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THE ABILITY OF FAST-GROWING TRANSGENIC AFRICAN CATFISH (*Clarias gariepinus*) ON PREDATOR AVOIDANCE

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ABSTRACT

Research Institute for Fish Breeding has produced transgenic African catfish (*Clarias gariepinus*) containing stripped catfish growth hormone gene (PccBA-*Ph*GH) with growth 19.86% faster than that of non-transgenic fish. This fish has high potential to be released and utilized for fish farming sector to increase national production. However, there is not yet information about environmental risk of this fish. One of the major fitness traits determining potential environmental risk is predator avoidance. This study aimed to determine the predator avoidance ability of transgenic African catfish in an experimental laboratory condition. In this study, thirty five individuals each of transgenic and non-transgenic with body weight of about 0.1 ± 0.019 g were communally stocked in 60 cm x 40 cm x 40 cm aquarium with limited feeding frequency (*ad libitum* twice a day). One day after the fish were stocked, the predators were added to each aquarium. The non-transgenic and transgenic with body weight of 1.0 ± 0.024 g were stocked as predators as many as five individual in each aquarium. After approximately two weeks of predation, all remaining fish were collected for transgenic verification by PCR method. Genomic DNA was isolated from fin tissue of individually survivors. The results of this study showed that the transgenic fish had worse predator avoidance and lower cannibal than non-transgenic ($P<0.05$). There was no significant difference in growth performance between transgenic and non-transgenic African catfish ($P>0.05$) in limited food. The transgenic fish may have lower fitness than non-transgenic.

KEYWORDS: **predator avoidance; transgenic; environmental risk; *Clarias gariepinus***

INTRODUCTION

African catfish (*Clarias gariepinus*) is one commodity which aquaculture development is favored by the Ministry of Marine Affairs and Fisheries. African catfish has long been grown in Indonesia. However, the genetic quality of African catfish in Indonesia has decreased in growth rate, so that the necessary requires genetic improvement. Genetic improvement in the establishment of a rapid growth strain of African catfish might be achieved through transgenesis. The phenotype of African catfish transgenic have not been shaped by many generation of selection. A single generation, the characteristics of the organism have been modified with no opportunity for selection and adaptation to their environment. Growth rate is one character that has been manipulated in transgenic fish. The fast-growing transgenic African catfish was produced using the method of sperms electroporation as a carrier PcCBA-PhGH plasmid in 2011 (Dewi, 2010). In the last study, the F_2 populations of transgenic African catfish produced 2-fold fast growing performance than non-transgenic (Marnis *et al*., 2015).

However, the fish from genetically modified is still controversial. Although many researches have done on transgenic fish such as growth performance of transgenic fish (Morales *et al*., 2001; Nam *et al*., 2001; Lu *et al*., 2002). Appetites, feeding motivation, nutritional set points, and feed conversion ratio of transgenic fish (Cook *et al*., 2000; Sundström *et al*., 2004; Raven *et al*., 2006; Oakes *et al*., 2007) development and earlier maturation age of transgenic fish (Devlin *et al*., 2004; Sundström *et al*., 2003), production of elevated GH of transgenic fish (Devlin *et al*., 2000; Kang & Devlin, 2004), transgene expression throughout the body (Mori & Devlin, 1999), only a little information is available regarding the environmental risks posed.

One of the methods that could be used for environment safety testing was by examining the predator avoidance. There are two important factors in determining whether an animal should risk exposure

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to a predator, namely: parameters that affect mortality rates (i.e. the probability of being killed by a predator over a discrete period) and growth rates (e.g. the life history characteristics of the fish, particularly the future fitness benefits associated with food) (Abrahams & Sutterline, 1999). This study aimed to determine the predator avoidance ability of transgenic African catfish in an experimental laboratory condition.

MATERIAL AND METHOD

Animal and Sample Collection

In this study, we used F_3 transgenic African catfish, carrying the pCcBA-*Ph*GH construct (Dewi, 2010). All fish were derived from a single family of African catfish (*Clarias gariepinus*) and originated from the collection in Research Institute for Fish Breeding, Sukamandi, Indonesia.

Predator Avoidance and Anti-Predator Responses of Transgenic African Catfish

In this study, thirty five individuals each of transgenic and non-transgenic with body weight about 0.1 ± 0.019 g were communally stocked in each 60 cm x 40 cm x 40 cm aquaria with limited feeding frequency (*ad libitum* twice a day). Normally, fish has feeding frequency *ad libitum* three to four times a day in regular culture. One day after the fish were stocked, the predators were added to the aquarium. The non-transgenic and transgenic African catfish with body weight about 1.0 ± 0.024 g were stocked as predators (five individuals each aquarium). After approximately two weeks of predation, all remaining fish were collected for transgenic verification and measured average body weight. Transgenic fish was identified using PCR method with DNA template that has been extracted from fin tissue.

