

TEKNIK PENAMBAHAN *Guanidinoacetic Acid* UNTUK PENINGKATAN PERTUMBUHAN IKAN PATIN (*Pangasius hypophthalmus*)

Technique of Dietary Supplementation of Guanidinoacetic Acid for Growth Improvement of Catfish (*Pangasius hypophthalmus*)

Aldi Huda Verdian^{1*}, Rio Yusufi Subhan², Nurul Fatimah²

¹Program Studi Budidaya Perikanan, Politeknik Negeri Lampung

²Program Studi Teknologi Pembenihan Ikan, Politeknik Negeri Lampung

*Corresponding author: aldihudaverdian@polinela.ac.id

ABSTRAK

Penelitian ini bertujuan untuk menentukan metode terbaik dalam suplementasi Guanidinoacetic acid (GAA) pada pakan untuk meningkatkan retensi penambahan GAA setelah perendaman di air dalam meningkatkan kinerja pertumbuhan ikan patin (*Pangasius hypophthalmus*). Benih ikan patin dengan rata-rata bobot 16.05 ± 0.47 g dan panjang 12.42 ± 0.30 cm dipelihara di dalam 9 unit kolam dengan ukuran (2 x 1 x 0,6 m). Ikan diberikan pakan selama 60 hari pemeliharaan tiga perlakuan yaitu; kontrol (tanpa penambahan GAA); perlakuan pelleting (penambahan 0,5% kg/GAA); dan perlakuan spray coating (penambahan 0,5% kg/GAA). Hasil penelitian menunjukkan bahwa perlakuan penambahan 0,5% kg/GAA dengan metode pelleting memberikan rata-rata bobot ikan dan biomasa paling tinggi dibandingkan dengan perlakuan lain dan kontrol ($p < 0.05$). Kesimpulan dari penelitian ini adalah suplementasi GAA pada pakan ikan patin dengan metode pelleting menunjukkan kinerja pertumbuhan terbaik jika dibandingkan dengan metode spray coating pada ikan patin.

Kata Kunci: Coating, Pelleting, Guanidinoacetic acid, Ikan Patin

ABSTRACT

The present study aims to determine the best technique of Guanidinoacetic acid (GAA) supplementation to improve retention of added GAA after immersion in water on the growth performance of *Pangasius catfish* (*Pangasius hypophthalmus*). *Pangasius catfish* juveniles with a mean body weight and length of 16.05 ± 0.47 g and 12.42 ± 0.30 cm were randomly distributed into 9 pond units (2 x 1 x 0,6 m). Three experimental diets were formulated to contain (0,5% kg⁻¹ GAA) with different technique of GAA supplementation, i.e. control, pelleting technique and spray-coating technique fed to the fish for 60 days of culture. The results showed that average body weight and biomass of fish fed diet with by GAA supplementation with the pelleting technique was higher ($p < 0.05$) than that of spray-coating technique and control. In conclusion, the consumption of diets containing GAA with pelleting technique resulted the best growth performance of *Pangasius catfish* (*Pangasius hypophthalmus*).

Keywords: Coating, Pelleting, Guanidinoacetic acid, *Pangasius Catfish*

INTRODUCE

Aquaculture industry is a rapidly growing sector playing a part in global

food safety and security. To meet the increased population demand, aquaculture or fish farming experiences significant

expansion and production is rapidly increased. One of the potential freshwater fish is *Pangasius catfish (Pangasius hypophthalmus)*. According to Hermiastutsi (2013), one strategy for increasing the production of *Pangasius catfish* is to produce inputs that meet the nutritional requirements of fish feed. The most important component that can be utilized for fish growth and survival is fish feed's nutritional requirements. If the essential amino acid content is also low, the feed is said to be of low quality. Since fish and shrimp bodies cannot synthesize or form essential amino acids, they should be present in the feed. Creatine is one of the supplements that can be added to the feed to promote growth, increase the protein content of catfish, reduce the amount of fat in fish, and improve production efficiency. One of the essential amino acids is creatine. According to a study (Zhang *et al.*, 2017), taking creatine supplements improved boiler growth and meat quality. However, creatine's shortcomings as a feed additive include its high price and instability (Yazdi *et al.*, 2017). The only creatine precursor with greater stability and lower cost is guanidinoacetic acid (GAA) (Liu *et al.*, 2015). Studies have found that appropriate GAA supplementation promoted growth in Nile tilapia (*Oreochromis niloticus*) and bullfrog *Rana (Lithobates) catesbeiana* (Aziza *et al.*, 2020; Zeng *et al.*, 2017). However, many studies concerned the effect of dietary supplementation of GAA, the current study about method of dietary supplementation GAA in fish are still limited. To the best of our knowledge, the present study is the first to elucidate the effects of GAA supplementation by different method in fish. This study was undertaken to better understand the optimization of GAA dietary supplementation on growth performance of *Pangasius catfish*.

