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Comparison of dietary butyric acid supplementation effect on growth performance and body composition of *Clarias gariepinus* and *Oreochromis niloticus* fingerlings

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Abstract

This study was conducted using butyric acid as a growth enhancer in the diets of *Clarias gariepinus* and *Oreochromis niloticus*. *C. gariepinus* (42.39 g±1.17) were fed diets containing varying inclusion levels of butyric acid (BA); 0 (CG1), 0.5 (CG2), 1.0 (CG3), 1.5 (CG4), 2.0 (CG5) g kg $^{-1}$, where, CG1 is the control or reference diet. *O. niloticus* (25.50 g±0.50) were also fed diets containing varying inclusion levels of butyric acid; 0 (ON1), 0.5 (ON2), 1.0 (ON3), 1.5 (ON4), 2.0 (ON5) g kg $^{-1}$, where, ON1 is the control or reference diet. Diets for *C. gariepinus* contained 40 % while those of *O. niloticus* had 35 % crude protein. Fish were fed to satiation twice daily in two equal portions for 12 weeks. At the end of the experiment *C. gariepinus* fed on diet CG5 (2 g kg^{-1} BA) had an improved growth of over 600 % while *O. niloticus* fed diet ON4 (1.5 g kg^{-1} BA) showed over 400 % improvement in growth when compared with those fed the control or reference diets. The highest carcass protein were also obtained in those fed BA diets when compared with their respective control diets. The results of the present study showed that there was better nutrient utilization in *C. gariepinus* and *O. niloticus* fed BA supplemented diets. Furthermore, there were no significant variations in the survival of fish fed BA diets when compared with their controls except for those fed ON4 which showed 100 % survival. Quality of water parameters were within the acceptable limits for the culture of fin fishes.

Keywords: Butyric acid, Growth enhancer, *Clarias gariepinus*, *Oreochromis niloticus*, Survival

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Introduction

Aquaculture remains the only viable alternative to bridge the gap between the demand and supply of fish to the growing world populace. The continual depletion of fish and fishery products in the wild has 'given birth' to numerous fish farms that are the last hope in recent times to ameliorate protein deficiencies in human diets. The constraints to aquaculture development are the high cost of feed production, quality fish seeds and good management practices (Jamiu and Ayinla, 2003).

Africa as a continent has begun to scale up its aquaculture production from 1.2 to 2.2 % in the last 10 years to boost the protein intake of people (FAO, 2012). The predominantly farmed fish species in Africa are the fin fishes; catfishes and tilapia.

The African mud catfish (*Clarias gariepinus*) is the most populous among fish farmers in Nigeria which is closely followed by Nile tilapia, (*Oreochromis niloticus)*. These two are the commonly used species in scientific research because of their adaptability to the tropical region. *C. gariepinus* is easy to culture, hardy, has a high feed conversion ratio, has the ability to make use of locally available feedstuff, has high market value and the ability to withstand harsh weather conditions; and hence, it is used in research (De Graaf and Jansen, 1996). The flesh of this species is also highly acceptable among the teeming populace due to its low cholesterol content. Taking into consideration the health risks associated with meat intake. *O. niloticus* is the second most farmed and researched fish species in Nigeria as it has a great potential for intensive culture.

The rapid decline in fish and fisheries resources coupled with the incessant outbreak of diseases has led nutritionists to proffering possible solutions through the use of dietary additives (Liem, 2009). These additives in the recent past have been antibiotics which aquaculture products have gradually developed resistance to, hence, the need for other alternatives (Ng and Koh, 2011). Some of these substitutes are; organic acids, pro and prebiotics, enzymes and nucleotides.

Organic acids are organic compounds with acidic properties and have been used as additives in the food and pharmaceutical industries for about a decade now but which are gradually gaining grounds in aquaculture (Dibner, 2004). Organic acids have continued to receive serious attention because of their uses in prophylactic treatments and antimicrobial properties against numerous pathogenic bacteria (Da Silva *et al*., 2013). They have also been reported to improve growth performance and nutrient utilization in farmed fish species (Omosowone *et al.*, 2015; Ng *et al.*, 2015). Furthermore, there has been the use of butyric acid and its salts in poultry and shrimp diets; however, there is a dearth of information on its use in fish diets. Therefore, this research work focused on the effect of dietary butyric acid on the growth performance and nutrient utilization in *C. gariepinus* and *O. niloticus.*

