



The growth performance and genotoxicity effect of dietary *Aloe vera* on *Oreochromis niloticus* juveniles

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ARTICLE INFO

Keywords:

Aloe vera
Growth performance
Oreochromis niloticus
Genotoxicity effects

Received: 27 February 2020

Accepted: 8 June 2020

Available online: 9 June 2020

DOI: 10.13170/ajas.5.2.16044

ABSTRACT

Growth and all activities of fish depend significantly on the food they consume. However, with the rising price of conventional feedstuffs, there is a need to investigate less expensive and readily available plant source as an additive. This research is designed to study the effect of dietary *Aloe vera* on the growth performance of *Oreochromis niloticus* juveniles (41.71 ± 0.78 g) and its effect on genotoxicity (MN: Micronucleus; LB: Lobed; BD: Bud; BL: Blebbied; BN: Binucleated; NT: Notched). This research investigated the growth performance and genotoxicity activity of *Oreochromis niloticus* juveniles in a tank culture system. *Aloe vera* (fine powder) was used as a test ingredient in the feed formulation at five inclusion levels ($T_1 = 5$ g, $T_2 = 10$ g, $T_3 = 15$ g, $T_4 = 20$ g, $T_5 = 25$ g) and the Control diet (C_0) without the test ingredient. Test diets were fed to *Oreochromis niloticus* juveniles, and each diet was assigned to the treatments and control overall in triplicates. Feeding with test diets was for twelve (12) weeks at 5% of their total body weight daily, which was divided into two and administered at 8 hrs and 16 hrs. The result showed that the highest growth rate (10.99 ± 5.49 g) and the lowest FCR (0.68 ± 0.08) were recorded in the fish fed with the Control diet (C_0). Better growth and nutrient utilization were achieved at low inclusion levels of *Aloe vera*, at 5g(T_1) (8.98 ± 4.49) and 10g (T_2) (6.73 ± 3.28) compared to the higher levels of incorporations of the test ingredient. The mean pH and temperature ($^{\circ}$ C) and dissolved Oxygen level mg/L are 6.50 ± 0.30 , 26.3 ± 0.60 and 4.48 ± 0.52 mg/L respectively. The values of micronuclei for the cultured *Oreochromis niloticus* juveniles were recorded to be ($T_1 = 5.50 \pm 0.50$, $T_2 = 2.00 \pm 1.00$, $T_3 = 2.00 \pm 0.00$, $T_4 = 2.50 \pm 1.50$, $T_5 = 4.00 \pm 1.00$, and $C_0 = 7.00 \pm 2.00$). Nuclear abnormalities were recorded in T_2 (BD = 1.00 ± 0.00), T_3 (NT = 1.00 ± 0.00), T_4 (BD = 1.33 ± 0.47 , BN = 3.33 ± 0.47), and T_5 (BN = 5.33 ± 0.47 , LB = 3.00 ± 1.00 , BL = 1.67 ± 0.47). However, no abnormality was recorded in the fish fed with the Control diet (C_0). This result showed that the slight addition of this *Aloe vera* to the diet of *Oreochromis niloticus* would enhance productivity.

Introduction

Aloe vera is well known plant not only in tribal community but modern lookout and also makes it therapeutic important. Since it is used in Ayurvedic, Homeopathic and Allopathic medicine because various research support that it contains vitamins, minerals, enzymes, amino acids, natural sugar and other bioactive compounds (Akhilesh and Rarawt, 2017). *Aloe vera* as an immunostimulant has been reported that it has the bactericidal and bacteriostatic activity (Mahdavi *et al.*, 2013). It was obvious that oral administration of aloe advanced the resistance of juvenile rockfish to the septicity of *Vibrio alginolyticus* (Kim *et al.*, 1999).