DNA Extraction

Genomic DNA of each sample was extracted using DNA extraction kit following the protocols recommended by manufacturer (GeneJet Genomic DNA Purification, Thermo Scientific). Briefly, the protocol consists series of steps including Cell lysis, DNA precipitation, binding DNA to the column, washing, and elution. Cell lysis was performed by weighing of approximately 10 mg of tissue sample and to check the success of genomic DNA extraction process, the sample was run on mini horizontal gel electrophoresis. The sample was loaded in to the 1.5% (w/v) agarose gel, powered with 65 volt electricity and run for 50 minutes. The gel was then stained with gelRed (*biotium*) 1 µg/ml and viewed using gel documentation system ultraviolet transillumination.

Polymerase Chain Reaction (PCR)

Amplification of transgene was performed using thermal cycling system (mycycler, Biorad). PCR standard was performed in a final volume of 25 µL, using a commercial kit master mix fast start PCR master (Roche, Germany). The PCR was used to amplify a 1,300-bp fragment transgene with primers ACT 107-F (5'- GTG TGT GAC GCT GGA CCA ACT – 3') and *Ph*GH2-R (5'CGA TAA GCA CGC CGA TGC CCA TTT-3 ') (Hidayani, 2009; Dewi, 2010), for 35 cycles: denaturing at 94°C for 30 secs, annealing at 55°C for 30 seconds, and extension at 72°C for one min. The amplification using the specific primer of ß-actin was performed as internal control of DNA loading. The ß-actin primers: bact-F (5'-TAT GAA GGT TAT GCT CTG CCC-3') and bact-R (5'-CAT ACC CAG GAA AGA TGG CTG-3') were designed from a catfish ß-actin sequence (Accession JF303887.1). The reaction was incubated at 94°C for 30 secs, annealing at 55°C for 30 secs, and extension at 72°C for one min for 30 cycle and the PCR products were then separated on 1.5% (w/v) agarose gel stained with gelRed (*biotium*) 1 µg/mL and visualized by ultraviolet transillumination.

Data Analysis

Mortality differences and mean body weights of transgenic and control of African catfish were compared using Student's *t* test.

RESULTS AND DISCUSSION

The result showed that the transgene was detected in juvenile of transgenic African catfish with the expected size product was 1,300-bp. The transgene was not detected in non-transgenic African catfish (Figure 1).

The result of this study showed that transgenic had worse predator avoidance and lower cannibal than that of non-transgenic ($P<0.05$) (Table 1). Similarly, the transgenic channel catfish (*Ictalurus punctatus*) containing growth hormone of salmon (RSVLTR-rtGH$_1$ DNA complementary, RSVLTR-rtGH$_2$, and RSVLTR-csGH cDNA) also had worse predator avoidance (*Micropterus salmoides* and *Lepomis cyanellus*) than that of non-transgenic (Dunham *et al.*, 1999). In addition, Sundström *et al.* (2004b) reported that transgenic Coho salmon (*Oncorhynchus kisutch*) had worse predator avoidance than that of non-transgenic. Later, Wei & Yan (2010) have demonstrated that the transgenic common carp had worse predator avoidance than that of non-transgenic.

Possibly, the swimming speed of transgenic African catfish was slower than that of non-transgenic, in order to be more vulnerable to be attacked by preda-