Materials and methods

Experimental diets

Feed ingredients used in experimental diets were purchased from a local market while the test ingredient (butyric acid) was obtained from the National Institute for Freshwater Fisheries Research (NIFFR), New-Bussa, Niger State, Nigeria. Two diets with crude protein levels (40 % and 35 %) were formulated for *C. gariepinus* and *O. niloticus* respectively. Five iso-nitrogenous diets with the same base ingredients (Tables 1 & 2) were prepared for each fish species. The test ingredient (butyric acid) was added at varying inclusion levels; 0, 0.5, 1.0, 1.5 and 2.0 g kg^{-1}. All ingredients were measured according to specifications, milled into fine powder (2 mm particle size), mixed thoroughly to obtain homogeneous dough and pelletized in a Hobart A-2007 mixing and pelleting machine (Hobart Ltd, London, UK). The resultant pellets were sun-dried (at temperatures between 32 and 34 ^{o}C for 72 h), packed in polythene bags and later refrigerated (at -20 ^{o}C) prior to use. Diets for *C. gariepinus* were designated as CG1 (control), CG2, CG3, CG4 and CG5, while those for *O. niloticus* were ON1 (control), ON2, ON3, ON4 and ON5. Proximate compositions of the prepared experimental diets were determined using AOAC (2005) methods. Growth indices were calculated using appropriate methods:

i. Weight gain (g)=W_2-W_1

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

ii. Specific Growth Rate (% d^{-1})

$$= \frac{100\,x\left(\ln[W_2]-\ln[W_1]\right)}{T}$$

Where; W_1 and W_2 are logarithms of initial and final fish weight, respectively, and T is the number of experimental days;

iii. Feed Conversion Ratio (FCR)

$$= \frac{amount\ of\ food\ given}{Total\ amount\ of\ fish\ produced\ (\ Weight\ gain\ )}$$

iv. Feed intake (g) = *quantity of feed fed x experimental period*

v. Percentage Weight Gain

$$= \frac{Final\ mean\ weight}{Initial\ mean\ weight}\ x\ 100$$

vi. Survival (%)

$$= \frac{No.\ of\ fish\ at\ the\ end\ of\ the\ \exp eriment}{Total\ No.\ of\ fish\ at\ the\ onset\ of\ the\ \exp eriment}$$

Table 1: Gross composition (g 100 g^{-1} dry matter) and proximate composition of experimental diets for *Clarias gariepinus* with crude protein values of 40%.

Ingredient	CG1 (Control)	CG2	CG3	CG4	CG5
Fishmeal	27.8	27.8	27.8	27.8	27.8
Soybean meal	27.8	27.8	27.8	27.8	27.8
Yellow maize	34.4	33.9	33.4	32.9	32.4
Veg. oil	4.0	4.0	4.0	4.0	4.0
*Vit./min. premixes	3.0	3.0	3.0	3.0	3.0
Starch	3.0	3.0	3.0	3.0	3.0
Butyric acid (g kg^{-1})	0.0	0.5	1.0	1.5	2.0
Proximate Composition (100 %)					
Dry matter	90.34	91.53	92.79	89.19	90.73
Protein	38.69	38.64	38.19	38.59	38.31
Lipid	14.46	15.05	17.90	15.38	15.75
Ash	6.79	6.95	7.46	7.31	7.59

Table 1 continued:					
Fibre	1.05	1.46	1.49	1.27	2.31
NFE	29.35	29.43	27.75	27.26	26.77

*Vitamin premix- A Pfizer livestock product containing the following per kg of feed: A = 4500 I, U, D = 11252 I.U, E = 71I.U,K3=2mg, B12=0.015mg, pantothenic acid = 5mg, nicotinic acid = 14 mg, folic acid = 0.4mg, biotin = 0.04 mg. choline = 150mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg, NFE = Nitrogen Free Extract= 100- (crude protein +crude fiber +lipid content +moisture content +Ash)

Table 2: Gross composition (g 100 g dry matter^{-1}) and proximate composition of experimental diets for *Oreochromis niloticus* with crude protein values of 35%.

