

Effect of various organic acid  
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Evaluation of growth, survival  
and feed utilization [version 1;  
referees: 3 approved]

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## RESEARCH NOTE

# Effect of various organic acid supplementation diets on *Clarias gariepinus* BURCHELL, 1822: Evaluation of growth, survival and feed utilization [version 1; referees: 3 approved]

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## Abstract

**Background:** The purpose of the current study was to determine the growth status, survival and feed utilization of catfish (*Clarias gariepinus* BURCHELL, 1822) fed various organic acid supplementations.

**Methods:** In total, 1600 fish were randomly distributed into 20 tanks and fed different types of diet: A, control diet without organic acid supplementation; B, control diet supplemented with 0.05% formic, acetic, and propionic acid; C, control diet supplemented with 0.1% formic, acetic, and propionic acid; D, control diet supplemented with 0.05% butyric acid; E, control diet supplemented with 0.01% butyric acid. The control diet was a commercial diet, containing 35% crude protein, 8.58% crude fat, and 2.75% fibre. All fish were fed using a satiation method, three times per day for 56 days. At the end of the trial, growth, survival and feed utilization were determined. Water quality parameters during the trial were also measured once a week.

**Results:** Fish fed diet type D had the significantly lowest ( $P < 0.05$ ) final weight (FW), weight gain (WG), and specific growth rate (SGR) of all diets. Survival, FW, WG, and SGR were found for fish fed diets A-C and E. Meanwhile, the feed conversion ratio, feed efficiency, and survival rate of fish were not affected by any types of diet. The water quality parameters were not significantly different between tanks and weeks: dissolved oxygen 6.79-6.81 mg L<sup>-1</sup>, pH 7.11-7.19, water temperature 28.97-29.32°C, nitrite (NO<sub>2</sub>) content 0.48-0.50 mg L<sup>-1</sup>, and ammonia (NH<sub>3</sub>) content 0.064-0.066 mg L<sup>-1</sup>.

**Conclusion:** The supplementation of 0.05% butyric acid in the diet of *C. gariepinus* for 56 days reduced the growth performance of the fish. However, supplementation of an organic acid in the diet of *C. gariepinus* had no impact on feed utilization, survival, and water quality parameters.

## Keywords

Organic acid, Growth, Survival Rate, Feed utilities, *Clarias gariepinus*

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## Introduction

Optimum and balanced nutrition, especially in fish culture, is a significant requirement and contributes up to 40–60% of production cost of farmed fish<sup>1,2</sup>. The balance of a commercial diet that enhances optimum fish growth and health has attracted much research to develop a specific diet formulation<sup>1</sup>. It is also well known that the use of antibiotics or chemical substances as a growth promoter in the feed of fish may help to improve growth, survival, and feed utilization. However, wider concerns regarding the negative effects to the environment has led to a ban of the use of such chemical substances in the field of aquaculture<sup>3</sup>.

Previous research stated that the use of non-chemical substances, such as acidifiers, to increase growth performance<sup>10</sup> has been performed in several fish. Dietary supplementation of citric acid/formic acid increases the bioavailability of minerals, including phosphorus, magnesium, calcium and iron in rainbow trout (*Oncorhynchus mykiss*), sea bream (*Pagrus major*) and Indian carp (*Labeo rohita*)<sup>4,5</sup>. Some researchers also claimed that dietary acidifiers in the feed of fish reduce the pH in the stomach and foregut, which help improve pepsin activity, enhancing protein metabolism and mineral intake<sup>12</sup> in the intestines<sup>16</sup>. In addition, these short-chain organic acids are generally absorbed through the intestinal epithelia by passive diffusion, providing energy for renewing the intestinal epithelia and maintaining gut health<sup>7</sup>.

Besides nutritional concern in aquafeed, generally aquaculture activities commonly produce waste, such as feed remains and feces, which can be converted into ammonia and nitrite. Further, the level of ammonia (NH<sub>3</sub>) and nitrite (NO<sub>2</sub>) increases rapidly in a closed culture system and can be harmful to fish<sup>8,9</sup>. Thus, water quality parameters are a major concern in the aquaculture system. Previous research revealed that the values of water quality parameters during fumaric acid feeding experiments on the African catfish (*Clarias gariepinus*) are relatively stable, providing a dissolved oxygen concentration 7.23–7.86 mg L<sup>-1</sup>, water temperature 25.13–25.27°C and pH 7.23–7.48<sup>10</sup>.

