of corn starch, contains approximately 60% protein and has potential to replace fish meal in diet because of its essential amino acid profile (Men *et al.* 2014).

The substances which reduce the reactive oxygen species are called as antioxidants (Majid *et al*. 2015). Under normal physiological conditions, the body of animals has capacity to produce suitable quantity of antioxidants that are able to prevent the process of oxidation. When there is higher rate of lipid per-oxidation, reactive oxygen specie is produced constantly. When free radicals exceed the limit of antioxidant potential of the body, stress is produced by oxidative response in body (Rahman *et al*. 2014). Vitamins are the organic compounds required by organisms in minute amount for proper growth and metabolism (Amlashi *et al.* 2012). Since most of fishes cannot synthesize vitamins at all or can only produce them in small quantities for normal

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growth and metabolism, they must be supplemented in the diet (Falahatkar *et al.* 2011). Vitamin E is required to maintain immunity, save cell membrane from peroxide damage and improves the tissue feature (Salinthone *et al.* 2013). Vitamin E is composed of four tocopherols and four tocotrienols having two rings and amongst them the most common and biologically dynamic molecule is *α*-tocopherol (Yang *et al*. 2012).

Fish fed with vitamin E showed significantly higher growth rate and feed efficiency (Kashani *et al*. 2010). Vitamin E requirement have been confirmed in many fish species. It was basically considered as a dietary component in nutrition of animals, which is very important for fish reproduction. In aquaculture, it is added in feed to enhance growth, and survival of fish against disease and increase stress resilience (Vismara *et al*. 2003). *C. catla* is an important carp specie; being cultured extensively for its fast growth rate, nutritional quality, good taste, acclimatization to laboratory conditions and high commercial value (Wahab *et al*. 2002). The present research work was conducted to evaluate the effect of α-tocopherol on growth performance, nutrient digestibility and antioxidant activity of *C. catla* fingerlings.

Materials and Methods

Present project was carried out in Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad, Pakistan. *C. catla* fingerlings of the equal body weight (7.05 ± 0.02 g) and same body length (6.05 ± 1.07 cm) were purchased from Government Fish Hatchery, Satiana road, Faisalabad.

Fish acclimatization and culture conditions

Fingerlings were kept in V-shaped tanks having 70 L volumetric capacity (specifically made for feces collection) and were acclimatized to experimental conditions for 15 days (Rowland 1991). Fingerlings were fed once with basal diet for two weeks (Allan and Rowland 1992). Before experiment, *C. catla* fingerlings were bathed in 0.5% NaCl solution, so as to free fish from fungal infection and ecto-parasites. On daily basis, temperature was measured with thermometer, pH with pH meter (Jenway 3510) and dissolved oxygen with DO meter (Jenway 970). By capillary method, aeration (24 h) was supplied to all tanks.

Feed ingredients and experimental diets preparation

Feed ingredients such as corn gluten meal, fish meal and soybean meal were grinded by passing through 0.5 mm sieve size. For the period of 5 min, all grinded feed ingredients were mixed in a specially designed tank while gradually adding fish oil. For making proper dough, 10–15% of water was also added (Lovell 1989). Likewise, to make pellets, previously made dough was passed through machine for pelleting. Prepared diet was supplemented with different levels of *α*-tocopherol as 100, 200, 300, 400, 500 and 600 mg/kg of body weight.

Feeding protocol

Feeding trial of 70 days was conducted and fingerlings were fed @ of 5% of their wet body weight, two times a day. Remaining diet was drained out from each tank after the feeding period of two hours on daily basis; by opening the valves of tanks. To remove the remaining diet particles, tanks were washed entirely and filled with water again. Feces were collected through fecal collecting tube of each tank through valve-I and II. To minimize the leakage of nutrients, fecal strings were collected carefully to avoid fecal dissolving. The collected strings were first dried in oven and then grinded for further chemical analysis.

Growth parameters

To study growth rate, fish in each tank were bulk weighed at the start and at the end of whole experimental period. Weight gain (WG %), FCR and specific growth rate (SGR) of fingerlings were checked by using the following standard formulae (National Research Council 1993) :

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

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

$$\text{SGR} = \frac{\text{Final Weight (g) - Initial Weight(g)}}{\text{No of trial days}} \times 100$$

Nutrient digestibility

Evaluation of apparent nutrient digestibility coefficients (ADC%) of experimental diets were measured by using the standard formula (National Research Council 1993):

$$\text{ADC}(\%) = 100 - 100 \times \frac{\% \text{ marker in diet}}{\% \text{ nutrients in feces}}$$

Antioxidant activity

To determine antioxidant status of *C. catla* fingerlings, percent inhibition of oxidation was calculated following Hussain *et al.* (2011) with minor modifications. 1 g dried and ground samples of fish body from each group were mixed with 10 mL of n-hexane in separate test tubes. 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 gently shaking, 200 μL from each test tube was picked and transferred to a new one so that 200 μL of 30% aqueous ammonium thiocyanate solution and 200 μL of 35% ferrous chloride solution can be mixed in it. At the

end, absorbance of solution by adding 10 mL of 95% ethanol in each test tube was measured using spectrophotometer at 500 nm. The percentage inhibition of oxidation was evaluated using the formula:

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

In formula, A (0) and A(s) are the sample's absorbance from 0 to 5 min, respectively.