Ingredient	**ON1 (Control)**	**ON2**	**ON3**	**ON4**	**ON5**
Fishmeal	23.25	23.25	23.25	23.25	23.25
Soybean meal	23.25	23.25	23.25	23.25	23.25
Yellow maize	43.50	43.00	42.50	42.00	41.50
Veg. oil	3.0	3.0	3.0	3.0	3.0
*Vit./min. premixes	4.0	4.0	4.0	4.0	4.0
Starch	3.0	3.0	3.0	3.0	3.0
Butyric acid (g kg^{-1})	0.0	0.5	1.0	1.5	2.0
Proximate Composition (100 %)					
Dry matter	94.52	94.19	93.50	93.57	93.78
Protein	37.22	36.25	38.30	38.71	37.38
Lipid	12.35	13.79	12.84	13.80	13.78
Ash	3.42	3.68	4.14	3.19	3.72
Fibre	7.80	7.43	6.89	8.45	7.10
NFE	33.73	32.37	32.23	29.42	31.80

*Vitamin premix- A Pfizer livestock product containing the following per kg of feed: A = 4500 I, U, D = 11252 I.U, E = 71I.U,K3=2mg, B12=0.015mg, pantothenic acid = 5mg, nicotinic acid = 14 mg, folic acid = 0.4mg, biotin = 0.04 mg. choline = 150mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg, NFE = Nitrogen Free Extract= 100- (crude protein +crude fiber +lipid content +moisture content +Ash)

Experimental design and setup

A completely randomized block design was used in which all the experimental units were homogenous with the test ingredient (butyric acid) as the only source of variation. Experimental fish samples; *C. gariepinus* (42.39±1.17 g) and *O. niloticus* (25.50±0.50 g) were obtained from the Fisheries and Aquaculture Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. Fish were certified pathogen free by the Veterinary Unit of the Department of Animal Production and Health of the University. Fish were acclimated to laboratory conditions for 10 days and fed their respective control diets to ensure uniformity before the commencement of the feeding trials. Fish were randomly distributed to 15 glass tanks of 50 L water capacity at the rate of 10 fish for each tank for *C. gariepinus* and 15 fish for each tank for *O. niloticus.* They were all fed to apparent satiation twice daily (8:00 – 9:00 and 16:00 – 17:00 h) in two equal portions for 12 weeks. Unconsumed feed particles and faeces were siphoned daily from tanks while culture water was partially (50 %) drained and changed to maintain good water quality. Three major water quality parameters (dissolved oxygen, temperature and pH) were monitored on a weekly basis. Dissolved oxygen was measured using

D – 5509 meter, water temperature was measured using a mercury-in-glass thermometer, while for water pH, a portable pH meter (Knick Portamess pH meter, Model 912) was used.

Growth trial

Fish were weighed fortnightly and change in weight was determined using a Citizen's electronic balance (maximum capacity; 5 kg). Experimental setups were checked daily for mortality, and when found, they were removed and the number recorded. Growth indices evaluated included; final weight, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), survival (%), feed intake, etc using appropriate formulae. Proximate analyses of experimental fish carcasses were also carried out using AOAC (2005) methods.

Statistical analysis

All data obtained were statistically assessed using analysis of variance (ANOVA, through the general linear model procedure of the SPSS 21.0 software). The values were expressed as means±standard error. Duncan's multiple range ad-hoc tests were used to test the significance of the difference between means by considering the differences significant at $p<0.05$.

Results

Experimental results showed that both fish species revealed significant differences in growth parameters measured between and within treatments (Tables 3 and 4). The highest increase in fish weight was obtained in *C. gariepinus* fed diet CG5 and in *O. niloticus* fed diet ON4. The weight gain in percentage was over 600 % in *C. gariepinus* and 400 % in *O. niloticus* when compared with the reference diets. Table 3 shows the body composition of *C. gariepinus* at the beginning and end of the experiment. Significant variations ($p<0.05$) were observed between the initial and final body compositions of fish during the experiment with respect to moisture, crude protein, lipid and ash. All fish had increased protein content over the initial value of 45.73 % but this was not different among treatments. *C. gariepinus* fed the test diets had higher lipid and ash contents compared with the initial. Table 4 revealed the body composition of *O. niloticus* at the beginning and end of the experiment. There were significant variations ($p<0.05$) between the initial and final body compositions of fish during the experiment with respect to dry matter, crude protein, lipid and ash. All fish had increased protein content over the initial value of 42.91 % but this was not different among treatments. Lower lipid and ash contents were recorded in the carcass of initial *O. niloticus* sampled when compared with those fed the test diets. The water quality parameters measured during the experimental period are shown in Figures 1 and 2. There were no significant variations ($p>0.05$) in dissolved oxygen and pH values in all treatments, however, significant variations were recorded in temperature for *C. gariepinus*. For *O. niloticus*, there were no significant

variations (p>0.05) in dissolved oxygen and pH values in all treatments but a slight variation was observed in the water temperature of those fed diet ON5.