Aloe vera extract has been reported to have effective influence on growth performance of *Cyprinus carpio*, it performs as a growth promoter, appetite stimulator and immunostimulant, reduce stress, reduce food losses, protect fish and enhance better growth of fish (Mahdavi *et al.*, 2013). Aquaculture is currently one of the fastest-growing food-producing sectors in the world. Its global importance is related to its contribution to the supply-demand gap of fish products. With the attendant decline in wild fish catches due to overexploitation of our fisheries resources, which leaves a yawning gap between demand and supply, aquaculture will be the only alternative to fill the

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gap. The projection for 2030 on total world fish production, with improvement in technology and high demand for fish and fisheries product, is expected to reach 201 million tonnes. This indicates an 18 per cent growth over 2016 compared to the observed growth of 2.3 per cent in 2013-2016. Aquaculture is expected to be higher than in other sectors to reach a projection of 109 million tonnes in 2030 with a 37 per cent growth rate over 2016 (FAO, 2018). The aquatic genetic resources (AqGR) are important for aquaculture development, conservation and food security. Accelerating and expanding genetic improvement of key AqGR represents a significant opportunity to enhance the efficiency of aquaculture production and sustainably grow production. (Graham et al., 2019). Nile Tilapia is of Africa origin and is the most important and highly consumed fish in the world today. It has been cultured widely to augment the capture fisheries (Ayoola and Abdul, 2016). They are widely cultured in the tropical region of the world. They are herbivorous species, Tilapia species feed on plankton, vegetation and algae. The high rate of culturing of Tilapia necessitates suitable formulation by using indigenous or local ingredients.

Fish feeds take about 60-70% of the total operational cost in aquaculture (Ayoola, 2010). Traditionally, fishmeal is the major constituents of feed formulations. It is the most preferred components of fish feed due to its balanced amino acid composition, growth potential and palatability. Fishmeal is expensive and this necessitates the researchers to investigate alternative sources of protein in order to reduce cost. This is due to the high demand for fishmeal that limits the supply of the product (Tacon and Metian, 2009; Muchlisin et al., 2016a).

The protein from plant sources seems to be the most appropriate alternative for protein sources in the preparation of the fish diet. The reliance on the addition of the plant-protein constituents in fish feed has increased because of its low - or no - cost and the presence of balanced amino acids (Gatlin et al., 2007).

Besides, the Nile Tilapia, *Oreochromis niloticus*, is naturally adapted to eating plant ingredients (Keenleyside, 1991). Hence, this study is targeted at determining the effect of *Aloe vera* on the growth performance of *Oreochromis niloticus* juveniles, as well as its genotoxicity effects.

Materials and Methods

Study area

The experiment was carried out in the Botanical and Zoological garden of the Faculty of Science, University of Lagos, Akoka. The University of Lagos is located in Lagos, Nigeria between the magnitudes of 6°03'0" N 3°23'10" E / 6.51667°N 3.38611°E.

Experimental design

Two hundred and fifty healthy juveniles of *Oreochromis niloticus* (41.71 ± 0.78 g) were purchased at a fish farm in Ibadan. The fish were acclimatized for four weeks and was fed with 2mm Coppens feed (40% crude protein) to satiation before the commencement of the experiment, and this was done in a tank with safety bars at the top to allow sufficient airflow.

After four weeks of acclimatization, 180 tilapia juveniles were stocked into plastic tanks at the rate of ten (10) fish per tank using a plastic sieve. The rectangular plastic tanks used for the culture has a dimension of length (41cm), width (28.5cm), and height (27 cm). The water level of 20cm depth was maintained in each of the tanks throughout the 12 weeks experimental period. The water supply to the culture tanks was sourced from a borehole, and to prevent pollution and fish mortality, the water in each tank was replaced at two days interval. Although, there were fluctuations in the interval when the water supply was limited. The weight of the fish was determined before the commencement of the experiment and afterwards, they were weighed every two weeks (bi-weekly) using a digital weighing scale.

Experimental diets and feeding trials

The fleshy leaf parts of *Aloe vera* were obtained from Iwaya market in Lagos. The plant was dried under the sun for three days and was ground into a fine powdery form using a home blender. It was kept in a dry container to prevent it from moulds.

Six experimental diets were formulated with *Aloe vera* added to other feed ingredients (100Kg) at 5g, 10g, 15g, 20g, and 25g respectively (Table 1). The crude protein content of the feed is 28%.

