

Effects of Varying Inclusion Levels of Oxalic Acid Supplemented Diets on Growth Performance and Carcass Composition of *Oreochromis Niloticus* Challenged with *Escherichia Coli*

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Abstract

The study investigated the efficacy of varying inclusion levels of oxalic acid on growth performance, carcass composition of *Oreochromis niloticus* and challenge test with *Escherichia coli*. *O. niloticus* of mean weight $7.00\text{g} \pm 0.04$ were fed with varying oxalic acid supplemented diets for 90 days. At the expiration of the feeding trials significant variations was recorded on growth performance indices and carcass composition of fish. The best growth was recorded in OAC4 which also had best protein retention. The culture water parameters (DO_2 , temperature, pH) were with the required limits. The study demonstrated that oxalic acid at 1.5% was able to improve growth and had highest survival in challenge test with *E. coli*.

Keywords: Oxalic acid; growth performance; carcass composition; *Oreochromis niloticus*

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Introduction

Aquaculture is one of the fastest growing food production sectors proposed to meet the increasing demand for animal protein, and to overcome human challenges related to diseases and low immunity as a result of protein deficiency. This sector is expected to increase human protein consumption to boost immunity against infections and related diseases. Aquaculture production is predicted to grow to between 400 - 500 million tonnes by 2030 and to keep up with demand, the potential solution to this move aquaculture to the next level, as suitable new locations for freshwater production become scarce (FAO, 2020). This can only be achieved with good water quality, nutritionally complete steady feeds of low cost that optimizes growth and reduces the probability of diseases and parasite infestations thereby improving fish health. Aquaculture was introduced to Nigeria in the 1950s with the culture of Tilapia [1]. The sector which started on a subsistence level has grown to be one of the most significant in the Sub-Saharan Africa. Nutrients in aquafeed play critical roles in aquaculture because, by influencing fish health,

growth, reproduction, production and management cost. Nutrients are substances that an organism needs to live and grow or a substance that provides nourishment for growth or reproduction, which must be taken from its environs or obtained from its food [2]. Nutrients are used to build and repair tissues, regulate body processes and convert energy (ATP) for daily muscle coordination. It has been reported that dietary supplementation of organic acids increases the bioavailability of minerals, including phosphorus, magnesium, calcium and amino acid in catfish and tilapia [3-5]. The search for feed additives is important point for aquaculture as wide varieties of natural growth promoters, including plant extracts, probiotics and organic acids have been applied globally with reasonable success [6-7]. The supplementation of organic acid as additive to basal ingredients in feed formulation has significantly increased body weight of aquatic organism and other animals. Oxalates are organic compounds with two carboxyl groups. They are made up of short chain fatty acids (SCFAs) and volatile fatty acids, or weak carboxylic acids similar to butyric, formic, citric, benzoic, lactic and malic acids (Koh et al, 2016). Oxalic acids in animal feed were reportedly used on

piglets to complement their limited capacity to maintain a low gastric pH and thus prevent digestive problems [8-10]. Therefore, oxalic acid or their salts would be a promising feed additive for aquatic organisms to improve growth performance and health status without the fear of resistance as a result of accumulation. Therefore, this work focused on growth performance, carcass composition of *O. niloticus* fed with varying inclusion levels of oxalic acid supplemented the diets and challenge with *E. coli*.

Materials and Methods

The research work was carried out at the Department of Fisheries and Aquaculture Technology Teaching and Research Farm, The Federal University of Technology, Akure, (FUTA) Ondo State, Nigeria. Proximate analysis of feed and fish were carried out at Animal Production and Health (APH) Laboratory, School of Agriculture and Agricultural Technology, FUTA.

Experimental Diets and Preparation

The test ingredient, Oxalic acid was procured from an accredited dealer in Nigeria. Five iso-nitrogenous diets of 35 % crude protein were formulated to contain oxalic acid at different inclusion levels of: 0 (OAC₁), 0.5 (OAC₂), 1.0 (OAC₃), 1.5 (OAC₄) and 2.0 (OAC₅) g100g⁻¹. Prior to diet preparation, ingredients were analyzed at the Central Research Laboratory, FUTA for proximate compositions. Basic ingredients such as fishmeal, soybean meal, groundnut cake (GNC), yellow maize meal, wheat offal, Cod-liver oil, vitamins and mineral premixes, and cassava starch (binder) were purchased from a licensed feed marketer. All ingredients were weighed based as indicated on the **Table 1**. Furthermore, ingredients were grinded using electric motor (KPG0154) and reweighed to precision using electronic Citizen's weighing balance (Model PB3002). Ingredients were then thoroughly mixed using Hammer mixer and pelleted following appropriate procedure in a Horbart A-2007 pelleting machine (Hobart Ltd, London, UK). Pellets product was sundried at ambient temperature (28-30°C) and later packed in polythene bags prior to use.