Table 3: Growth performance and nutrient utilization (Mean±S.E) of *Clarias gariepinus* during the experimental period.

Parameter	CG1 (Control)	CG2	CG3	CG4	CG5
IW	41.90±0.46^{a}	42.60±0.82^{a}	43.10±0.68^{a}	42.77±1.04^{a}	41.60±0.12^{a}
FW	199.30±1.77^{a}	208.50±1.73^{b}	240.50±1.66^{c}	220.57±1.01^{b}	314.20±2.28^{d}
WG	157.40±1.71^{a}	165.93±0.98^{b}	197.40±1.72^{c}	177.80±1.11^{b}	272.60±2.27^{d}
WG (%)	376.50±44.05^{a}	389.73±5.75^{a}	459.19±45.97^{c}	417.36±34.76^{b}	655.20±54.04^{d}
SGR	2.83±0.22^{a}	2.90±0.05^{a}	3.12±0.11^{b}	2.99±0.06^{a}	3.66±0.08^{b}
FCR	2.68±0.02^{a}	2.68±0.02^{a}	2.68±0.02^{a}	2.68±0.03^{a}	2.67±0.02^{a}
FI	422.62±46.04^{a}	445.53±2.62^{b}	530.02±46.08^{c}	477.39±29.71^{b}	731.93±61.05^{d}
% survival	96.67±3.33^{a}	96.67±3.33^{a}	96.67±3.33^{a}	96.67±3.33^{a}	96.67±3.33^{a}

Means in a given column with the same letter were not significantly different at (p< 0.05)
IW=Initial weight (g), FW=Final weight (g), WG=Weight gain (g), WG (%) =Weight gain (%), SGR=Specific growth rate, FCR=Feed conversion ratio and FI=Feed intake (g)

Table 4: Growth performance and nutrient utilization (Mean±S.E) of *Oreochromis nilloticus.*

Parameter	ON1 (Control)	ON2	ON3	ON4	ON5
IW	25.10±0.20^{a}	24.90±0.12^{a}	25.23±0.16^{a}	25.19±0.32^{a}	25.75±0.25^{a}
FW	119.89±3.86^{a}	129.52±3.27^{b}	119.71±5.00^{a}	134.60±1.80^{c}	124.20±4.27^{b}
WG	94.79±3.13^{a}	104.62±3.86^{b}	94.48±4.69^{a}	109.41±4.76^{c}	98.48±4.13^{a}
WG (%)	377.65±32.43^{a}	420.16±43.12^{c}	374.47±30.41^{a}	434.34±47.60^{d}	382.47±43.03^{b}
SGR	2.35±0.51^{a}	2.40±0.11^{b}	2.35±0.20^{a}	2.43±0.56^{b}	2.37±0.32^{a}
FCR	4.01±0.75^{c}	3.77±0.19^{b}	4.03±0.38^{c}	3.65±0.41^{a}	3.82±0.63^{b}
FI	380.39±7.56^{a}	394.04±8.40^{c}	380.88±5.87^{b}	399.81±1.72^{c}	376.58±3.05^{a}
Survival	96.67±3.33^{a}	96.67±3.33^{a}	96.67±3.33^{a}	100.00±0.00^{b}	96.67±3.33^{a}

Mean in a given column with the same letter were not significantly different at (p<0.05)
IW=Initial weight (g), FW=Final weight (g), WG=Weight gain (g), WG (%) =Weight gain (%), SGR=Specific growth rate, FCR=Feed conversion ratio and FI=Feed intake (g)

Table 5: Whole body composition (Mean±S.E) of *Oreochromis niloticus* carcass fed varying levels of butyric acid supplemented diets.