Statistical analysis

One way analysis of variance (ANOVA) was applied on the data of growth performance, nutrient digestibility and anti-oxidant status. The differences between means were compared by applying Tukey's Test (Snedecor and Cochran 1991). For statistical analysis, the Co-Stat computer software (version 6.303, Monterey, CA, PMB 320, 93940 USA) was used.

Results

Growth parameters

The growth performance of *C. catla* fingerlings fed on corn gluten meal-based diets supplemented with different levels of α-tocopherol as 0, 100, 200, 300, 400, 500 and 600 mg/kg diet is shown in Table 1, 2. In terms of weight gain %, diet IV with α-tocopherol at 300 mg/kg of diet showed maximum values. In all treatments, initial weight of fingerlings was almost same, but weight of fingerlings measured after experiment was significantly different than others. The maximum weight gain (16.31 g), weight gain % (230%) was noted in fish fed at 300 mg/kg of α-tocopherol supplementation, followed by fish (15.51 g; 219%) fed at 200 mg/kg of α-tocopherol. These values were found to be significantly ($P < 0.05$) different when compared with control diet (12.92 g; 183.35%) and other test diets. Beyond 300 mg/kg, further increase in α-tocopherol level did not result in improved results. Its maximum concentration improved weight gain only up to 171%. Lowest value of FCR (1.80) was noticed in fish fed diet having 300 mg/kg α-tocopherol which rendered most of the feed portion to be converted in body flesh. Alternatively, highest FCR value (1.99) was obtained by using control diet having no supplementation of vitamin E. Supplementation of α-tocopherol in corn gluten meal-based diet at the level of 300 mg/kg had a vital role in promoting growth of fish.

Nutrient digestibility

The analyzed composition of nutrients (crude protein, crude fat and gross energy) in diet and feces of *C. catla* fingerlings fed on graded concentrations of α-tocopherol supplemented corn gluten meal-based diet is shown in Table 3 and 4, respectively. It was observed that α-tocopherol at 300 mg/kg supplementation in corn gluten meal-based diet played a vital role in improving apparent digestibility coefficient (ADC%). Results showed that increasing trend was observed in nutrients digestibility up to level IV having 300 mg/kg of α-tocopherol in diet where it reached its maximum, while further increase in α-tocopherol supplementation resulted in reduced digestibility of nutrients. In current study, it was observed that at 300 mg/kg level, the digestibility value of crude fat (76.54%), crude protein (75.79%) and gross energy (61.03%) were found to be highest and significantly ($P < 0.05$) different than all other test diets (Table 5). However, lowest digestibility value of nutrients, crude fat (64.68%), crude protein (55.36%) and gross energy (40.62%) was noted at control diet (0 mg/kg α-tocopherol). These results revealed that supplementation of α-tocopherol at the level of 300 mg/kg of diet is the best for maximum digestibility of nutrients in body of *C. catla* fingerlings and resulted in reduced discharge of nutrients in environment.

Antioxidant activity

The α-tocopherol's antioxidant activity at various levels (0, 100, 200, 300, 400, 500 and 600 mg/kg) was checked as shown in Table 6. Decreasing trend of percentage of oxidation was observed with increasing level of α-tocopherol in all fish groups. It means that antioxidant activity of α-tocopherol was best described at its highest level. Experimental diet VII with 600 mg/kg of α-tocopherol was observed to be the best ($P < 0.05$) of all other test diets because the percentage of oxidation was lowest (5.46%) when compared with other test diets. Second good results (18.22%) were found at 500 mg/kg level of α-tocopherol. On the other hand, 100% oxidation was observed in control group that fed on diet supplemented with 0 mg/kg of α-tocopherol.

