Application of organic acids in *Clarias gariepinus* Burchell, 1822 aquafeed: impacts on fish relative growth rate, condition factor and cannibalism ratio

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Abstract. The present research was performed to determine the relative growth rate (RGR), condition factor (K) and cannibalism ratio (CR) of Catfish (*Clarias gariepinus* Burchell, 1822) fed different level and types of organic acid addition in the fish. Eighty fish were randomly assigned into five group triplicates, reared, and fed different type organic acid supplemented viz: control diet with no organic acid addition (B1); mixture of formic, acetic and propionic acid at level 0.05% (B2); mixture of formic, acetic and propionic acid at level 0.1% (B3); 0.05% butyric acid (B4); 0.1% butyric acid (B5), for 8 weeks. At the end of the 8th week, RGR, K, and CR were determined. Fish fed 0.1% mix of formic, acetic and propionic acid or 0.1% butyric acid in the diet had the significantly higher (*p*<0.05) RGR than control or other groups. Meanwhile, the K and CR of fish during the trial were not affected by any various and levels of the organic acid. In conclusion, the supplementation of 0.1% mix organic acid or 0.1% butyric acid in the diet of *C. gariepinus* for 8 weeks enhanced the RGR of the catfish but no effect on the condition factor and cannibalism ratio.

1. Introduction

In fish culture, optimum and balanced nutrition are the very important aspect which contributes up to 40–60% of total cost production in farmed fish [1, 2]. Achieving the balance of nutrition in a commercial diet that supports optimum fish growth and health has a main goal of the farmer and much researcher. Further, many researchers have conducted some studies to develop a specific diet formulation [1]. It is already a major concern that the application of antibiotics or chemical substances can be used as a growth booster in the fish fed which may help to enhance growth, survival, and feed utilization. Nevertheless, many concerns regarding the negative effects of antibiotics and/or chemical substances to the environment have led to an avoid or even a ban of the application of chemical substances in aquaculture practice [3].

A past study revealed that the use of non-chemical compound, namely acidifiers, to enhance growth performance has been applied in many fish. The application of citric acid/formic acid enhances the metabolism minerals, such as phosphorus, magnesium, calcium and iron in *Oncorhynchus mykiss*, sea *Pagrus major* and *Labeo rohita*[4, 5]. Some researchers also stated that supplementation acidifiers in the diet of fish decrease the pH in the digestive system such as stomach and foregut, which
increasing pepsin activity, boosting protein catabolism and mineral absorption by intestines [4, 6]. Moreover, a short-chain organic acid is commonly absorbed by the intestinal epithelia through passive diffusion, resulting in energy for maintaining the intestinal epithelia and gut health [7].

One of the strains of African catfishes, namely Clarias gariepinus Burchell, 1822, is currently an important species for fish culture industry in Asian countries. In Indonesia, the production of catfish placed in rank two after tilapia, which was from 144,755 MT in 2009 to 644,221 MT in 2013 [8]. The catfish is a pseudo-lungs, has relatively long bodies and a high capacity to produce mucus as a form of protective adaptation to living in stagnant environments or drought conditions. Moreover, the catfish is grouped in omnivorous fish which consumes plant material, plankton, arthropods, molluscs, fish, reptiles, and even amphibians [9]. The catfish is also more resistant to diseases and has a high growth performance [10]. In addition, cannibalistic behaviour eventually occurs at the larval and juvenile stages of the catfish which impacts on the economic benefit [11].

To boost growth status, such as relative growth rate of this catfish, aquaculturists and researchers have supplemented various compound to the diet of catfish [12-14]. Moreover, the condition factor of the catfish which is a method to understand their physical condition in the well-being of an individual fish should be known. However, the information regarding dietary addition of organic acid (formic, acetic, propionic and butyric acid) on the relative growth rate (RGR), condition factor (K), and cannibalism ratio (CR) in catfish is very limited. Thus, the purpose of the present research was to determine the RGR, K, and CR of catfish fed different types of diet, containing an organic acid.

2. Materials and Methods

2.1. Site and time
The research was done at PT Suri Tani Pemuka Unit Research and Development, Ciranjang, West Java, Indonesia from March to May 2018. All C. gariepinus were obtained from PT Suri Tani Pemuka, Cisarua, Tegal Waru, HIAT Purwakarta Regency, West Java 41165, Indonesia. The fishes were maintained in the oxygenated polythene bag and transported by truck to PT Suri Tani Pemuka, Research and Development Farm, Ciranjang West Java, Indonesia. The fish had been acclimated and grown under the PT Suri Tani protocols and conditions.

