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Eni Kusrini, Alimuddin, Muhammad Zairin Jr., and Dinar Tri Sulistyowati (Aquaculture Post-graduate program, Department of Aquaculture, FPIK, IPB)

Gene transfer on *Betta imbellis* through transfection method with different DNA concentration

Indonesian Aquaculture Journal Volume 11 Number 1, 2016, 1-7

Big size betta (giant) have a high economic value compared to normal size betta, and over expression of growth hormone gene can produce a giant fish. As an initial step of giant transgenic betta production, this study was conducted to obtain DNA plasmid concentration which provides higher hatching and survival rates of betta larvae. This study used pCcBA-PhGH gene construct that containing growth hormone gene of Siamese catfish (PhGH) and it is controlled by the common carp b-actin (CcBA) promoter. *Betta imbellis* broodstocks were spawned naturally, and embryos were collected 1-2 minutes after spawning time. One hundred embryos were dipped in 2 mL of transfectan X-treme gene which contained the *Cc*BA-*Ph*GH expression vector (50 μg/mL), on room temperature for about 30 minutes. Treatments on this study were different transfectant : DNA plasmid ratios, namely: A (0.75 μL : 0.25 μL); B (0.75 μL : 0.50 μL); C (0.75 μL : 0.75 μL), D as control 1 (without transfectant, 0.25 μL DNA); E as control 2 (0.75 μL transfectant, without DNA), and F as control 3 (without transfectant and without DNA). Each treatment was repeated three times. Transfection embryos were hatched on a container (1 L volume). The results showed that hatching rate (HR) and larval survival rate (SR) (at 4 days after hatching) on treatment A were the same as the control, but slightly higher than B and C treatments. PCR analysis with DNA template showing that PhGH gene were found on embryos and larvae (pooled sample) of treatment A, B, and C. Furthermore, RT-PCR analysis showed the existence of PhGH mRNA expression on the pooled samples of embryos and larvae. Therefore, embryo transfection with the mixture of 0.75 μL transfectant and 0.25 μL gene expression vector can be used to generate betta transgenic.

KEYWORDS: gene transfer; growth hormone; *B. imbellis*; DNA concentration; transfection *polyphekadion*

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Huria Marnis, Bambang Iswanto, Imron, Selny Febrida, and Raden Roro Sri Pudji Sinarni Dewi (Research Institute for Fish Breeding)

The ability of fast-growing transgenic African catfish (*Clarias gariepinus*) on predator avoidance

Indonesian Aquaculture Journal Volume 11 Number 1, 2016, 1-7

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*

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Bambang Iswanto, Huria Marnis, and Rommy Suprapto (Research Institute for Fish Breeding)

Response to selection for body weight in the third generation of mass selection of the African catfish (*Clarias gariepinus*) at research institute for fish breeding Sukamandi

Indonesian Aquaculture Journal Volume 11 Number 1, 2016, 15-21

Genetic improvement of the African catfish (*Clarias gariepinus*) in Indonesia for increasing growth performance has been conducted by Research Institute for Fish Breeding at Sukamandi through mass selection. Collection and characterizations of the founder populations, building the synthetic base population, first generation and second generation through mass selection were conducted during 2010-2013. Later, in 2014 it was followed by building the third generation. The present study aimed to find out the genetic gain in the third generation in term of response to selection for body weight. Fifty pairs of the selected (fast growing) individuals from the second generation were mated to produce the third generation. As a comparison, five pairs of average-sized individuals were mated to produce the control population, as a second generation representative. Larval rearing, nursery and grow-out phases were respectively held for 25 days in the aquaria, 30 days in the concrete tanks and 60 days in the concrete ponds. At the end of each phase, individual samplings of body weight were undertaken. The results showed that mean body weight of the third generation was higher than that of control population at the end of larval rearing phase (0.21 ± 0.26 g *versus* 0.20 ± 0.15 g), nursery phase (6.12 ± 2.93 g *versus* 5.80 ± 3.50 g) and grow-out phase (198.67 ± 82.82 g *versus* 165.22 ± 71.09 g). Those results revealed that response to selection for body weight of the third generation was positive, *i.e.* about 20.24% (33.45 g).

KEYWORDS: African catfish (*Clarias gariepinus*); body weight; mass selection; response to selection; third generation

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Asda Laining, Usman, and Rachman Syah (Research Institute for Coastal Aquaculture)

Induction of gonadal maturation of pond cultured male tiger shrimp, Prnaeus monodon with different dosages of gonadotropin releasing hormone analogue against eye stalk ablation

Indonesian Aquaculture Journal Volume 11 Number 1, 2016, 23-30

Very low naturally mating rate of pond-reared tiger shrimp broodstock is probably due to the slow maturation of the male stock. The aim of this study was to evaluate the salmon gonadotrophin releasing hormone analoque (sGnRHa) in stimulating the gonadal maturation of male stock of pond-reared tiger shrimp. The treatments were three dosages of sGnRHa at 0.1 (OV-1), 0.2 (OV-2), and 0.3 (OV-3) mL/kg of shrimp weight and control was eye stalk ablation (AB). The sGnRHa was administered via injection three times with one week interval. Male stocks with average initial body weight of 82.1 g were randomly distributed into four of 10 m^3 concrete tanks, 26 males for each tank. Variables observed were performances of spermatophores and profiles of amino acid and fatty acid of muscle of the male stocks. After induction, number of male maturing indicated by spermatophores releasing from terminal ampullas was higher in shrimp induced with OV-1 (80.8%) compared to control which was only 46.1%. Furthermore, shrimp treated OV-2 had the highest spermatophore weight of 0.16 g compared to control (0.11 g) and other two groups. Amino acid profiles improved as the dose of sGnRHa increased up to 0.2 mL/kg from 61.23% for ablated male becoming 71.27% for OV-2. Total fatty acid also tended to improve by increasing the dose of hormone injection, however, the ablated male had higher total fatty acid content than that of OV-1. The present finding demonstrated that the dose of sGnRHa to stimulate the gonadal maturation of pond-reared male tiger shrimp could be applied at range between 0.1-0.2 mL/kg of shrimp weight.

