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EFFECTS OF DIETARY SUPPLEMENTATION OF FUMARIC ACID ON GROWTH PERFORMANCE OF AFRICAN CATFISH *Clarias gariepinus* AND *Aeromonas sobria* CHALLENGE

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ABSTRACT

Five iso-nitrogenous (39.0% crude protein) and iso-caloric diets (510 kJ gross energy) were prepared with fumaric acid added at varying inclusion levels: 0 (D_1), 0.5 (D_2), 1.0 (D_3), 1.5 (D_4) or 2.0 (D_5) g kg^{-1} of diet. Diets were fed to triplicate groups of *Clarias gariepinu*s (mean initial weight 68.14±1.5 g) for 84 days in 50 liters glass tanks. At the end of the experiment, fish fed diet D_3 gave significantly higher growth indices closely followed by the control diet (D_1). There were significant differences in weight gain, specific growth rate, food conversion ratio and percentage survival in fish fed diet D_3 when compared with those fed diet D_5. The haematological parameters of experimental fish revealed significant variations among treatments. The highest haemoglobin, packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) values were recorded in fish fed diet D_2 while the lowest were recorded in those fed diets D_3. Challenge test showed that mortality was 100% in the control while it was between 0 and 86.67% in other treatments. Inclusion of 1.0 g kg^{-1} of fumaric acid in *C. gariepinus* diets boosted growth. Inclusion of 0.5 g kg^{-1} of fumaric acid improved fish haematological parameters. In overall, incorporation of fumaric acid in *C. gariepinus* diets improved fish survival after *Aeromonas sobria* challenge.

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INTRODUCTION

Fumaric acid is a weak, non-toxic organic acid used as food additive since 1946 (Malone, 2000; Xie et al., 2003). It has been used in food preservation because of its effect in reducing bacteria in feed (Luckstadt, 2008). Organic acids have been used in pig feed production for over two decades but there is a paucity of information on their use in aquaculture (Jensen, 1998). Dietary organic acids and their salts are able to inhibit microbial growth in food and consequently preserve the microbial balance in the gastrointestinal tract (Freitag, 2007). In the intestinal tract of the animal, acidifiers act in two ways, reducing the pH level in the stomach and dissociating acid from the bacterial cell (Liu, 2001). Organic acids also improve pancreatic secretions thereby increasing nutrient digestibility (Luckstadt, 2008).

The response of various fish species to different acidifiers has shown their potential alternative to antibiotics in managing their gut micro-flora, promoting growth and survivability (Browdy et al., 2011). African catfish *Clarias gariepinus* is widely cultivated in Nigeria because of its ability to utilize various feedstuffs as food. The fish is a good converter of feed to flesh and is able to withstand harsh environmental conditions. Another characteristic that makes *C. gariepinus* a good experimental fish is its resistance to diseases and fast growth rate. The economic importance of this species increased over the years because of its extensive use in aquaculture (De Graaf and Jansen, 1996).

The uses of dietary organic acids enhanced growth performance of various fish species and have been used as alternatives to other feed additives in aquaculture diets (Liu, 2001, Ramli et al., 2005, Owen et al., 2006, Freitag, 2007, Ng et al., 2009, Luckstadt and Kuhlmann, 2011). Despite these achievements, there is a dearth of information on the effect of dietary fumaric acid on the growth of *C. gariepinus*. Thus, the aim of the present study was to evaluate the response of *C. gariepinus* to fumaric acid supplemented diets challenged with pathogenic bacteria, *Aeromonas sobria*.

MATERIALS AND METHODS

Experimental set up

An indoor feeding experiment was conducted to evaluate the effect of fumaric acid on the growth performance of *C. gariepinus*. Fifteen plastic tanks of 50-litre capacity, each filled with 25 litres of water, were aerated continuously using an air compressor.

Experimental diets

All feedstuffs used in diet preparation were locally purchased from a market in Akure, Nigeria. The active ingredient (fumaric acid) was obtained from the National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa, Niger State, Nigeria. Five iso-nitrogenous (39% crude protein) and iso-caloric (510 KJ gross energy) diets were prepared and designated as D_1 (control), D_2, D_3, D_4 and D_5. Amounts of 0 (control), 0.5, 1.0, 1.5 and 2.0 g kg^{-1} of fumaric acid were taken and mixed with a basal feed (39% crude protein) (Table 1).