MATERIAL AND METHODS

Experimental Design

This study was carried out on July until September 2022 at the Fish Pond of the Department of Aquaculture, Politeknik Negeri Lampung, Lampung, Indonesia. Nine pond unit with a dimension of 2 x 1 x 0,6 m assigned for control and GAA treatment with different method of supplementation i.e. spray-coating method and pelleting method (3 replicates). All experimental ponds were supplied with dechlorinated tap water through a water pipeline system and were supplied with air through air pipeline using air blower. Fish feces and feed wastes were removed everyday by siphoning. The study lasted for 60 days after two weeks adaptation.

Experimental Diet

Pangasius catfish were fed a commercial diet (30% crude protein) enhanced with different additives GAA (0,5%/kg diet) with different method of supplementation i.e. coating and pelleting. GAA was from Behn Meyer Chemical. Pelleting method was conducted by the milled pellet and then GAA are mixed. After the mixing process, water is added to homogeneous material as much as 20-40% then mixed until homogeneous and can be fisted. The dough mixture is steamed for 20 minutes and printed in a pellet machine according to the size of the fish's mouth opening. The mold is accommodated on a tray and the pellet is dried in an oven with a temperature of 60 ° C for 12-24 hours (Arif *et al.*, 2018). Otherwise, spray-coating method was conducted by 1 kg of feed were spread as a single layer on stainless steel and sprayed by 0,5%/kg of GAA.

Fish Rearing

Catfish juveniles from Polifish hatchery at respective average body

length dan weight of 12.42 ± 0.30 cm and 16.05 ± 0.47 g were acclimatized for 7 days and stocked at density of 10 ind/m³. Commercial feed (30% crude protein) was provided three times a day i.e. at 07.00; 12.00; and 18.00 at the rate of 5-7% of wet body weight per day for 60 days of culture. Water temperature, pH and dissolved oxygen (DO) were measured on a daily basis, whereas the total ammonia nitrogen, nitrite nitrogen and nitrate nitrogen concentrations, alkalinity and hardness were measured on a weekly basis following the procedures described in American Public Health Association (APHA, 1998). The water quality parameters in the experimental units were adjusted to the normal ranges for the optimum growth and survival of *Pangasius catfish*

Measurement of Fish Growth and Feed Utilization

At the beginning of the experiment and at intervals of once every two weeks throughout the duration of the experiment, each fish was individually weighed to the nearest 0.1 g. At the time of weighing, the mortality of the fish was recorded every two weeks. The growth performance and feed utilization efficiency were calculated as following:

Body weight gain (BWG) = final weight (g/fish) – initial weight (g)

Daily weight gain (DWG) = body weight gain (BWG) / period

Survival rate (SR) % = (No. of fish survived at the end of the experiment / whole number of fish at the beginning) ×100

Feed conversion ratio (FCR) = feed intake (g) /body weight gain (g)

Statistical Analyses

The growth parameters were analyzed by one-way analyses of variance (ANOVA). Duncan's post-hoc test was subsequently performed to determine the difference between the treatments. Statistical analyses were performed using a statistical software SPSS 26 and Microsoft Excel 2019.

RESULTS AND DISCUSSION

Table 2 displays the average initial weight, final body weight, body weight gain, and daily weight gain of *Pangasius catfish* fed by various GAA supplementation methods. There were no significant differences in the initial weight between any of the treatment groups ($P > 0.05$). Means of body weight at the beginning of the experiment in this study indicate that the distribution of each fish between the two treatments was completely random. GAA supplementation with the pelleting technique treatment resulted in significant ($P < 0.01$) increases in final body weight, body weight gain, and daily weight gain (124.29 ± 0.68^a g), respectively; Spray-coated treatment came next (117.73 ± 0.89^b g), followed by control (112.36 ± 0.47^c g), respectively.

Table 1. Table of Water Quality

Treatments	Suhu (°C)	DO (mg/L)	pH	NH₃ (mg/L)
Control	27.4 – 30.2	4.40 – 6.33	7.1 – 7.4	0 - 0,012
Spray Coating	27.4 – 30.2	4.73 – 6.30	7.0 – 7.4	0 - 0,013
Pelleting	27.4 – 30.2	4.66 – 6.56	7.0 – 7.4	0 - 0,012

Table 2. Table of Growth Performance

Parameters	Treatment (method of supplementation)		
	Control	Spray Coating	Pelleting
Initial Weight (g)	16.05 ± 0.01 ^a	16.11 ± 0.12 ^a	16.10 ± 0.01 ^a
Final Weight (g)	128.41 ± 0.47 ^c	133.84 ± 0.77 ^b	140.39 ± 0.67 ^a
Body Weight Gain (g)	112.36 ± 0.47 ^c	117.73 ± 0.89 ^b	124.29 ± 0.68 ^a
Daily Weight Gain (g/d)	1.87 ± 0.01 ^c	1.96 ± 0.01 ^b	2.07 ± 0.01 ^a
Specific Growth Rate (%)	3.53 ± 0.01 ^c	3.59 ± 0.02 ^b	3.68 ± 0.01 ^a
Survival Rate (%)	90.00 ± 5.00 ^b	93.33 ± 2.89 ^{ab}	98.33 ± 2.89 ^a
FCR	1.63 ± 0.05 ^c	1.41 ± 0.04 ^b	1.30 ± 0.05 ^a