Experimental Fish

Three hundred (300) apparently healthy Nile tilapia (*Oreochromis niloticus*) fingerlings of mean weight 7.00g ± 0.04g were procured from the Teaching and Research Farm, Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Akure. Experimental fish were acclimated to laboratory conditions for 14 days, and fed with farm-processed feed before the commencement of the feeding trials. Fish were starved for 24 hours, prior to being placed on experimental diets, with proximate compositions also analyzed.

Experimental Design and Set up

The experimental design was a complete randomized design (CRD) with all experimental samples homogenous, while the only source of variation is the test ingredient (Oxalic acid). An indoor experimental set-up of fifteen (15) glass tanks (70 x 45 x 45) cm with 60-litres water capacity were used for the experiment following standard bioassay procedures (APHA, 1990). Each unit was fitted with 50Hz aerator of 120W, (Coresun model: ACO-008) and powered electrically for an average of 15-18 hours per day. The source of culture water was from borehole situated on the Teaching and research farm.

Experimental Procedure

Apparently healthy fish with mean weight 7.00±0.04g were distributed at a rate of 15 fish per tank, into 15 glass aquarium tanks in triplicates. Fish were fed at 5% body weight in two equal portions twice daily between 08.00 – 09:00 and 16:00 – 17:00 hours GMT for 90 days. Uneaten feeds were siphoned every morning using a pressure sucking pipe. Water was partially (25 %) drained and replaced with fresh water daily and totally drained twice a week. Fish in experimental units were batch weighed at 2 weeks interval and feeding regimen readjusted based on change in body weight. Some water quality parameters were monitored on weekly basis using appropriate instruments. Temperature was

Table 1. Gross Composition of the Experimental Diets (g/100g) for *O. niloticus* (dry matter).

Ingredient	Dietary Treatment				
	Control(D ₁)	D ₂	D ₃	D ₄	D ₅
Fish meal	28.13	28.13	28.13	28.13	28.13
Soybean meal	18.75	18.75	18.75	18.75	18.75
Groundnut cake	9.38	9.38	9.38	9.38	9.38
Maize	20.25	20.25	20.25	20.25	20.25
Wheat	13.50	13.50	13.50	13.50	13.50
Cod liver-oil	3.00	3.00	3.00	3.00	3.00
Vit. & Mineral mixture/Premix	2.00	2.00	2.00	2.00	2.00
Cassava starch	5.00	4.50	4.00	3.50	3.00
*Oxalic acid (gkg ⁻¹)	0.00	0.50	1.00	1.50	2.00
Proximate Analysis Composition (100 %)					
Moisture content	14.04	10.97	10.63	11.05	11.45
Crude Protein	35.16	35.11	35.21	35.30	35.11
Crude Ash	12.19	12.35	12.37	12.85	13.21
Crude Lipid	9.72	8.27	7.53	7.87	8.50
Crude fibre	6.06	5.96	6.74	5.21	6.01
NFE	22.83	27.30	27.54	27.91	25.55

measured using Search Tech Instrument thermometer, pH; Pen type pH meter and Dissolve oxygen; DO Meter Type (Labtech model: AVI – 660).

Experimental Analyses: At the expiration of the feeding trials growth performance evaluation were carried out using the following indices;

$$i. \text{ Weight Gain (WG) (g)} = W_2 - W_1$$

Where; W_2 and W_1 are the final and initial body weight of fish respectively;

$$ii. \text{ Specific Growth Rate (SGR)}$$

This was calculated from data on changes of body weight over a given time intervals

$$SGR (\%d^{-1}) = \frac{100 \times (ln[W_2] - ln[W_1])}{T}$$

Where; W_1 and W_2 are logarithms of initial and final fish weight respectively, and T is the number of experimental days. $\%d^{-1}$

$$iii. \text{ Feed Intake (FI)}$$

It was obtained by adding biweekly mean feed intake (DFI) of the fish under each treatment for the experimental period.

$$FI(g) = \text{quantity of feed fed} \times \text{experimental period}$$

$$iv. \text{ Feed Conversion Ratio (FCR)}$$

$$FCR = \frac{\text{Amount of food given (feed intake)}}{\text{Total amount of fish produced (weight gain)}}$$

$$v. \text{ Feed Efficiency Ratio (FER)}$$

$$FER = \frac{\text{weight gain}}{\text{feed intaken}}$$

$$vi. \text{ Percentage Weight Gain (PWG)}$$

$$PWG = \frac{\text{Final Weight} - \text{initial weight}}{\text{Initial weight}} \times 100$$

$$vii. \text{ Survival (\%)}$$

$$\frac{\text{Number of fish at the end of the experiment}}{\text{Total Number of fish before experiment}} \times 100$$

Carcass Composition

Samples of fish were taken from each treatment tank at the end of acclimated and feeding trial for the initial and final proximate

carcass analyses respectively. The proximate analysis was carried out according to the standard methods of AOAC (2005).