A strain of African catfish, *Clarias gariepinus* BURCHELL, 1822, is a popular species for aquaculture industry in Asian countries. In Indonesia, the production of catfish is been the second largest after tilapia, reaching a production from 144,755 MT in 2009 to 644,221 MT in 2013<sup>11</sup>. Catfish has pseudolungs, long bodies and a high capacity to produce mucous as a form of adaptation to live in stagnant environments or drought conditions. It is omnivorous, feeding on various feeds, such as plant material, plankton, arthropods, molluscs, fish, reptiles, and amphibians<sup>12</sup>. Compared to other species, catfish is more resistance to diseases, handles stressors well and has a high growth performance<sup>13</sup>. To increase growth performance, aquaculturists and researchers have added various supplementations to the diet of catfish<sup>14–16</sup>. However, the information regarding supplementation of organic acid (formic, acetic, propionic and butyric acid) in the diet of catfish is very rare<sup>11</sup>. Thus, the aim of the current experiment was to evaluate the growth performance, feed utilization, and survival of catfish fed different types of diet, containing organic acid.

## Methods

### Site and time

The research was performed at PT Suri Tani Pemuka Unit Research and Development, Ciranjang, West Java, Indonesia from March to May 2018. All *C. gariepinus* were provided by PT Suri Tani Pemuka (Cisarua, Tegal Waru, HIAT Purwakarta Regency, West Java, Indonesia). The fish were kept in oxygenated polythene bags and transported by truck to PT Suri Tani Pemuka, Research and Development Farm, Ciranjang West Java, Indonesia. Then, the fish had been adapted and grown under farming conditions.

The study was carried out within The PT Suri Tani ethical protocols of the farm.

### Experimental design

Five groups in five separate tanks, namely: A, control diet without organic acid supplementation; B, control diet supplemented with 0.05% formic, acetic, and propionic acid; C, control diet supplemented with 0.1% formic, acetic, and propionic acid; D, control diet supplemented with 0.05% butyric acid; E, control diet supplemented with 0.01% butyric acid. The control diet which was provided from a commercial diet (provided by PT Suri Tani Pemuka, Purwakarta, West Java, Indonesia), containing 35% crude protein, 8.58% crude fat, and 2.75% fibre. The study was repeated for 30 times. All fish were maintained in a plastic tank (vol. 520 L) at a stocking density of 80 fish per tank and reared for 56 days.

### Fish culture and feeding trial

In total 1600 fish with an initial average weight 8.78 g were randomly assigned into 20 plastic tanks (80 fish/tank) with a volume of 520 L. Each tank was filled with fresh water up to 500 L and the fish were stocked at the density of 80 fish tank<sup>-1</sup>. The fish were fed with diets A–E three times per day (01:00, 05:00 and 09:00 GMT) using satiation methods for 56 days.

### Measured parameters

Biomass (g) of the fish per tank were measured at the beginning and the final day of the study. Meanwhile, the weight gain was calculated using equation:

$$W = (W_t/N_t) - (W_0/N_0)$$

where W is weight gain (g), W<sub>t</sub> is the weight of the fish at the end of trial (g), and W<sub>0</sub> is the weight of fish at the beginning of the trial (g). The feed utilization and survival rates were determined following equations that were previously used by Muchlisin<sup>17</sup> and Nugroho<sup>18</sup>:

$$\text{Feed efficiency (FE)} = 1/\text{FCR} \times 100\%$$

where FCR = feed conversion ratio:

$$\text{FCR} = F / (W_t - W_0)$$

where F = total feed intake (g).

$$\text{Survival rate (SR)} = (N_t/N_0) \times 100\%$$

where N<sub>t</sub> is total fish at the end of experiment and N<sub>0</sub> is total fish at start of experiment.