Discussion

The present study showed that α-tocopherol is an important feed additive to enhance antioxidant activity, improve growth and nutrient digestibility. The higher weight gain, weight gain values were observed in fingerlings fed on 300 mg/kg of alpha tocopherol supplemented corn gluten meal-based diet. Results resembling to our study were reported by Muchlisin *et al*. (2016) who described that 150 mg/kg of *α*-tocopherol is an optimum dosage for better growth of keureling (*Tor tambra*). Galaz *et al*. (2010) found that increasing the dose of dietary α-tocopherol (up to 500 mg/kg) enhanced growth in *Vibrio anguilarum* challenged fish (Nekoubin *et al*. 2012). Gao *et al*. (2012) demonstrated that vitamin C supplementation in red sea bream at 500 mg/kg level could improve growth parameters and can reduce oxidative stress. However, in contradiction to current results, Gao *et al.* (2014) reported that the increasing dietary vitamin E level from 100 to 200 mg/kg decreased growth performance in

Table 1: Ingredients 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)	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

Table 2: Growth performance of *C. catla* fingerlings fed α-tocopherol supplemented corn gluten meal-based diets

Growth parameters	Test Diet-I (Control diet)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI	Test Diet-VII
	α-tocopherol Levels (mg/kg)						
	0	100	200	300	400	500	600
IW (g)	7.05 ± 0.02^{a}	7.07 ± 0.03^{a}	7.06 ± 0.02^{a}	7.09 ± 0.02^{a}	7.09 ± 0.03^{a}	7.05 ± 0.05^{a}	7.06 ± 0.02^{a}
FW (g)	19.97 ± 0.02^{e}	21.41 ± 0.03^{c}	22.58 ± 0.06^{b}	23.40 ± 0.04^{a}	21.46 ± 0.06^{c}	20.94 ± 0.05^{d}	19.19 ± 0.03^{f}
WG (g)	12.92 ± 0.03^{e}	14.35 ± 0.01^{c}	15.51 ± 0.05^{c}	16.31 ± 0.03^{b}	14.36 ± 0.04^{a}	13.89 ± 0.05^{c}	12.12 ± 0.02^{b}
WG (%)	183.35 ± 0.78^{c}	203.02 ± 0.64^{b}	219.63 ± 0.86^{a}	230.00 ± 0.58^{a}	202.49 ± 0.50^{b}	196.89 ± 0.09^{b}	171.64 ± 0.42^{d}
WG (fish/day) g	0.18 ± 0.00^{d}	0.20 ± 0.00^{d}	0.22 ± 0.00^{cd}	0.23 ± 0.00^{b}	0.21 ± 0.00^{a}	0.20 ± 0.00^{bc}	0.17 ± 0.00^{d}
FI	0.37 ± 0.02^{bc}	0.39 ± 0.03^{cd}	0.41 ± 0.02^{ab}	0.42 ± 0.01^{a}	0.38 ± 00.2^{a}	0.39 ± 0.01^{ab}	0.34 ± 0.01^{c}
FCR	1.99 ± 0.08^{ab}	1.89 ± 0.12^{abc}	1.85 ± 0.10^{ab}	1.80 ± 0.05^{ab}	1.84 ± 0.08^{ab}	1.97 ± 0.05^{a}	1.98 ± 0.04^{ab}

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, FCR= Feed Conversion Ratio, FI= Feed Intake

Table 3: Analyzed compositions (%) of CP, EE and GE in feed of *C. catla* fingerlings fed on corn gluten meal-based diet with α-tocopherol supplemented test diets

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

CP= crude protein, EE= Ether Extract, GE= Gross Energy
Means within columns having different superscripts are significantly different at $P < 0.05$
Data are means of three replicates

juvenile Japanese flounder, *Paralichthys olivaceus*.

The accessibility of nutrients to the fish is basically related with its digestibility values. In current study, it was observed that at 300 mg/kg level, the digestibility values of crude fat (76.54%), crude protein (75.79%) and gross energy (61.03%) were found to be highest and significantly different from all other test diets. Muchlisin *et al.* (2016) reported that dietary vitamin E significantly affected protein retention in the carcass and protein digestibility of fish. Chae *et al.* (2006) concluded that supplementation of 100 and 200 mg/kg of α-tocopherol increased the nutrient digestibility in chicks. Data about nutrient digestibility in fish fed α-tocopherol or vitamin E is rare.

Vitamin E possesses effective antioxidant activity that protects fish tissues from oxidative damage (Rainis *et al.* 2007). Ciocoiu *et al.* (2007) deduced that α-tocopherol performed major antioxidant activity in per oxidation of lipids in mammalian plasma membrane. Significant role of α-tocopherol in tissues appeared to be protecting the membrane polyunsaturated fatty acids from oxidation of free radicals. Tocher *et al.* (2002) concluded that decreased level of α-tocopherol in tissues of fish reduced the activity of liver antioxidant enzymes and increased the activity of lipid peroxide enzymes in juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and red sea bream (*Sparus aurata* L.). From these results, it can be concluded that vitamin E played an important role against oxygen free radicals to protect biological membranes.