Challenge test with Enterotoxigenic Bacteria (*Escherichia coli*)

An immuno-competence test was conducted at the end of the feeding trials. Fifteen fish were randomly selected from each of the treatments (5 fish per tank) and introduced into 50 ml/l of broth culture of pathogenic strain of *E. coli* (1.7×10^9 cells/ml). Clinical signs, post-mortem lesions and mortalities was monitored for 9 days and recorded.

Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) and general linear mode function of Statistical Package for Social Sciences (SPSS version 23.0). The treatment means were separated where there was a significant different using Duncan's multiple range test (Duncan 1955) at significance level of ($p \leq 0.05$) and values were expressed as means \pm standard error.

Results

The effects of oxalic acid on growth performance and nutrient utilization of *O. niloticus* fed oxalic acid supplemented diets is presented on **Table 2**. There were no significant variations ($p > 0.05$) between the initial weights of the fish, which means that the weights were homogenous, no biasness was introduced from the initial weights. There were significant variations ($p < 0.05$) in the growth evaluation indices measured in fish fed on the control and test diets. Significant variations ($p < 0.05$) were observed in the final weight, weight gain, feed conversion ratio (FCR), feed efficiency ratio (FER) and specific growth rate (SGR) in OAC1, OAC2 and OAC5, but there was no significant different between the treatment OAC3 and OAC4 ($P > 0.05$). Mortality was recorded in fish fed OAC1 and OAC2, OAC3 while OAC4 and OAC5 percentage survival is 100% the challenge test (**Figure 1**). **Table 3** revealed the whole body composition of *O. niloticus* at the beginning and at the end of the feeding trial. There were significant variations ($p < 0.05$) between the initial and final body compositions of fish with respect to moisture, lipid, fat and crude protein. There were no significant variations ($p > 0.05$) in crude lipid of the fish on tested diets, except OAC4 which was different from other treatments. The initial body composition of *O. niloticus* had the highest moisture content, closely followed by OAC5 and

Table 2. Growth performance of *Oreochromis niloticus* fed various levels of Oxalic Acid supplemented diets (Mean \pm SE).

Parameter	OAC1	OAC2	OAC3	OAC4	OAC5
Initial Mean Weight (g)	7.01 \pm 0.01 ^a	7.00 \pm 0.01 ^a	7.00 \pm 0.01 ^a	7.00 \pm 0.01 ^a	7.01 \pm 0.01 ^a
Final Mean Weight (g)	19.23 \pm 0.16 ^a	22.07 \pm 0.17 ^c	23.87 \pm 0.20 ^d	26.06 \pm 0.14 ^d	20.62 \pm 0.13 ^b
Mean Weight Gain (g)	12.22 \pm 0.16 ^a	15.07 \pm 0.17 ^c	16.88 \pm 0.75 ^d	19.06 \pm 0.05 ^d	13.61 \pm 0.13 ^c
Mean Feed Intake (g)	31.60 \pm 0.07 ^a	32.20 \pm 0.05 ^a	32.42 \pm 0.04 ^{ab}	32.88 \pm 0.04 ^b	31.05 \pm 0.03 ^a
FCR (g)	2.58 \pm 0.04 ^d	2.09 \pm 0.03 ^b	1.87 \pm 0.03 ^a	1.65 \pm 0.01 ^a	2.32 \pm 0.02 ^c
FER (g)	0.38 \pm 0.59 ^a	0.47 \pm 0.57 ^c	0.53 \pm 0.75 ^d	0.60.55 \pm 0.39 ^d	0.43 \pm 0.39 ^b
SGR (%day ⁻¹)	0.49 \pm 0.00 ^a	0.55 \pm 0.00 ^c	0.59 \pm 0.01 ^d	0.63 \pm 0.00 ^d	0.52 \pm 0.01 ^b
PWG(%day ⁻¹)	1.94 \pm 0.03 ^a	2.39 \pm 0.03 ^c	2.68 \pm 0.0 ^d	2.74 \pm 0.02 ^d	2.16 \pm 0.02 ^b
Survival (%)	85.33 \pm 3.84 ^a	90.78 \pm 2.22 ^b	95.78 \pm 2.22 ^{ab}	97.78 \pm 2.22 ^b	85.22 \pm 2.22 ^a