Values in the same column with different superscripts are significantly different at $p < 0.05$

As an alternative to natural creatine, the present study demonstrates that the Pelleting technique can reduce water leaching losses from diets by adding 0.5 percent/kg of GAA. This study shows that pelleting technique give the best final weight of 140.39 ± 0.67 g. It may due to pelleting technique is a method of administering GAA by mixing it with fish feed during the pellet manufacturing process. GAA is mixed with other feed ingredients and then processed with a pellet machine to form fish feed pellets. This study shows that there are several advantage of pelleting technique, such as intensive processing: with the pelleting process involves high pressure and heat, allowing GAA to mix well and bind with other feed ingredients; Stability and durability: Fish feed pellets have better stability and durability compared to powdered feeds, ensuring that GAA remains intact and is not easily degraded during storage; Ease of fish feeding: Fish feed pellets are easier to handle and provide to fish in their intact form. However, there are some drawbacks to the pelleting method such as potential degradation, the heat during pellet manufacturing can affect the stability of GAA, and there is a possibility of GAA degradation during the pellet processing.

Then uneven distribution, the mixture of GAA in feed ingredients for pelleting method may not be uniformly distributed throughout the pellets, leading to variations in GAA content among

individual pellets. Meanwhile, there are several drawbacks with the spray coating technique such as potential loss of GAA: Some GAA in the spray solution may be wasted or detached from the surface of the fish feed particles before being consumed by the fish, which can reduce the effectiveness of administration; Limited penetration: Spray coating may not be able to penetrate well into the inner parts of the fish feed particles, especially in dense feeds. This can affect the absorption and efficiency of GAA utilization by the fish (Huang & Nitin, 2019).

In other hand, even though creatine as a feed supplement plays an important role for optimal growth and nutrient utilization in humans, the improvement in growth performance seen with commercial GAA supplementation in comparison to Cr may be attributed and various farm animals, such as pigs (Michiels *et al.*, 2012), chicken (Ringel and colleagues, 2007) and poultry meat quality (Stahl *et al.*, 2003). According to Wyss & Kaddurah-Daouk (2000), approximately 95% of the Cr pool is found in muscle. However, in comparison to GAA, which is more stable and less expensive, synthesis of Cr in the kidney and liver is insufficient for animals' optimal supply and exhibits some disadvantages such as instability through storage and at lower pH values due to its chemical properties (Baker, 2009). Because the compound is quickly absorbed from the gastrointestinal tract

and transformed into Cr, which safeguards the amino acids (arginine and glycine) involved in its synthesis (Ostojic *et al.*, 2015). The differences in dietary formulation and experimental conditions (Zeng *et al.*, 2017) may be to blame for the conflicting results, which are in line with the findings of the current study. According to Ostojic (2016), an increase in creatine synthesis may be to blame for these outcomes, which result in more energy for cellular bioenergetics. In a similar vein, it has been demonstrated that creatine causes cell hydration, resulting in increased cell volume, total body water, and muscle volume (Haussinger, 1996). (Bekara *et al.*, 2007) discovered that when cells are hydrated, protein synthesis is made easier, protein decomposition is prevented, and glycogen synthesis is improved.

This study shows that there are significant effect of feed conversion ratio (FCR) by difference technique. It shows that pelleting technique proved to be effective in facilitating fish growth, leading to a body weight gain of 124.29 ± 0.68^a g. (Fu *et al.*, 2015) found that Jian carp (*Cyprinus carpio*) FCR significantly decreased when supplemented with 0.5g GAA kg^{-1} diet (Wang *et al.*, 2012), as well as (Mousavi *et al.*, 2013) anticipated that increasing the amount of guanidine acetic acid in the diet would increase the animals' performance at all times, which contributes to Cr formation, and conserve arginine, which could then be utilized by the body for other functions like protein anabolism.

The results of this study also indicate that the supplementation of GAA at normal dosage did not have a significant impact on water quality during the rearing of fish. The absence of significant effects suggests that the observed water quality parameters remained within acceptable ranges despite GAA supplementation.

CONCLUSION

In general, present study show that adding 0,5% kg^{-1} of GAA by pelleting technique in *Pangasius catfish* diets improves growth performance, feed utilization and survival rate of *Pangasius catfish* as alternative for natural creatine. GAA supplementation dietary with Pelleting technique can minimize the leaching losses from diets in water.

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