The water quality parameters such as dissolved oxygen (DO) and temperature were measured using a digital water checker (YSI™ Model 550A Dissolved Oxygen Meter; Fisher Scientific, USA). pH was measured with a pH-meter (CyberScan pH 11; EuTech Instruments, Singapore). Meanwhile, NO<sub>2</sub> and NH<sub>3</sub> were detected using Sera test kit (Sera GmbH D52518, Heinsberg, Germany). All the water quality parameters were measured once a week.

#### Data analysis

Results are expressed as means  $\pm$  standard error (SE) and data were analysed using SPSS version 22 (SPSS, Inc., USA). The data of survival (%) was transformed using arc sine before statistical analysis. Meanwhile, growth analysis and water quality were subjected to analysis of variance (ANOVA), followed by Duncan post hoc test to evaluate significant differences among the groups of treatments. All significant tests were at  $P > 0.05$ .

#### Results

Based on the statistical analysis, the present results showed that the control diet (A) and the supplementation organic acid diet of *Clarias gariepinus* (B–E) had no significant effect ( $P > 0.05$ ) on the feed conversion ratio (FCR), feed efficiency (FE), and survival rate (SR). The trial also showed that fish fed diet D had the significantly lowest ( $P < 0.05$ ) final weight, weight gain, and specific growth rate (SGR), but a similar final weight, weight gain, and SGR were found on fish fed diets A–C, and E (Table 1).

The water quality parameters during the study showed that the supplementation organic acid in the diet of *Clarias gariepinus* had no effects on the water quality culture. Dissolved oxygen ranged 6.81–6.88 mg L<sup>-1</sup>; pH 7.12–7.21, and water temperature 27.07–29.50°C. Meanwhile, nitrite (NH<sub>2</sub>) content ranged from 0.045 to 0.057 mg L<sup>-1</sup> and the ammonia (NH<sub>3</sub>) content ranged from 0.372 to 0.50 mg L<sup>-1</sup> (Table 2).

The data showing the growth parameters such as initial and final weight, total weight gain, and total feed consumed by fish for every

experimental group, and water quality parameters can be seen in Dataset 1.

**Dataset 1. The initial and final weight, body weight gain, survival, and total feed consumed by fish for every experimental group (A–E) and water quality parameters**

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#### Discussion

The present results revealed supplementation of organic acid in the diets had no significant effect ( $P > 0.05$ ) on the feed conversion ratio (FCR), feed efficiency (FE), and survival rate (SR). However, dietary supplementation of 0.05% of butyric acid in the diet of *C. gariepinus* resulted in a significantly lower ( $P < 0.05$ ) final weight, weight gain, and SGR compared with other diets. A similar final weight, weight gain, and SGR were also found for fish fed a control diet (A), and those fed with 0.05% (B) and 0.1% (C) mix of formic, acetic, and propionic acid, and 0.1% (E) of butyric acid. These findings are in line with a previous study where dietary 0.5 g kg<sup>-1</sup> butyric acid supplementation in the diet of *Clarias gariepinus* found no significant difference in weight gain, SGR, SR, and FCR. In contrast, weight gain, SGR, SR, FE and FCR of *Oreochromis niloticus* were significantly improved after being fed 0.5 g kg<sup>-1</sup> butyric acid supplementation in the diet<sup>16</sup>.

According to Da Silva *et al.*<sup>19</sup>, butyrate acid in shrimp diets could be feed attractants for fish, which improve feed intake. Organic acids such as butyric acid improve the feed intake, gut and gastrointestinal tract activity of a red hybrid tilapia, *Oreochromis* sp., by the reduction in pH<sup>20</sup>. The other benefits of butyric acid for improving growth is attributed to the aroma which acts as an attractant in the diet of shrimp<sup>21</sup>. However, a past study found that the increasing levels of dietary organic acid such as fumaric acid (1.5–2 g kg<sup>-1</sup>) in the diet of *C. gariepinus* significantly reduced growth performance and feed utilities and improved survival rate after a challenge test with bacteria<sup>10</sup>. These findings might be correlated with pH balance in the gut of the fish fed dietary high

**Table 1. Mean  $\pm$  SE of growth and feed utilities of *Clarias gariepinus* BURCHELL, 1822 fed organic acid supplementation in the diet for 56 days.**