After formulation, the feed was pelletized to 2mm using a pelletizing machine at Royal Farms and Veterinary Enterprise, Oba-Agege, Lagos state. Afterwards, the feeds were stored in plastic bottles. Feeding of the fish was done at the early hours of the morning before sunrise (7 am – 8 am)

and in the evening before sunset (4 pm – 6 pm) for 12 weeks. The fishes were fed to satiation.

Table 1. Composition of experimental diets used in this study

Ingredients	C ₀ (kg)	T ₁ (kg)	T ₂ (kg)	T ₃ (kg)	T ₄ (kg)	T ₅ (kg)
Fish meal	10.00	10.00	10.00	10.00	10.00	10.00
Soya bean	21.00	21.00	21.00	21.00	21.00	21.00
Rice bran	26.00	26.00	26.00	26.00	26.00	26.00
Maize	20.00	20.00	20.00	20.00	20.00	20.00
Groundnut cake	21.00	21.00	21.00	21.00	21.00	21.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50	0.50
<i>Aloe vera</i>	-	0.005	0.01	0.015	0.020	0.025

Note: C₀=control, T₁-T₅=Treatment

Growth Parameter index

Weight gain (biweekly gain in weight, average weight gain per fish and tank), specific growth rate (SGR), and feed conversion ratio (FCR) using the formulae indicated below:

Mean weight gain (MWG): The mean weight gain was calculated using the formula (Putra *et al.*, 2016) as follow:

$$\text{Mean weight gain (g)} = W_f - W_i$$

Where: W_f = Final average weight (g); W_i = Initial average weight (g)

Specific Growth Rate

This is the percentage rate of change in the logarithm body weight. It was computed according to Muchlisin *et al.* (2016b). The SGR was calculated using the formula below:

$$\text{SGR (\% day}^{-1}\text{)} = \frac{(\ln W_t - \ln W_o) \times 100}{t}$$

Where SGR = Specific growth rate (% day⁻¹), t = experimental period (days), W_o = initial weight (g), W_t = final weight (g).

Feed conversion ratio

This is the amount of unit weight of food that specimens were able to convert into unit muscle. It was determined by the formula below:

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Total weight gain (g)}}$$

Survival rate

The survival rate was calculated by this following formula based on Muchlisin *et al.* (2016a):

$$\text{Survival rate (\%)} = \frac{N_o - N_t}{N_o} \times 100$$

Where, N_o = total fish at start of experiment, N_t = total fish dead during of experiment.

Protein intake (PI), Protein intake was calculated using the formula:

PI = Feed intake X Percentage (%) protein in the diet.

Extraction of blood from fish

At the end of the entire study, the haematological analysis was carried out. Blood samples were collected via caudal vein puncture. The fishes were taken from each tank using a sieve and each of the fish was held firmly then placed belly upward to show the ventral region using a dry towel. The blood samples were collected from the caudal peduncle with the aid of a 2 ml plastic syringe caudal vein using 5ml ethylenediaminetetraacetate (EDTA) as anticoagulant (AOAC, 1995). Blood, 2.0 ml, was decanted in heparinized bottles for determination of blood parameters.

Genotoxicity evaluation

A single drop of blood is placed on the surface of a clean and grease-free microscope slide at a distance of 2cm from one end. The blood smear is created by carefully consistently extending this drop of blood with the edge of another clean slide held at angle 45-degree to the first. Once prepared, the blood smear slide is dried by gently waving it in the air (Ayoola and Omoile, 2019). The micronucleus was scored according to the method adopted by Ayoola *et al.* (2012).

Proximate analysis of *Aloe Vera*

A sample of the grounded *Aloe Vera* was taken to the Livestock Feed Plc. to evaluate the protein, fat, fibre, ash, moisture content of *Aloe Vera*. The results of the Proximate Composition of *Aloe vera* analysis is in Table 2.