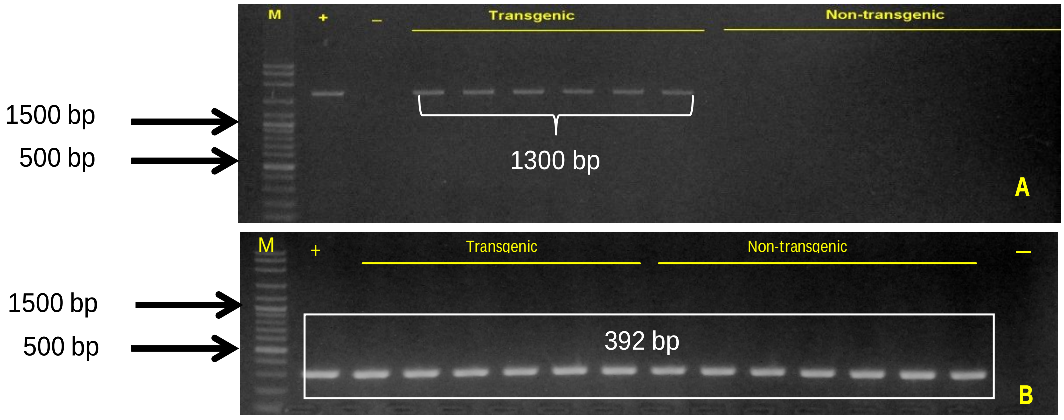

Figure 1. Detection of transgene (*Ph*GH) and β-actin gene in African catfish (*Clarias gariepinus*) transgenic and non-transgenic juvenile using PCR method; A). detection transgene, B). detection β-actin gene, (-) indicates a negative control, (+) indicates a positive control. The expected size of *Ph*GH gene the amplified fragment is 1,300 bp and the expected size of β-actin the amplified fragment is 392 bp

tors. Several experiments reported that the swimming speed of transgenic fish containing the growth hormone were slower than that of non-transgenic (DeLiang *et al*., 2007; Lee *et al*., 2003; Farrell *et al*., 1997). The swimming speed of each type of fish determines its ability to survive in the environment (Swanson *et al*., 1998). Furthermore, predator avoidance of transgenic African catfish correlated with willingness to risk exposure to a predator. Abrahams & Sutterline (1999) reported that transgenic salmon had rate of feed consumption that were approximately five times than that of the control fish and rate of movement approximately double than that of controls. Transgenic salmon also spent significantly more time feeding in the presence of the predator, and consumed absolutely more food at that location. When there was a real risk of mortality, control fish almost completely avoided the dangerous location. Transgenic fish continued to feed at this location, but at a reduced level. These data demonstrate that the growth enhancement associated with the transgenic manipulation increases the level of risk these fish are willing to incur while for aging.

If transgenic African catfish was accidentally released into nature, they would most likely decrease in number because of their increased susceptibility to predators, and eventually the transgenic African catfish would be eliminated. Alternatively, if the transgenic African catfish was not totally eliminated, the population fitness could be lowered because of the increased vulnerability to predation. Dunham *et al*. (1999) reported that the selection theory would predict transgenic fish elimination, especially because the fitness trait is predation avoidance and predation pressure is severe in the natural environment for transgenic fish. These research findings indicate that transgenic African catfish may have lower fitness than that of non-transgenic.

There was no significant difference in growth performance between transgenic and non-transgenic African catfish ($P > 0.05$) (Table 1). These results suggest that transgenic African catfish would not exhibit their fast growth in limited food. Possibly, transgenic African catfish did not express their growth potential where food was limited. Dunham *et al*. (1999) reported that limited food did not allow the transgenic channel catfish (*Ictalurus punctatus*) to express their growth. Likewise, the growth performance of triploid oysters would not increase where food was limited (Dunham, 1996). In Addition, Gregory & Wood (1999) reported that negative growth effects were more severe in the fish with food limitation.

CONCLUSION

The result of this study showed that transgenic African catfish had worse predator avoidance and lower cannibal than non-transgenic significanly. There was no significant difference in growth performance between transgenic and non-transgenic African catfish where treated with limited feeding frequency. These research findings indicate that the ecological risk of transgenic African catfish would be low or non-existent. Moreover, the transgenic fish may have lower fitness than non-transgenic.

Table 1. The survival rate of transgenic African catfish and average body weight in predator avoidance test

Predator	Survival rate				Body weight (g)	
	Transgenic		Non-transgenic		Transgenic	Non-transgenic
	N	%	N	%		
Non-transgenic	5	7,14[a]	15	21,43[b]	0.08 ± 0.03[ns]	0.09 ± 0.04[ns]
	3	4,29 [a]	27	38,57 [b]	0.07 ± 0.03[ns]	0.07 ± 0.05[ns]
	2	2,86 [a]	24	34,27 [b]	0.06 ± 0.02[ns]	0.05 ± 0.02[ns]
Transgenic	30	42,86[ns]	32	45,71[ns]	0.06 ± 0.02[ns]	0.05 ± 0.02[ns]
	33	47,14[ns]	33	47,14[ns]	0.08 ± 0.04[ns]	0.09 ± 0.04[ns]
	28	40,00[ns]	27	38,57[ns]	0.09 ± 0.04[ns]	0.08 ± 0.04[ns]
None	35	50,00[ns]	34	48,57[ns]	0.09 ± 0.04[ns]	0.10 ± 0.04 [ns]
	33	47,14[ns]	34	48,57[ns]	0.09 ± 0.04[ns]	0.08 ± 0.04[ns]
	32	45,71[ns]	33	47,14[ns]	0.07 ± 0.03[ns]	0.07 ± 0.04[ns]

Remark: ns = non significance

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