Parameters	Diets				
	A	B	C	D	E
Initial weight (g)	702.50 $\pm$ 2.50 <sup>a</sup>	702.50 $\pm$ 2.50 <sup>a</sup>	702.50 $\pm$ 2.50 <sup>a</sup>	702.50 $\pm$ 2.50 <sup>a</sup>	700.00 $\pm$ 2.50 <sup>a</sup>
Final weight (g)	7065.00 $\pm$ 136.16 <sup>ab</sup>	7007.50 $\pm$ 161.10 <sup>ab</sup>	7307.50 $\pm$ 199.30 <sup>a</sup>	6702.50 $\pm$ 206.37 <sup>b</sup>	7205.00 $\pm$ 163.01 <sup>ab</sup>
Weight gain (g)	87.07 $\pm$ 2.09 <sup>a</sup>	84.42 $\pm$ 2.59 <sup>ab</sup>	87.09 $\pm$ 3.06 <sup>a</sup>	78.78 $\pm$ 1.78 <sup>b</sup>	87.96 $\pm$ 2.11 <sup>a</sup>
SGR (% day <sup>-1</sup> )	4.26 $\pm$ 0.04 <sup>a</sup>	2.21 $\pm$ 0.05 <sup>ab</sup>	4.26 $\pm$ 0.05 <sup>a</sup>	4.10 $\pm$ 0.03 <sup>b</sup>	4.29 $\pm$ 0.03 <sup>a</sup>
FCR	1.16 $\pm$ 0.03 <sup>a</sup>	1.14 $\pm$ 0.03 <sup>a</sup>	1.11 $\pm$ 0.03 <sup>a</sup>	1.15 $\pm$ 0.02 <sup>a</sup>	1.12 $\pm$ 0.02 <sup>a</sup>
FE (%)	86.41 $\pm$ 2.23 <sup>a</sup>	87.71 $\pm$ 2.30 <sup>a</sup>	90.20 $\pm$ 2.74 <sup>a</sup>	86.73 $\pm$ 1.45 <sup>a</sup>	89.25 $\pm$ 1.95 <sup>a</sup>
SR (%)	92.18 $\pm$ 1.72 <sup>a</sup>	94.06 $\pm$ 1.72 <sup>a</sup>	95.31 $\pm$ 0.59 <sup>a</sup>	95.62 $\pm$ 1.19 <sup>a</sup>	93.12 $\pm$ 0.36 <sup>a</sup>

Different alphabets (a, b) indicate significantly different means for different group of diets at  $P < 0.05$ . A = control diet without organic acid supplementation; B = supplemented-control diets with 0.05% mix of formic, acetic, and propionic acid; C = supplemented-control diets with 0.1% mix of formic, acetic, and propionic acid; D = supplemented-control diets with 0.05% of butyric acid; E = supplemented-control diets with 0.1% of butyric acid; SGR = Specific growth rate, FCR = Feed conversion ratio, FE = Feed efficiency, SR = Survival rate. The control diet was a commercial diet, containing 35% crude protein, 8.58% crude fat, and 2.75% fibre.



**Table 2.** Mean  $\pm$  SE water quality parameters the cultured media of *Clarias gariepinus* BURCHELL, 1822 during the trial.