Table 2. Proximate Composition of *Aloe vera*

Ingredients	Percentage of composition (%)
Moisture	11.00
Ash	9.00
Crude lipid	2.00
Crude protein	7.38
Crude fibre	1.50
Carbohydrate	69.26
Total	100.14

The experimental feed was formulated with varying inclusion levels of *Aloe vera* at 5g, 10g, 15g, 20g, and 25g.

Statistical analysis

The data obtained were statistically evaluated using the one-way analysis of variance (ANOVA). The data included means and standard deviations.

Results

The mean water quality parameters of the cultured environment are represented in Table 3. The mean temperature values range from 25.6 – 27.0°C (26.30 ± 0.60), while pH values range between 6.1 – 6.9 (6.50 ± 0.30). The dissolved oxygen, on the other hand, range from 4.48 – 4.61mg/L (4.5 ± 0.6).

Table 3. Mean Water Quality Parameters of the Experimental Tanks

Parameters	Holding tank	Experimental Tanks
pH	6.54 ± 0.33^a	6.50 ± 0.30^a
Temperature(°C)	26.1 ± 1.59^a	26.3 ± 0.60^a
Dissolved oxygen (mg/L)	4.61 ± 0.60^a	4.48 ± 0.52^a

Table 4. Growth parameters of *Oreochromis niloticus* fed with the various diet.

	T ₁ (g)	T ₂ (g)	T ₃ (g)	T ₄ (g)	T ₅ (g)	C ₀ (g)
IMW (g)	41.71 ± 0.78^a	41.87 ± 1.19^a	41.48 ± 1.00^b	41.21 ± 0.93^a	41.65 ± 2.25^a	41.45 ± 1.16^a
FMW(g)	50.69 ± 0.86^b	48.24 ± 1.60^a	51.69 ± 1.26^b	60.42 ± 1.09^a	51.78 ± 4.11^a	63.44 ± 1.60^a
AMW(g)	8.98 ± 4.49^a	6.37 ± 3.18^c	10.21 ± 2.10^a	19.21 ± 2.10^a	10.13 ± 1.86^c	21.99 ± 5.49^a
FCR (g)	0.85	1.06	1.35	1.31	1.40	0.68
SGR	0.22 ± 0.10^a	0.16 ± 0.08^{ab}	0.10 ± 0.05^c	0.08 ± 0.17^a	0.08 ± 0.04^a	0.18 ± 0.09^a
Survival rate (%)	100	100	100	100	100	100

*IMW: Initial Mean Weight, FMW: Final Mean Weight, AMW: Average Mean Weight, FCR: Food Conversion Ratio, SGR: Specific Growth Rate

Genotoxicity Activity

The Control blood sample observed contained elliptical nuclei, which were considered as normal micronucleus. For Test diet 1 (5g of *Aloe vera*), Test diet 2 (10g of *Aloe vera*), and Test diet 3 (15g of *Aloe vera*), the normal micronucleated cells were also observed. However, in the Test diet 3 (15g of *Aloe vera*), there were a few notched nuclei (nuclei with slits that extends well into the nuclear envelope). Deviation from the normal shape was also observed in Test diet 4 (20g inclusion of *Aloe vera*), which

Growth Performance of *Oreochromis niloticus*

Fish fed with the Control diet (C₀) gave the best growth performance. On the other hand, fish fed with Test diet 5 (25g inclusion of *Aloe vera*) resulted in the lower growth rate (Table 4).

The highest body weight gain (10.99 ± 5.49) was also recorded for fish fed with the Control diet (C₀), while the least weight gain (3.73 ± 1.86) was recorded for fish fed with Test diet 5 (25g inclusion of *Aloe vera*).

The highest and lowest FCR was recorded for fish fed with Test diet 5 (25g inclusion of *Aloe vera*) and fish fed with the Control diet (C₀) respectively. Hence, the Control has the best FCR, because the lower the FCR, the higher the body weights gain.

contained binucleated cells (cells with two or more nuclei, and nuclear buds) which are small invagination of the nuclei envelope resembling a micronucleus. In Test diet 5 (25g of *Aloe vera*), a few binucleated cells were also observed, while the other cells appeared to be blebbed (a bulge or protrusion of the cells, characterized by a spherical, bulky morphology) and lobed (roundish and flattish projection) (Table 5 and Figure 1a-e).