Conclusion

Results of present research work suggested that supplementation of α-tocopherol in corn gluten meal-based diet improved the growth parameters, nutrient digestibility and antioxidant activity of *C. catla*. Maximum values of growth performance, nutrient digestibility and antioxidant activity analysis in *C. catla* were observed at 300 mg/kg of α-tocopherol level.

Table 4: Analyzed compositions (%) of CP, EE and GE in feces of *C. catla* fingerlings fed on corn gluten meal-based diet with α-tocopherol supplemented test diets

Experimental diets	α-tocopherol Levels (mgkg^{-1})	CP (%)	EE (%)	GE (kcalg^{-1})
Test Diet-I (Control)	0	14.28 ± 0.10^{a}	2.92 ± 0.054^{a}	2.69 ± 0.16^{a}
Test Diet-II	100	9.18 ± 0.03^{e}	2.70 ± 0.02^{b}	2.26 ± 0.08^{ab}
Test Diet-III	200	9.27 ± 0.02^{e}	2.20 ± 0.03^{e}	2.07 ± 0.64^{ab}
Test Diet-IV	300	8.41 ± 0.05^{f}	1.97 ± 0.05^{f}	1.82 ± 0.10^{b}
Test Diet-V	400	11.48 ± 0.34^{d}	2.15 ± 0.02^{e}	1.96 ± 0.01^{b}
Test Diet-VI	500	12.87 ± 0.09^{c}	2.46 ± 0.03^{d}	2.27 ± 0.06^{ab}
Test Diet-VII	600	13.75 ± 0.10^{b}	2.57 ± 0.02^{c}	2.46 ± 0.13^{ab}

CP= crude protein, EE= Ether Extract, GE= Gross Energy
Means within columns having different superscripts are significantly different at $P < 0.05$
Data are means of three replicates

Table 5: Apparent digestibility coefficient (%) of CP, EE and GE of *C. catla* fingerlings fed on corn gluten meal-based diet with α-tocopherol supplemented test diets

Experimental diets	α-tocopherol Levels (mgkg^{-1})	CP (%)	EE (%)	GE (%)
Test Diet-I (Control)	0	55.36 ± 0.19^{g}	64.68 ± 0.33^{d}	40.62 ± 0.78^{b}
Test Diet-II	100	71.13 ± 0.24^{c}	66.20 ± 0.86^{d}	51.21 ± 0.23^{ab}
Test Diet-III	200	72.82 ± 0.19^{b}	72.82 ± 0.57^{b}	54.21 ± 0.47^{ab}
Test Diet-IV	300	75.79 ± 0.72^{a}	76.54 ± 0.64^{a}	61.03 ± 0.39^{a}
Test Diet-V	400	66.82 ± 0.89^{d}	74.29 ± 0.35^{ab}	57.58 ± 0.60^{a}
Test Diet-VI	500	62.84 ± 0.71^{e}	70.28 ± 0.39^{c}	50.97 ± 0.73^{ab}
Test Diet-VII	600	59.89 ± 0.56^{f}	65.92 ± 0.80^{d}	46.45 ± 0.85^{ab}

CP= crude protein, EE= Ether Extract, GE= Gross Energy
Means within columns having different superscripts are significantly different at $P < 0.05$
Data are means of three replicates

Table 6: Antioxidant activity of α-tocopherol of *C. catla* fingerlings fed on corn gluten meal-based diet with α-tocopherol supplemented test diets

Experimental diets	α-tocopherol Levels (mgkg^{-1})	Absorbance	Oxidation (%)
Test Diet-I (Control)	0	0.0283 ± 0.00012	100.00 ± 0.00
Test Diet-II	100	0.0274 ± 0.00010	97.81 ± 0.35
Test Diet-III	200	0.0261 ± 0.00019	93.34 ± 0.74
Test Diet-IV	300	0.0244 ± 0.00013	84.28 ± 0.82
Test Diet-V	400	0.0078 ± 0.00022	33.25 ± 0.44
Test Diet-VI	500	0.0054 ± 0.00015	18.22 ± 0.34
Test Diet-VII	600	0.0013 ± 0.00011	5.46 ± 0.24

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

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Author Contributions

Syed Makhdoom Hussain Conceived and designed the project and provided facilities for research work; Muhammad Arshad Conducted trial, collected data and wrote this manuscript; Shafaqat Ali Helped in writing, revising and improvement of manuscript; Muhammad Moazam Jalees Helped in writing the manuscript; Farooq Ahmad helped in writing, revising and improvement of manuscript; Fatima Bashir Helped in compiling results; Aqsa Sharif Helped in nutrients analysis; Zeeshan Yousaf Helped in conducting feeding trial and formulating fish feed.

Conflict of Interest

All authors declare no conflict of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding autho

Ethical Approval

Not applicable in this paper.

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