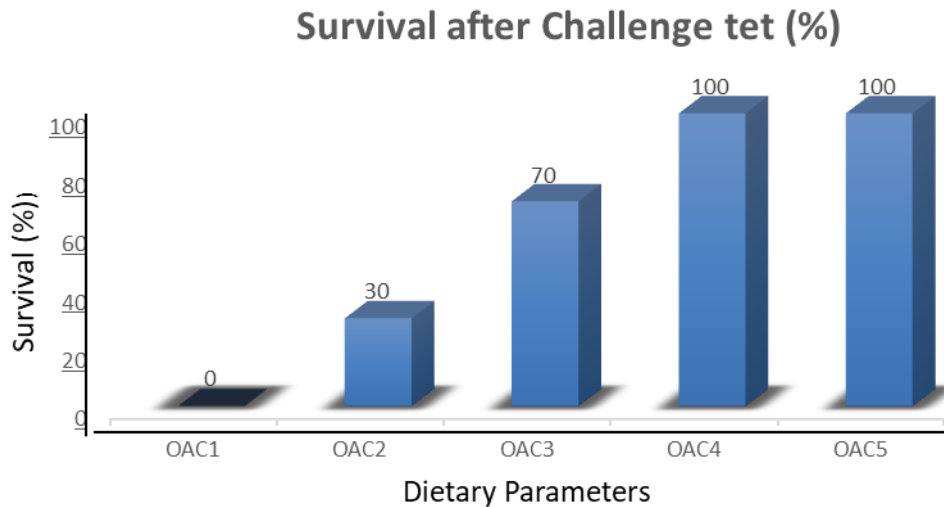


Figure 1 Percentage survival of *O. niloticus* challenge with *E. Coli*.

Table 3. Whole body composition of *Oreochromis niloticus* (dry matter) fed varying levels of oxalic acid supplemented diets (Mean \pm S.E).

Parameters	Initials	OAC1	OAC2	OAC3	OAC4	OAC5
Moisture	6.83 \pm 0.01 ^c	7.80 \pm 0.01 ^e	7.94 \pm 0.01 ^f	6.32 \pm 0.01 ^a	6.66 \pm 0.04 ^b	6.91 \pm 0.01 ^d
Ash	15.77 \pm 0.01 ^e	15.77 \pm 0.01 ^e	14.61 \pm 0.06 ^c	15.13 \pm 0.01 ^d	13.83 \pm 0.01 ^a	14.10 \pm 0.02 ^b
Fat	12.17 \pm 0.02 ^a	12.67 \pm 0.03 ^{ab}	13.50 \pm 0.28 ^c	12.32 \pm 0.01 ^b	13.4 \pm 0.01 ^c	13.24 \pm 0.02 ^{bc}
Protein	47.05 \pm 0.02 ^b	46.13 \pm 0.02 ^a	47.69 \pm 0.02 ^c	50.35 \pm 0.01 ^e	52.02 \pm 0.01 ^f	49.39 \pm 0.01 ^d
NFE	18.18 \pm 0.02 ^d	17.65 \pm 0.49 ^d	16.07 \pm 0.39 ^c	15.65 \pm 0.36 ^b	14.07 \pm 0.03 ^a	16.38 \pm 0.05 ^c

Table 4. Water quality parameters in experimental units *Oreochromis niloticus* fed Oxalic acid supplemented diets.

Parameter	OAC1	OAC2	OAC3	OAC4	OAC5
DO ₂ (mg/l)	6.00 \pm 0.12 ^a	6.17 \pm 0.09 ^a	6.73 \pm 0.03 ^b	6.53 \pm 0.09 ^b	6.53 \pm 0.09 ^b
pH	8.60 \pm 0.06 ^b	8.07 \pm 0.15 ^a	8.20 \pm 0.11 ^a	8.40 \pm 0.03 ^{ab}	8.03 \pm 0.14 ^a
Temperature (C)	27.4 \pm 0.06 ^{ab}	27.43 \pm 0.67 ^{ab}	27.50 \pm 0.06 ^b	27.33 \pm 0.03 ^{ab}	27.30 \pm 0.06 ^a

Table 5. Challenge test using *O. niloticus*

Parameter	OAC1	OAC2	OAC3	OAC4	OAC5
Stock	15	15	15	15	15
Mortality	15	10	5	0	0
Survival (%)	0	30	70	100	100

the least in OAC3 (Tables 3-5). Also results of water parameters obtained during the experimental trials were: dissolved oxygen concentration, 6.00 – 6.53mg/l, pH, 8.03 – 8.60 and temperature, 27.30 – 27.64.