Experimental fish

C. gariepinus (N=225) with an average initial body weight (68.14 ± 1.5 g) were obtained from the teaching and research farm of the Federal University of Technology, Akure. The fish were acclimated to laboratory conditions for 14 days during which they were fed a basal diet (39.0% crude protein) at 5% body weight at 8:00-9:00 h and 16:00-17:00 h. The amount of feed given to fish was adjusted weekly based on changes in body weight.

Experimental procedure

The feeding trial lasted 84 days. De-chlorinated tap water was supplied into tanks and filled up to 25 liters level. Stocking of fish was 15 fish / tank with each treatment replicated thrice. One third of the water in each tank was changed daily using hose to avoid deterioration of water quality as a result of accumulation of unconsumed feed and fish.

Table 1. Ingredients (g kg^{-1}) and proximate composition (g) of experimental diets supplemented with fumaric acid

Ingredient	Dietary Treatment				
	D_1 (Control)	D_2	D_3	D_4	D_5
Fish meal	30	30	30	30	30
Soybean meal	30	30	30	30	30
Yellow maize	30	30	30	30	30
Vegetable oil	4	4	4	4	4
Vitamin Premix*	5	5	5	5	5
Starch	1	1	1	1	1
Fumaric acid *(g /kg diet)*	0	0.5	1.0	1.5	2.0
Proximate Composition (% of dry matter)					
Dry matter	93.29	93.42	93.23	93.08	92.79
Ash	15.36	15.25	15.98	16.20	16.73
Fat	12.42	13.02	12.33	13.04	13.61
Protein	39.56	39.28	39.15	39.09	39.08
Fibre	0.85	0.61	0.44	0.38	0.26
NFE	25.10	25.26	25.33	24.37	23.11

*A Pfizer livestock product containing the following per kg of feed: A = 4500 I. U, D = 11252 I.U, E = 71 I.U, K_3 = 2 mg, B_{12} = 0.015 mg, panthothenic acid = 5 mg, nicotinic acid = 14 mg, folic acid = 0.4 mg, biotin = 0.04 mg, choline = 150 mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20 mg, iodine = 0.6 mg, selenium= 2.2 mg, zinc = 20 mg, antioxidant = 2 mg, Nitrogen Free Extract (NFE), Dry Matter (DM), NFE = Nitrogen + Free Extract

Analytical procedure

Water temperature (mercury-in-glass thermometer), pH (Jenway 350) and dissolved oxygen (D − 5509) concentration were routinely monitored in all tanks. At the beginning and end of the feeding trial, pooled samples of 15 fingerlings were analyzed for carcass composition (AOAC, 2005): dry matter (DM) after drying in an oven at 105°C until constant weight; crude protein (N = 6.25) by Kjeldahl digestion and distillation after acid digestion; crude lipid by petroleum ether extraction in a Soxtec apparatus; ash by incineration in a muffle furnace at 550°C for 8 to 12 h.

Fish performance was based on growth performance and nutrient efficiency indices. At the end of the feeding trial, the following indices were calculated: weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) using the following formulae:

Average WG = final average weight (g) – initial average weight (g);

% Weight gain = Initial weight / final weight x 100;

SGR (% d^{-1}) = 100 × (ln *W*t – ln *W*o)/*t*

where *W*t and *W*o represent final and initial body weights of fish, respectively, and *t* represents the duration of the feeding trial; FCR = dry weight of feed (g) / wet weight gain by fish (g); and PER = wet weight gain by fish (g) / protein intake (g)

Where protein intake (g) = protein (%) in feed × total weight (g) of diet consumed / 100.

NFE = 100 − (the addition of the values of protein, fat, ash, moisture and fiber contents)

Twelve fish (four fish / replicate) were used for blood analysis and 5 ml blood samples from each treatment were collected by cardiac puncture using 5 ml disposable syringes into treated Bijou bottles. The blood was stored at –40°C prior to analysis following the methods described by Svobodova et al. (1991).