Table 5. Mean and Standard Error of Micronucleus and Nuclear Abnormalities

	MN	LB	BD	BL	BN	NT
5g	5.50 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10g	2.00 ± 1.00	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15g	2.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00
20g	2.50 ± 1.50	0.00 ± 0.00	1.33 ± 0.47	0.00 ± 0.00	3.33 ± 0.47	0.00 ± 0.00
25g	4.00 ± 1.00	3.00 ± 1.00	0.00 ± 0.00	1.67 ± 0.47	5.33 ± 0.47	0.00 ± 0.00
C ₀	7.00 ± 2.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*MN: Micronucleus; LB: Lobed; BD: Bud; BL: Blebbed; BN: Binucleated; NT: Notched

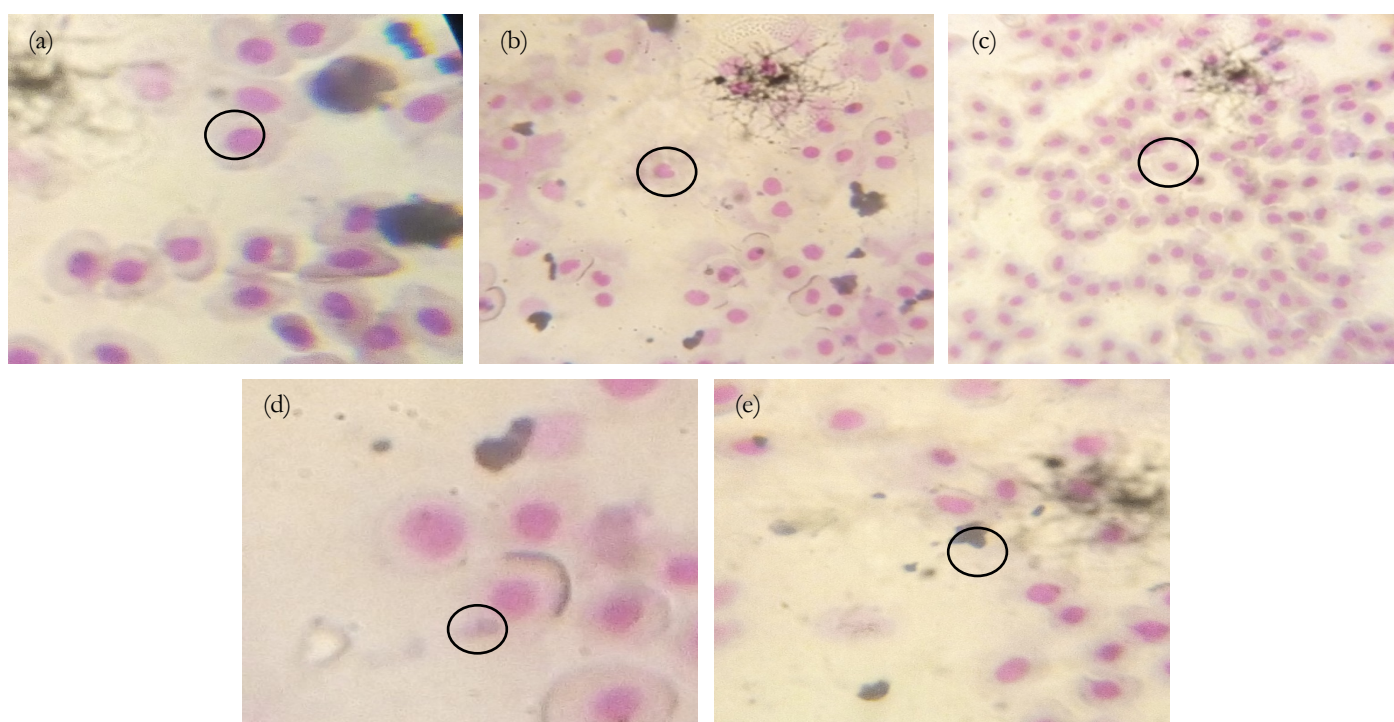


Figure 1. (a) Slide showing micronuclei; (b) notched nucleus; (c) nuclear bud; (d) binucleated cell; (e) blebbed nuclei under a light microscope.