Parameters	Diets				
	A	B	C	D	E
Dissolved oxygen (mg L <sup>-1</sup> )	6.81 $\pm$ 0.01 <sup>a</sup>	6.81 $\pm$ 0.01 <sup>a</sup>	6.78 $\pm$ 0.01 <sup>a</sup>	6.81 $\pm$ 0.01 <sup>a</sup>	6.79 $\pm$ 0.01 <sup>a</sup>
pH	7.17 $\pm$ 0.04 <sup>a</sup>	7.21 $\pm$ 0.05 <sup>a</sup>	7.06 $\pm$ 0.04 <sup>a</sup>	7.11 $\pm$ 0.04 <sup>a</sup>	7.19 $\pm$ 0.04 <sup>a</sup>
Temperature (°C)	29.33 $\pm$ 0.21 <sup>a</sup>	29.53 $\pm$ 0.20 <sup>a</sup>	28.71 $\pm$ 0.22 <sup>a</sup>	29.05 $\pm$ 0.26 <sup>a</sup>	29.11 $\pm$ 0.18 <sup>a</sup>
NH <sub>3</sub> (mg L <sup>-1</sup> )	0.06 $\pm$ 0.001 <sup>a</sup>	0.06 $\pm$ 0.001 <sup>a</sup>	0.06 $\pm$ 0.001 <sup>a</sup>	0.06 $\pm$ 0.001 <sup>a</sup>	0.06 $\pm$ 0.001 <sup>a</sup>
NO <sub>2</sub> (mg L <sup>-1</sup> )	0.46 $\pm$ 0.02 <sup>a</sup>	0.48 $\pm$ 0.01 <sup>a</sup>	0.50 $\pm$ 0.00 <sup>a</sup>	0.50 $\pm$ 0.00 <sup>a</sup>	0.50 $\pm$ 0.00 <sup>a</sup>

Mean  $\pm$  SE followed by same superscript letter (a) indicated not significantly different at  $P < 0.05$ . Water quality parameters were measured once a week during the study. A = control diet without organic acid supplementation; B = supplemented-control diet with 0.05% mix of formic, acetic, and propionic acid; C = supplemented-control diets with 0.1% mix of formic, acetic, and propionic acid; D = supplemented-control diets with 0.05% of butyric acid; E = supplemented-control diets with 0.1% of butyric acid. The control diet was a commercial diet, containing 35% crude protein, 8.58% crude fat, and 2.75% fibre.

levels of organic acid. Furthermore, various concentrations of the organic acids such as propionic acid and acetic acid, have been determined to have effects on the feeding behaviour of *Oreochromis niloticus*. The supplementation of propionic acid at  $10^{-4}$ – $10^{-6}$  M can stimulate feeding<sup>22</sup>. However, dietary propionic acid at  $10^{-3}$  M may suppress feeding. In addition, past research has also found that dietary supplementation of acetic acid at  $10^{-5}$  M had no effect on fish feeding. Lim *et al.*<sup>23</sup> revealed that the beneficial of the organic acid supplementation in the diet of fish may vary among fish and tend to be inconsistent, depend on the dietary ingredient, culture system, and water quality.

It is clear that feed remains in the water medium might change the water quality. Current findings stated that the water quality parameters during the trials showed no effects on the medium fish culture during the present study (Table 2). These findings are consistent with a past study by Omosowone and Adeparusi<sup>16</sup>, stating that water quality parameters such as temperature, dissolved oxygen and pH measured in a similar current experimental setups are all within the accepted range for the culture of fin fishes in tropical regions, as recommended by National Research Council (USA)<sup>24</sup>.

## Conclusion

The inclusion of organic acid in the diet of *C. gariepinus* had no impact on the feed utilities, survival, and water quality

parameters in the present study. However, the inclusion of 0.05% butyric acid in the diet of *C. gariepinus* for 56 days reduced growth performance and feed utilization. Further research needs to be conducted to evaluate the effects of organic acid supplementation in the diet of fish on digestive enzyme activity, gut bacteria population, and fillet proximate analysis.

## Data availability

Dataset 1: The initial and final weight, body weight gain, survival, and total feed consumed by fish for every experimental group (A–E) and water quality parameters. DOI, 10.5256/f1000research.15954.d216486<sup>25</sup>.

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<http://www.doi.org/10.5256/f1000research.15954.d216486>

## Open Peer Review

Current Referee Status:   

Version 1

Referee Report 09 October 2018

doi:10.5256/f1000research.17425.r38343



**Indra Suharman** 

Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Riau, Pekanbaru, Indonesia

This papers reports on the growth performance of fish fed supplementation of organic acid in the diets of catfish. From this point of view the article is very interesting and deserves to be accepted for publication.

The Abstract is quite clear and provide a concise conclusion of the research work. However, it is better to state the initial average weight of fish in the "Abstract".

The Introduction is strong supported by the literature cited and the objective of this study is clear stated.

The Methods is quite clear and the analysis correctly explained what has been obtained from the designed work and appropriately reflects the topic studied.