An immuno-competence test was conducted four days after the end of the feeding trials for 14 days. Fifteen fish were randomly selected from each of the treatments and introduced into 2 ml/l of a 24 hour broth culture of pathogenic strain of *Aeromonas sobria* (1.7 x 10^9 cells/ml). Clinical signs, post-mortem lesions and mortalities were evaluated as described by El-Attar and Moustafa (1996).

Statistical analysis

All data obtained were subjected to one-way analysis of variance (ANOVA) test and using the general linear mode function of Statistical Package for Social Science (SPSS), version 16.0. When appropriate, dietary means were subjected to Duncan's Multiple Range test (Duncan, 1955), set at $p<0.05$.

RESULTS

Analysis of the carcass composition of the fish fed diet D_3 indicated highest protein content (56.06%) while the same parameter was lowest in fish fed D_5 (52.33%). The highest DM, NFE and fat contents (96.08, 6.93, and 23.86%) were recorded in fish fed diet D_5 with the lowest fat (21.47%) and DM (97.08%) contents recorded in those fed diet D_1. However, the highest ash content (15.24%) was recorded in those fed diet D_1 and the lowest in D_5 (12.96%) (Table 2).

Values of water quality parameters monitored during the experiment were: dissolved oxygen concentration, 7.23 − 7.86 mg/l, water temperature, 25.13 − 25.27°C and pH, 7.23 − 7.48. At the end of the feeding trials, there were significant changes in the growth indices of fish (Table 3). Mortalities were recorded in fish fed diets D_2, D_4 and D_5 while in diets D_1 and D_3 percentage survival was 100% (Figure 1). It was observed that the inclusion of fumaric acid in the feed of *C. gariepinus* at different varying inclusion levels gave different results. The highest weight gain (97.37 g) was recorded in fish fed diet D_3 while the lowest (48.57 g) was in those fed diet D_5.

The haematological parameters of the experimental fish are shown in Table 4. Statistical analysis revealed significant differences among treatments with highest haemoglobin, PCV, RBC and WBC values recorded in fish fed diet D_2 while the lowest were recorded in those fed diets D_3. The MCHC val-

Table 2. Proximate composition of whole body of *C. gariepinus* fingerlings fed experimental diets D_1 to D_5 (wet basis)

Parameters	Dietary Treatment				
	D_1 (Control)	D_2	D_3	D_4	D_5
Ash	15.24±0.07^{d}	14.47±0.04^{c}	13.89±0.09^{b}	13.68±0.27^{b}	12.96±0.06^{a}
Fat	21.47±0.17^{a}	21.98±0.12^{ab}	22.55±0.35^{b}	23.25±0.59^{c}	23.86±0.36^{c}
Protein	55.75±1.04^{c}	53.64±0.46^{b}	56.06±0.57^{c}	54.59±0.31^{b}	52.33±0.33^{a}
NFE	4.62±1.11^{a}	6.53±0.56^{bc}	3.66±0.32^{a}	4.83±0.60^{ab}	6.93±0.13^{c}

Values in the same row with different superscript are significantly different ($p<0.05$); NFE = Nitrogen free extract

Table 3. Mean growth performance and nutrient utilization of *C. gariepinus* fingerlings fed experimental diets D_1 to D_5 for 84 days