Discussion

Acceptability of the feed by fish is one of the problems encountered when alternative feed sources of plant origin are used in feeding fish, is frequently associated with palatability. This study revealed that *Oreochromis niloticus* juveniles accepted the experimental diet, which indicates that the level of incorporation of Aloe vera, does not affect the palatability of the diets. The total feed intake was highest in the control diet. The lowest and best FCR was also recorded in the Control diet (C_0) which showed that it was able to convert the feed fed into fish flesh. Test diet 5 (25g inclusion of *Aloe vera*) showed a poor growth performance and Food Conversion Ratio. Nutrient utilization and Better growth were achieved in 20g inclusions of *Aloe vera* when compared to other levels of incorporations in the test ingredients.

The body weight recorded in fish fed with the Control diet (C_0) was higher while the feed conversion ratio (FCR) was the lowest than those of the other fish. These align with the work of Ayoola and Omoile (2019) who observed a better growth response on fish fed protein sources than those containing plant protein sources, which are also having a lower FCR.

The best FCR obtained in the Control diet (C_0) could be attributed to the non-inclusion of *Aloe vera* with a lower level of fibre in the diet. The FCR obtained in diets containing *Aloe vera* could be as a result of palatability sources, which result in high FCR in the diet and reduced growth (Ayoola and Omoile, 2019). Suppressed growth and feed conversion ratio in lake trout, *Salvelinus namaycush*, fed with a high level of protein incorporation were also reported by Poston (1986).

The water quality parameters of the experimental setup were within the range recommended for the culture of *Oreochromis niloticus*. This agrees with the work of Getabu (1992) when he worked on the "Mortality and growth parameters in *Oreochromis niloticus*".

Nuclear abnormalities were identified as reported by Ayoola and Bamiro (2017). that, a micronucleus is a round cytoplasmic inclusion having a diameter one-tenth to one-third that of the primary nucleus. The nuclear abnormalities other than micronuclei are classified into five groups, which are: binucleated cells, cells with blebbed nuclei, cells with lobed nuclei, cells with notched nuclei, and nuclear buds or nuclear membranes (Al-Sabt and Metcalfe, 1995, Graham et al., 2019). The erythrocyte abnormalities regularly reoccurring in a cell population is dependent on the kinetics of cell

proliferation (Ayoola and Bamiro, 2017). Other factors such as diet and exposure pathways may also play a role (Al-Sabt and Metcalfe, 1995). This study observed that Blebbed nuclei or nuclear buds have small evaginations of the nuclear envelope resembling a micronucleus, however, are attached by a small thread-like stalk. Lobed nuclei have large evaginations of the nuclear envelope that have no clear shape or definition. A binucleated cell contains two nuclei that are not attached and relatively similar in size. Notched nuclei have clear slits that extend well into the nuclear envelope.

The Control diet had the highest value for micronuclei (7.00 ± 2.00), while Test diet 2 (2.00 ± 1.00) and Test diet 3 (2.00 ± 0.00) had the least value of micronuclei. No abnormalities were recorded in the fish fed with the Control diet. Increase in nuclear abnormalities would signify necrosis or myocardial infarction as reported by Ayoola and Taiwo (2017) or hepatic metabolism, which are all indicators of poor protein quality of the diets.

Conclusions

The result of this study revealed that 20g inclusion of *Aloe vera* to the diet of *Oreochromis niloticus* affected the overall health status of the fish, and this might be as a result of the laxative effect of *Aloe vera*, which increases intestinal water content, as well as peristalsis. However, at a much lower level of inclusion, the growth performance of the fish was moderate. Hence, with the easy access of *Aloe vera*, it could be recommended that the slight addition of this plant to the diet of *Oreochromis niloticus* would enhance growth performance and health status because of its medicinal value.

Declarations of interest

The author(s) declare that there is no conflict of interest with regards to the research, authorship and/ or publication of this article.

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