The Discussion is clear explained and the Conclusion has justified the basis of the results.

Overall, the paper is well written and data well analyzed. I have found very interesting results as the effect of organic acid supplementation in the diets on growth performace and feed utilization of catfish.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**



Yes

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** Aquatic animal nutrition (Aquaculture)

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 01 October 2018

doi:10.5256/f1000research.17425.r38357



**Ilham Alimin**

Feed and Nutrition Research Group, Department of Aquaculture, Jakarta Fisheries University (Sekolah Tinggi Perikanan Jakarta), Jakarta, Indonesia

Manuscript titled "Effect of various organic acid supplementation diets on *Clarias gariepinus* BURCHELL, 1822: Evaluation of growth, survival and feed utilization" could be acceptable. Study design, data processing, writing, etc. are relatively good. However, some revisions need to be made to index the manuscript.

#### **Abstract**

Results:

...were found for fish fed diets A-C and E - Kindly replace A-C with A,B,C

...were not affected by any types of diet - write type instead of types

Keywords:

.... Survival rate, ...*Clarias gariepinus* should be in *italics*

#### **Introduction**

Paragraph 2:

Previous research stated that the use of ..... - whose research? citation?

... which help improve pepsin activity, **thus** enhancing...

Paragraph 3:

Since the word "generally" also means "commonly", please use one of those words

... Previous research revealed that the values.... - whose research? citation?

Paragraph 4:

A strain of African catfish, *Clarias gariepinus* BRUCHELL, 1822 ... - The species should be written as *C. gariepinus*

.... on various feeds, such as plan material,... - material should be in plural form, please add 's'

The role of organic acids should also be mentioned in the "Introduction" section.

#### **Methods**

Site and time:

Then, the fish had been adapted and .... - should be "Then, the fish **were** adapted and .....

Experimental design

Is it true that the study was repeated four times? Or do you mean "all treatments were designed in four replicates"?

Fish culture and feeding trial:

Again, kindly write diets A,B,C,D and E instead of diets A-E

Regarding feed intake, how did you measure the feed intake? Was there any uneaten feeds?

Measured parameters:

Kindly replace "W0" with "Wo" and "N0" with "No" in the formula

### Results

When you rewrite the scientific name of the species i.e. *Clarias gariepinus*, kindly use *C. gariepinus*. The same concern also applicable in Discussion section. Also check it in the Table 1.

In Table 1 and Table 2, letter(s) ie. a,b,c should be written only when there was significant difference(s) among treatments. Therefore, FCR, FE and SR should not be highlighted with common superscript (a) since they were all statistically insignificant.

### Discussion

Paragraph 2:

... and tend to be inconsistent, depend on the .... - kindly write "depending on" instead of "depend on"

**Is the work clearly and accurately presented and does it cite the current literature?**

Partly

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 19 September 2018

doi:10.5256/f1000research.17425.r38341

**Gitartha Kaushik**

Department of Zoology, North-Eastern Hill University (NEHU), Shillong, Meghalaya, India

The paper is scientifically sound in its current form and only minor, if any, improvements are suggested:

Kindly make these following corrections.

1. Introduction: It is also well known that the use of antibiotics or chemical substances as a growth promoter in the feed of fish may help to improve growth, survival, and feed utilization ---- **kindly cite the article stating this statement.**

2. Introduction: Previous research stated that the use of non-chemical substances such as acidifiers, to increase growth performance has been performed in several fish ---- **kindly cite some previous reports**

3. Introduction: Besides nutritional concern in aquafeed, generally aquaculture activities commonly produce waste, such as feed remains and feces, which can be converted into ammonia and nitrite ---- **Who stated this? Kindly cite the article**

4. Introduction: *Clarias gariepinus* BURCHELL, 1822 kindly write the nomenclature following FishBase. <https://www.fishbase.de/summary/1934>

5. Methods: All *C. gariepinus* were provided: **Kindly check the spelling of the species.**

The paper has well experimented. It can be accepted after these minor corrections.

**Is the work clearly and accurately presented and does it cite the current literature?**

Partly

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Referee Expertise:** Fishbiology, molecular Taxonomy and adaptive modifications

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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