Parameters	Dietary Treatment				
	D_1 (Control)	D_2	D_3	D_4	D_5
Average initial weight (g)	67.08±0.66^{ab}	68.65±0.67^{bc}	69.14±o.69^{c}	66.64±0.69^{a}	69.20±0.69^{c}
Average final weight (g)	161.20±1.17^{b}	155.92±1.28^{b}	166.52±1.55^{c}	128.39±1.61^{a}	117.77±1.66^{a}
Average weight gain (g)	93.97±0.48^{b}	87.28±0.61^{b}	97.37±0.87^{b}	61.75±0.93^{a}	48.57±0.97^{a}
Weight gain (%)	240.29±1.70^{b}	227.31±1.92^{ab}	240.95±1.20^{b}	192.83±1.40^{a}	170.16±1.40^{a}
SGR (day^{-1})	1.18±0.12^{a}	1.18±0.12^{a}	1.17±0.12^{a}	1.18±0.12^{ab}	1.18±0.12^{b}
Feed intake (g)	239.62±1.16^{d}	222.56±1.15^{d}	197.66±0.17^{c}	150.05±1.20^{b}	111.23±1.08^{a}
PER	0.98±0.04^{bc}	0.98±0.04^{bc}	1.23±0.06^{c}	1.03±0.06^{ab}	1.09±0.06^{a}
FCR	2.55±0.12^{ab}	2.55±0.37^{ab}	2.03±0.38^{a}	2.43±1.20^{b}	2.29±0.91^{c}
Survival (%)	100±0.00^{b}	97.33±2.66^{b}	100±0.00^{b}	86.67±6.66^{ab}	79.33±6.66^{a}

Values in the same row with different superscript are significantly different ($p<0.05$); PER = protein efficiency ratio, FCR = feed conversion ratio

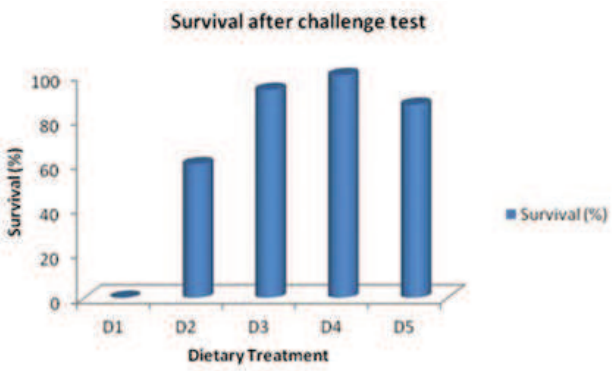

Fig 1. Percentage survival of *C. gariepinus* challenged with *A. sobria*

ues ranged between 33.4 and 33.7 gm/100ml, MCH (29.3 and 30.3 pg) and MCV (87.8 and 91 fl).

Some clinical signs of experimentally infected fish include poor appetite, loss of equilibrium, erratic movement of fish, swimming upside down and loss of all reflexes. Prior to the death of fish, other signs noticed were bleeding of barbels and fins, excess mucus, ulceration on the gill cover, whitening of skin and rotting of tail. Mortality did not occur until the third day and was recorded in fish fed diets D_1, D_2, D_3 and D_5 (Table 5). The mortality recorded in the control was 100% while 0% was recorded in those fed diet D_4. The percentage mortality in D_2 was 60%, D_3 had 93.33% and D_5 recorded 86.67%.

Table 4. Haematological characteristics of *C. gariepinus* fed experimental diets

Parameters	Dietary Treatment				
	D_1 (Control)	D_2	D_3	D_4	D_5
Hb (*g/dl*)	9.7±0.03^{b}	11.7±0.05^{c}	8.1±0.01^{a}	8.4±0.00^{a}	8.7±0.01^{a}
PCV (%)	29±0.50^{b}	35±0.50^{c}	24±0.50^{a}	25±0.50^{a}	26±0.50^{ab}
WBC (x 10^3/mm^3)	5.9±0.50^{b}	6.5±0.50^{c}	4.2±0.50^{a}	5.6±0.50^{b}	4.8±0.50^{a}
RBC (*μ/l*)	3.30±0.03^{c}	3.85±0.02^{b}	2.65±0.01^{a}	2.80±0.01^{a}	2.95±0.03^{a}
MCHC (*g/dl*)	33.4±0.00^{a}	33.4±0.05^{a}	33.7±0.00^{b}	33.60±0.01^{b}	33.4±0.05^{a}
MCH (*pg*)	29.30±0.05^{a}	30.30±0.05^{b}	30.00±0.05^{ab}	30.00±0.05^{ab}	29.4±0.05^{a}
MCV (*fl*)	87.8±0.05^{a}	91.0±0.05^{b}	88.8±0.05^{ab}	89.30±0.05^{ab}	88.1±0.05^{a}
Neutrophils (%)	64±0.20^{d}	62±0.25^{c}	49±0.20^{a}	51±0.25^{a}	60±0.45^{b}
Lymphocytes (%)	36±0.35^{a}	38±0.35^{b}	51±0.45^{e}	47±0.15^{d}	40±0.20^{c}
Monocytes (%)	1±0.00^{a}	1±0.00^{a}	1±0.00^{a}	2±0.00^{b}	1±0.00^{a}