Parameter (%)	ON initial	ON1 (Control)	ON2	ON3	ON4	ON5
Moisture	8.16	6.47±0.07^{ab}	6.50±0.00^{b}	6.40±0.20^{b}	6.60±0.00^{b}	6.04±0.09^{a}
Protein	42.91	46.22±0.32^{a}	47.81±0.68^{a}	46.41±0.62^{a}	48.12±0.92^{a}	47.90±0.75^{a}
Lipid	20.34	24.29±0.26^{ab}	22.35±0.08^{a}	24.12±1.14^{ab}	26.27±1.17^{b}	25.51±1.78^{ab}
Ash	11.21	13.21±0.18^{b}	13.12±0.20^{b}	13.17±0.29^{b}	12.85±0.34^{ab}	12.13±0.11^{a}
NFE	17.38	9.81±0.41^{a}	10.31±0.92^{a}	9.90±1.83^{a}	6.16 ±1.73^{a}	8.42±2.63^{a}

Means with the same superscripts in the same row are not significantly different at p<0.05

Table 6: Carcass composition (Mean±S.E) of *Clarias gariepinus* carcass fed varying levels of butyric acid supplemented diets.

Parameter (%)	CG initial	CG1 (Control)	CG2	CG3	CG4	CG5
Moisture	12.28	9.95±0.01^{c}	5.40±0.03^{a}	5.79±0.11^{b}	5.86±0.02^{b}	5.61±0.26^{a}
Protein	45.73	49.33±0.51^{a}	49.00±0.07^{a}	50.84±0.26^{b}	50.07±0.95^{a}	51.70±0.65^{b}
Lipid	23.55	27.36±0.14^{a}	28.01±0.39^{a}	27.72±0.55^{a}	27.85±1.04^{a}	30.66±0.28^{b}
Ash	8.16	10.05±0.42^{a}	11.47±0.14^{b}	11.69±0.02^{b}	11.07±0.44^{b}	12.16±0.04^{c}
NFE	10.28	6.77±0.37^{b}	4.55±0.14^{ab}	3.96±0.20^{ab}	4.80±1.53^{ab}	2.34±1.10^{a}

Means with the same superscripts in the same row are not significantly different at p<0.05

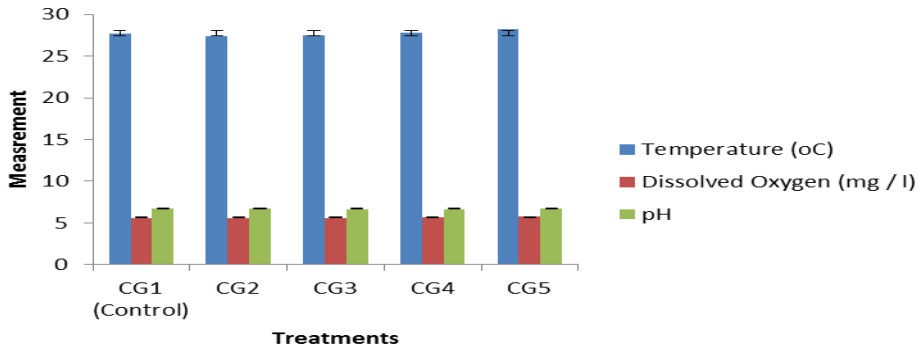

Figure 1: Water parameters of *Oreochromis niloticus* fed butyric acid supplemented diets.

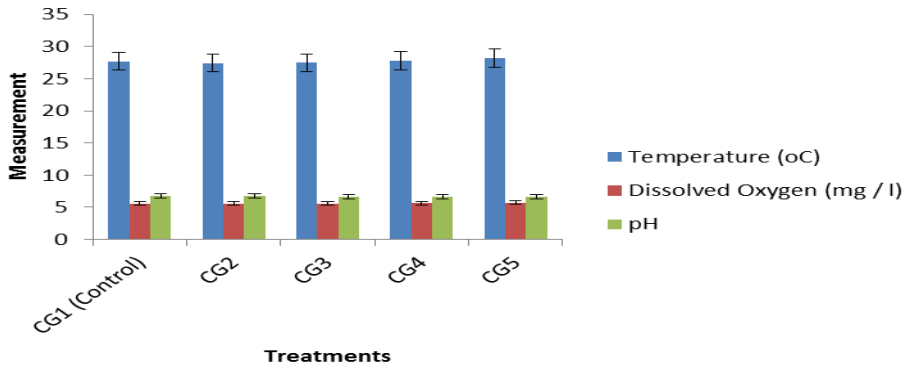

Figure 2: Water parameters of *Clarias gariepinus* fed butyric acid supplemented diets.

Discussion

The mean initial stocking weight of *C. gariepinus* and *O. niloticus* were 42.39±1.17 g and 25.50±0.50 g, respectively. The result of the study revealed significant differences (p<0.05) in all the growth indices measured. It was discovered that *C. gariepinus* fed a BA diet at CG5 had the highest growth performance compared with the control in respect to final weight, weight gain, percentage weight gain, specific growth rate and feed intake.