2.2. Experimental design
Five groups in five triplicates separate tanks, viz: B1, control diet without organic acid addition; B2, control diet added with 0.05% formic, acetic, and propionic acid; B3, control diet added with 0.1% formic, acetic, and propionic acid; B4, control diet added with 0.05% butyric acid; B5, control diet added with 0.1% butyric acid. The control diet was formulated from a commercial diet which was obtained from PT Suri Tani Pemuka, Purwakarta, West Java, Indonesia. The commercial diet contained 35% crude protein, 8.58% crude fat, and 2.75% fibre. All fishes were reared in a plastic tank (vol. 520 L) at a stocking density of 80 fish per tank for 8 weeks.

2.3. Fish culture and feeding trial
In total 1200 fishes (initial average weight 8.78 g) were placed randomly into 15 plastic tanks (80 fish/tank) with a volume of 520 L. Each tank was filled with fresh water up to 500 L. The fishes were fed with B1-B5 three times per day (01:00, 05:00 and 09:00 GMT) using satiation methods for 8 weeks.

2.4. Measured parameters
Biomass (g) fishes per tank were determined on the first day and the final day of the experiment. The RGR was calculated using equation as previously used by Dharmaraj and Dhevendaran [15]: [(final mean mass–initial mean mass)/(initial mean mass)] x 100. Meanwhile, the K was calculated according to the equation proposed by Hun-Han [16]: K = (Wx100)/L3, Where, W=weight of fish (g), L=Length of fish (cm). Meanwhile, the survival rate (SR) was measured by calculating live fish using equations
that were previously used by Muchlisin et al and Nugroho et al [17, 18] as SR = (Nt/N0) x 100%, where ), Nt is total fish at the final day of trial and N0 is total fish at the first day of the trial.

2.5. Data analysis
The data of RGR, K, and CR are expressed as means ± standard error (SE) and 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 $P<0.05$. All analysis was performed using SPSS version 22 (SPSS, Inc., USA).

3. Results
Present findings revealed that the application of 0.1% mix formic, acetic and propionic acid or 0.1% butyric acid in the diet of fish resulted higher RGR than control. However, any various of organic acid supplementation in the diet of fish had no effects on the K and CR (Table 1).

Table 1. Mean and standard error of relative growth rate, Condition Factor and cannibalism of Mutiara catfish (Clarias gariepinus Burchell, 1822) fed various organic acid supplementation for 56 days

| Parameters | Diets          |
|------------|----------------|
|            | B1             | B2             | B3             | B4             | B5             |
| RGR        | 15.91 ± 0.42a  | 16.49 ± 0.08ab | 17.20 ± 0.52b  | 15.75 ± 0.03a  | 16.98 ± 0.21b  |
| K          | 0.77 ± 0.01a   | 0.77 ± 0.02a   | 0.79 ± 0.01a   | 0.79 ± 0.01a   | 0.75 ± 0.06a   |
| CR         | 6.25 ± 1.91a   | 4.58 ± 2.73a   | 3.75 ± 0.72a   | 2.08 ± 1.50a   | 5.00 ± 0.00a   |

Different alphabets (a, b) indicate significantly different means for different group of diets at $p<0.05$. B1 = Control diet without organic acid supplementation; B2 = supplemented control diets with 0.05% mix formic, acetic and propionic acid; B3 = supplemented control diets with 0.1% mix formic, acetic and propionic acid; B4 = supplemented control diets with 0.05% butyric acid; B5 = supplemented control diets with 0.1% butyric acid; RGR = Relative growth rate; K = Condition factor; CR = Cannibalism rate.

4. Discussion
Organic acid has been used as supplementation in practical fish culture [19, 20]. Past researcher Da Silva et al.[21] found that the use of butyrate acid in shrimp culture has been applied as feed attractants which enhance feed utility. The butyric acid has also improved the feed utility, digestive tract activity and reduced pH milieu in the gastrointestinal tract of a red hybrid tilapia, Oreochromis sp. [3, 22]. In addition, butyric acid has a property as attractant in the diet of shrimp, enhancing growth rate [23]. In contrast to current finding, the use of fumaric acid (1.5-2 g kg$^{-1}$) for C. gariepinus significantly culture decreased not only growth statues but also feed utilities [24]. These results can be correlated with pH balance in the digestive tract of the fish fed with high levels of fumaric acid. In addition, the supplementation of propionic acid at $10^{-3}$ M may decrease feeding activity of the fish. Meanwhile, previous research also revealed that the addition of acetic acid at $10^{-5}$ M had a negative effects on the fish feeding activity. As the results of the effects of organic acid on the fish is different from fish to fish, Lim et al.[25] stated that the organic acid supplementation in the diet of fish may differ from fish to fish, inconsistent, depending on the feed ingredient, culture model, and water quality parameters.
5. Conclusion
This research highlighted the addition of organic acid in the diet has positive results on the relative growth rate but no effects on the condition factor and cannibalism ratio. The supplementation of mixture of 0.1% formic, acetic and propionic acid; or 0.1% butyric acid in the diet of C. gariepinus is suggested to increase relative growth rate.

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