Discussion

Growth is one of the major factors used in aquaculture for evaluating nutrient utilization in fish nutrition. It was observed in this study that growth slowed down at the onset of the experiment with gradual increase in weight gain. There were significant variations ($p < 0.05$) in most of growth indices measured at the end of the feeding trial. The highest weight was recorded in *O. niloticus* fed diets 4, signifying better nutrient utilization when compared to other treatment, the trend fish growth was proportional to weight gain, feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) of butyric acid

supplemented diets on *O. niloticus* at 1.5% inclusion shows highest significant growth parameters when compared with those fed with controls diet [11]. The FCR and SGR also proportionate to *C. gariepinus* fed varying levels of ascorbic acid and iron nanoparticles that indicates a significant different $p < 0.05$ in protein efficiency ratio [12]. In addition; Hassaan *et al.* (2014) put reported that *O. niloticus* fed with 1 % ca-lactate supplemented diet had higher growth, increased FI, and improved FCR and PER than the control group. Furthermore Ng *et al.* (2009) submitted that the use of butyric acid improved the feed intake, gut and gastrointestinal tract activity of red hybrid tilapia. Ramli *et al.* (2005) also opined that the inclusion of potassium salt of formic acid at 2 % in tilapia diets led to an increase in weight gain by 18.6%. This positive weight gain trend in this study is attributed to oxalic acids supplementation in fish diet, which reduced the pH level in the stomach, thereby providing a remedy to the problem of feed digestion. Other factors such as species and physiological age, experimental fish, type and level of organic acids, diet composition, and culture conditions may influence growth-promoting effects in aquaculture fish species. However, this finding is contrary to that of Magdy *et al.* (2017) who reported that *O. niloticus* fed with malic acid blend and organic salt was not significant different at 0.5%, 1.0% and 1.5% supplementation in

terms of final weight, weight gain and specific growth rate. According to Da-Neves (2020), the effect of organic acids or organic salts depend on fish species, sources and inclusion levels which probably reflect in different nutrient utilization or digestive processes.

Addition of oxalic acid in *O. niloticus* diets revealed that growth was not linear as fish fed at 2.0% supplementation level had a lower weight gain when compare with those fed at 1.5%. Proximate or chemical analysis is frequently used to determine the influence of feed on fish body composition. The results of proximate analysis of whole fish body composition showed significant variations ($p < 0.05$) when compared in the parameters with the initials and CTR. The low moisture content in fish body is a good quality of preservation of their shelf life. A higher protein composition is an indication that the protein to energy ratio used in the diets was in the right proportion, so there was no sparing of protein for energy in the feeding trial. More protein indicates an added value to the nutritional quality of the fish fed with oxalic-acid supplemented [13]. Reported significant variation in whole body composition of *O. niloticus* carcass fed with varying levels of butyric acid supplemented diets. This finding is similar to that of [14] who reported lower fat and ash contents in *O. niloticus* fed fumaric supplemented diets when compared with the control. Magdt *et al.* (2017) also reported that the use of oxalic and malic acid blend (OAB 1:1) in *O. niloticus* at 1.0% gave the highest protein and ash contents and lowest lipid content when compared with the control.

Water is the habitat of the fish, where different function take place, hence its importance in every experiment. Water parameters measured during experiment varied dissolved oxygen ranges 6.00 – 7.23mg/l, pH (7.02 – 8.03) and Temperature (26.17

– 27.50). These variations were within the acceptable ranges recommended for the culture and rearing of tilapia [15,16]. It could therefore be inferred that experimental diets did not affect the water quality negatively nor led to water deterioration in all the experimental units.

Conclusion

This study had demonstrated that supplementation of oxalic acid probably increased the epithelial layer of intestine and thus increased diffusion of nutrients into the tissue to improve fish growth and did not pose any negative impact on carcass quality. Hence supplementation of *O. niloticus* diets with oxalic acid at 1.5% indicates the best protein retention, survival and percentage weight gain per day. Carcass analysis of the whole body illustrated that the dietary organic acid influenced the nutrient retention in experimental fish. Fish farmers should therefore be enlightened on the use of organic acids in fish diets. Also government should be encouraged to reduce tax on Organic acid or make it available at subsidized rates to fish farmer as plant fertilizers. Subsequent research should be on the use of oxalic acid on other fishes to establish further comparative analysis of its effect in aquaculture fish species and a combination of oxalic acids and prebiotics could also be explored.

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Conflict of interest

Author declares no conflict of interest.

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