Values in the same row with different superscript are significantly different ($p<0.05$)
PCV = packed cell volume, Hb = haemoglobin estimation, WBC = white blood cell count, RBC = red blood cell count, MCHC = mean cell haemoblobin concentration, MCH = mean corpuscular haemoglobin, MCV = mean corpuscular volume

Table 5. Challenge test using *Clarias gariepinus* exposed to *Aeromonas sobria*

Parameter	Dietary Treatment				
	D_1	D_2	D_3	D_4	D_5
No. of fish before challenge test	15	15	15	15	15
No. of fish after challenge test	0	9	14	15	13

DISCUSSION

The proximate compositions of the experimental diets analyzed were within the acceptable range for commercial fish. US Nutrition Data Base (1999) reported that most of the commercial fish feed for catfish feed contain 32 - 35% crude protein and 10 − 15% lipid level. Santiago and Lovell (1988) estimated the protein requirement for tropical catfish to be 35 − 40%. Daramola and Osanyinlusi (2006) recommended that moisture content in fish diets should not be more than 10% for optimal storage and prevention of spoilage. Moisture content experimental diets were between 6.58 − 7.21% which is within the recommended range. Lipids are primarily included in formulated diets to maximize their protein sparing effect being a source of energy. The observed lipid values were in line with Ross (1985) who reported that in general 10 − 20% of lipid in diets for some of the freshwater fish families (Claridae, Cichlidae, Clupeidae, Cyprinidae) gives optimal growth without producing an excessively fatty carcass.

Jauncey (2000) indicated that fiber content of more than 12% is not desirable in most of fish diets. High inclusion rate of fiber in fish diets would consequently result in decrease in nutrient quality. The analyzed crude fiber contents of all the experimental diets were within the recommended dietary limit for most freshwater fishes. The addition of fumaric acid in the experimental diets had no negative effects on the whole-body composition of *C. gariepinus* (Table 2). Moisture, protein, lipid and ash contents were not significantly affected by dietary treatment. Similar results were recorded by Sarker et al. (2012) who used six blends of organic acids in diets of *Oreochromis niloticus* and reported that organic acid led to slight increase in ash content of *O. niloticus*, which suggests that total mineral uptake might be enriched through diet supplementation by organic acids (Ng et al., 2009).

Water parameters were within the acceptable ranges recommended for rearing and culturing most of the tropical fishes (NRC, 1996). At the end of the feeding trial, there were significant differences ($p<0.05$) in final weight (FW), specific growth rate (SGR), weight gain (WG), percentage weight gain (PWG), protein efficiency ratio (PER), feed conversion ratio (FCR) and survival rate (SR). Fish fed on diet D_3 had the highest growth performance and was consistently followed by those fed diet D_1 (control). Fish fed on diet D_5 performed poorly in all of the growth parameters.

Owen et al. (2006) demonstrated that 1.0% fumaric acid inclusion level in poultry feed led to a higher weight gain than 1.5 or 2.0% inclusion level. This report agrees with the result obtained in this research as 1.0 g of fumaric acid / kg in diet of *C. gariepinus* led to better nutrient utilization when compared to others that have higher inclusion levels. Luckstadt and Kuhlmann (2011) also indicated that 1.0% fumaric acid in broiler diets resulted in higher body weight gain. Protein efficiency ratio is a measure of growth using the dietary protein as an index, therefore, high PER value will be obtained if the weight gain is high compared to the dietary protein intake. In this study, the highest mean PER value was observed in *C. gariepinus* fed diet D_3; this may be a result of the balance in amino acids as observed by Sarker et al. (2012).