The FCR and survival do not show any significant differences (p>0.05). The increase in weight was proportional to increase in test ingredient up to CG3, but there was a decline at CG4 while a sky rocketed increase was observed in *C. gariepinus* fed diet CG5. For the *O niloticus*, the highest growth performance, SGR, FCR and survival were observed in those fed diet ON4 although there was no significant difference (p>0.05) in their feed intake. It had been demonstrated that the inclusion of a blend of butyrate glycerides in broiler diets at 2g kg^{-1} had a beneficial effect on their live performance (Antongiovanni *et al.*, 2007). Omosowone *et al.* (2015) also reported that the inclusion of fumaric acid in the diets of *C. gariepinus* led to a higher growth. Similarly, Romano *et al.* (2015) also reported increased weight in shrimp fed a diet supplemented with organic acids blend. Increased growth in fish fed organic acid supplemented diets is believed to

be enhanced by nutrient and mineral digestibility.

An experiment by De Wet (2005) demonstrated that rainbow trout fingerlings fed on 10 and 15 kg acidifier 1000 kg^{-1} feed had significantly higher final weights compared to the control group after three months of feeding on artificial growth promoters (AGP). Owen *et al.* (2006) tested the sodium salt of butyric acid as a feed additive in the omnivorous tropical catfish (*C. gariepinus*) at 2 kg 1000 kg^{-1} in a fish meal based diet and a defatted soya concentrate diet. No significant variations were found in *C. gariepinus* fed the defatted soya concentrate diets when compared with the control. However, in the catfish fed on a fish meal diet, the weight gain was observed to be slightly higher (from 131.3% in control to 141.4% for sodium butyrate group). Furthermore, Da Silva *et al.* (2013) opined that butyrate salts in shrimp diets acted as feed attractants which led to significantly increased feed intake when compared with the control. Similar findings of improved growth were recorded when *Labeo rohita* was fed an organic acid containing phosphate salt (Baruah *et al.*, 2007).

Organic acids affect the feed, gut and gastrointestinal tract of the animal by the reduction in pH (Liu, 2001; Freitag, 2007; Luckstadt, 2008; Ng *et al.*, 2009). Another reason for improved growth is attributed to the aroma of butyric acid which is used as an attractant in the diet of shrimp (Da Silva *et al.*, 2015; Ng *et al,* 2015). This tends to increase feed intake, thus, increasing their growth and feed efficiencies over the experimental period. No mortality was recorded in *O. niloticus* fed diet ON4 which was significantly (p<0.05) different from others. Mortalities recorded was generally low across all treatments. As such, this cannot be attributed to the incorporation of butyric acid in fish diets as they were recorded even in control diets.

The inclusion of butyric acid in the experimental diets had no negative effects on the whole-body composition of *C. gariepinus*. The crude protein content recorded in the carcass of *O. niloticus* did not reveal any significant differences as they were within the same range. This is however different in *C. gariepinus* as there was a slight variation in the crude protein of fish carcass. Also, there was no variation in the lipid content of *C. gariepinus* carcass except in those fed diet CG4. While for the *O. niloticus*, those fed diets ON4 and ON2 showed the highest and lowest lipid contents, respectively. The variation observed in fish carcass lipid content in this study is slightly different from what was observed by Romano *et al.* (2015) where lipid contents of shrimp fed organic acid blends decreased with an increase in inclusion levels of organic acid blends.

Water quality parameters (temperature, dissolved oxygen and pH) measured in both experimental setups were all within the accepted range for the culture of fin fishes in tropical regions as recommended by NRC (1996).

This study has shown organic acids as a viable means of boosting fish

growth and enhancing its health. They can therefore serve as a better and perfect replacement to antibiotics.

This study had demonstrated that incorporating butyric acid improved fish growth and did not pose any negative impact on carcass quality nor the environment. Hence, organic acids are better alternatives to antibiotics in fish diets.

It is no longer news that organic acids could be used to replace antibiotics, in terms of fish growth and health and feed preservation. Fish farmers should therefore be encouraged to incorporate them in fish diets. Governments should also be encouraged to purchase them in bulk to give fish farmers at subsidized rates as they do to agricultural fertilizers given to farmers. Also, subsequent research should be geared at extracting some active herbal ingredients that have organic acid properties to make them readily available to fish farmers.

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