Analyses of the fish carcass indicate more protein retained in the body at the end of the experiment. This suggests that the protein to energy ratio used in the feed was at the right level and as a result there was no sparing of protein for energy (Browdy et al., 2011). No mortality was recorded in fish fed diets D_1 and D_3 when compared to others. Mortalities recorded in other treatments might be a result of higher inclusion levels of fumaric acid in their diets. However, this is not linear because no mortality was recorded using diet D_3 which had a higher inclusion than D_2.

There are wide ranges of various factors which influence the variability of most haematological parameters. Such factors include fish species, age, gender, water quality, temperature, feed, sampling methods, etc. Bello-Olusoji et al. (2006) reported significant means of evaluating physiological condition of a cultured fish through its blood. Also, blood analysis could be considered in assessing fish feed (Adeparusi and Ajayi, 2004). There were significant differences ($p<0.05$) in all the blood indices measured when compared with the control. The Hb, PCV, WBC and RBC of fish fed diet D_2 were higher than the others. Furthermore, in other immunological parameters such as MCHC, MCH, MCV and differential counts, those fish fed fumaric acid performed better when compared with the control. This could be attributed to the fact that organic acids prevent the proliferation of pathogenic bacteria and modulate indigenous bacteria so that health and immune status of fish is improved (Ravindran, 2006).

The challenge test with *Aeromonas sobria* revealed the resistance ability of *C. gariepinus* fed different levels of fumaric acid in diet. Mortality did not occur until the third day. The result is in line with the report of Ramli et al. (2005) that hybrid tilapia (*Oreochromis spp.*) fed potassium diformate in diets showed higher survival when challenged with *Vibrio anguillarium*.

CONCLUSION

Inclusion of 1.0 g kg^{-1} fumaric acid in *C. gariepinus* diets boosted growth without having any negative effect on car-

cass composition. Inclusion of 0.5 g kg^{-1} of fumaric acid improved fish haematological parameters. Generally, incorporation of fumaric acid in *C gariepinus* diets improved fish survival after *Aeromonas sobria* challenge. The response of *C. gariepinus* to fumaric acid indicates its potential as alternative to antibiotics in boosting fish growth through better nutrient utilization.

Sažetak

UTJECAJ FUMARNE KISELINE KAO HRANIDBENOG DODATKA NA PERFORMANSE RASTA AFRIČKOG SOMA *Clarias gariepinus* I IZLOŽENOST PATOGENU *Aeromonas sobria*

Za potrebe istraživanja pripremljeno je pet hranidbenih smjesa izjednačenih prema hranidbenom sastavu (39% sirovih proteina, 510 kJ bruto energije) u koje je nadodana fumarna kiselina s različitim udjelima: 0 (D1), 0,5 (D2), 1,0 (D3), 1,5 (D4) ili 2,0 (D5) g kg^{-1} od ukupne smjese. Afrički somovi su podijeljeni u triplikate te hranjeni 84 dana u 50 litarskim bazenima. Na kraju pokusa, ribe hranjene hranidbenom smjesom D3 imale su značajno više indekse rasta, usporedive sa sličnim rezultatima kontrolne hranidbe (D1). Utvrđene su značajne razlike u masi, specifičnoj stopi rasta, konverziji hrane i postotku preživljavanja kod usporedbe riba hranjenih hranidbenim smjesama D3 i D5. Hematološki parametri ukazuju na značajne varijacije između tretmana. Najviše vrijednosti hemoglobina, PCV, eritrocita i leukocita zabilježene su kod riba hranjenih s D2, a najniže kod riba hranjenih s D3 hranidbenom smjesom. Pri izlaganju riba patogenu, zabilježen je 100% mortalitet u kontrolnoj skupini te između 0 – 86,67% u ostalim tretmanima. Možemo zaključiti da je uključivanje 1,0 g kg^{-1} fumarne kiseline u hranidbu afričkog soma unaprijedilo rast riba dok je inkluzija od 0,5 g kg^{-1} poboljšala krvne pokazatelje. Ugradnja fumarne kiseline u hranidbu afričkog soma poboljšala je preživljavanje nakon izloženosti patogenu *Aeromonas sobria*.

Ključne riječi: organska kiselina, preživljavanje, hematološki parametri, som, patogene bakterije

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