KEYWORDS: sGnRHa; hormone dosage; spermatophore; reproduction; tiger shrimp

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Sekar Ayu Chairunnisa, Sri Nuryati, Alimuddin, Sri Murtini, Ayi Santika, and Dwi Hany Yanti (Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor)

Efficacyof GP-11 KHV DNA vaccine in *Cyprinus carpio haematopterus*

Indonesian Aquaculture Journal Volume 11 Number 1, 2016, 31-39

Koi herpesvirus (KHV) is one of the major pathogen for koi and common carp causing high mortality and economic losses for the farmers. The purpose of this study was to determine the efficacy of glycoprotein-11 (GP-11) KHV DNA vaccine and compared to GP-25 KHV DNA vaccine. The vaccine in the form of naked DNA plasmid was delivered by intramuscularly injection to the three-month-old koi. The fish were divided into six groups, i.e. unvaccinated group (negative control C^- and positive control C^+), and vaccinated group (2.5 mg/100 mL of GP-11 (group 1), 7.5 mg/100 mL of GP-11 (group 2), 12.5 mg/100 mL of GP-11 (group 3), and 12.5 mg/100 mL of GP-25 (group 4)). At day 42 post vaccination, all fish of each group were challenged by injecting KHV titre 10^{-3} FID_{50}. Number of dead fish was counted everyday after the challenge until 30 days. The results showed that vaccinated fish had survival rate of 83.33%-93.33% (group 2, 3, and 4). It showed that GP-11 KHV DNA vaccine had high efficacy. As a conclusion, the application of 7.5 mg/100 mL of GP-11 DNA vaccines have an equal efficacy with 12.5 mg/100 mL GP-25 related to the immune response activity against KHV infection.

KEYWORDS: *Cyprinus carpio* koi; DNA vaccine; injection; koi herpesvirus

UDC 639.2.09

Lili Sholichah, Munti Yuhana, Angela Mariana Lusiastuti, and Tri Heru Prihadi (Graduate School of Aquaculture Science, Bogor Agricultural University)

Potency and afficacy test of a vaccine in addition with adjuvant against koi herpesvirus koi (*Cyprinus carpio*)

Indonesian Aquaculture Journal Volume 11 Number 1, 2016, 41-47

Koi Herpesvirus (KHV) is a malignant virus infecting the goldfish and koi in all stadia and cause mortality up to 95%. The purpose of this study was to determine the potency and efficacy of inactivated-vaccine in addition with adjuvant against KHV in koi fish. The viral propagation was done using a KF-1 cell line in 25 cm^2 flask. The cultured virus was harvested on 12 days post inoculation, and then the harvested virus was inactivated with 0.1% formalin as inactivated-vaccine. Three hundred of test fish (10.38 ± 1.25 g) maintained in 126 L of plastic containers with aeration, and fed with pellets twice a day. After 14 days of adaptation, the fish were divided into five treatments (A = vaccine; B = vaccine + *Complete Freund's Adjuvant*; C = vaccine + *Incomplete Freund's Adjuvant*; K+ = positive control, and K- = negative control) and each treatment has four replicates. Vaccine was given by injecting intramuscularly of 0.1 mL per fish. All fish were challenged by injecting intramuscularly of 0.1 mL of KHV virus with concentration of $10^{4.58}$ $TCID_{50}$/mL after 21 days post vaccination. The results showed that the B treatment had higher ($P<0.05$) values of hematocrit level, lysozyme activity, and titer of antibody compared with positive control. In addition, the survival of fish in B treatment also had the highest percentages and significantly different compared to other treatments ($P<0.05$). The conclusion of this research was the application of inactivated KHV vaccine in 0.1% formalin with the addition of *Complete Freund's Adjuvant* through the injection dose 0.1 mL $fish^{-1}$ in $10^{4.58}$ $TCID_{50}$/mL capable to enhance the immune responses and raised the optimal protection of KHV antibody in koi fish.

KEYWORDS: inactivated vaccines; adjuvants; immune responses; *Cyprinus carpio*; KHV

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I Nyoman Adiasmara Giri*)#, Ketut Sugama), Alimuddin***), and Anang Hari Kristanto****)**

*) Research and Development Institute for Mariculture, Gondol

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Table 1. Response to selection and final mean body weight of the third generation compared to the control population of the African catfish *Clarias gariepinus* at the end of larval rearing, nursery and grow-out phases

Phases	Periods (days)	Final mean body weight (g)		Response to selection	
		Third generation	Control	Gram (g)	Percentage (%)
Larval rearing	25	0.19 ± 0.10	0.19 ± 0.07	-	-
Nursery	30	6.12 ± 2.93	5.80 ± 3.50	-	-
Grow-out	60	198.67 ± 82.82	165.22 ± 71.09	33.45	20.24

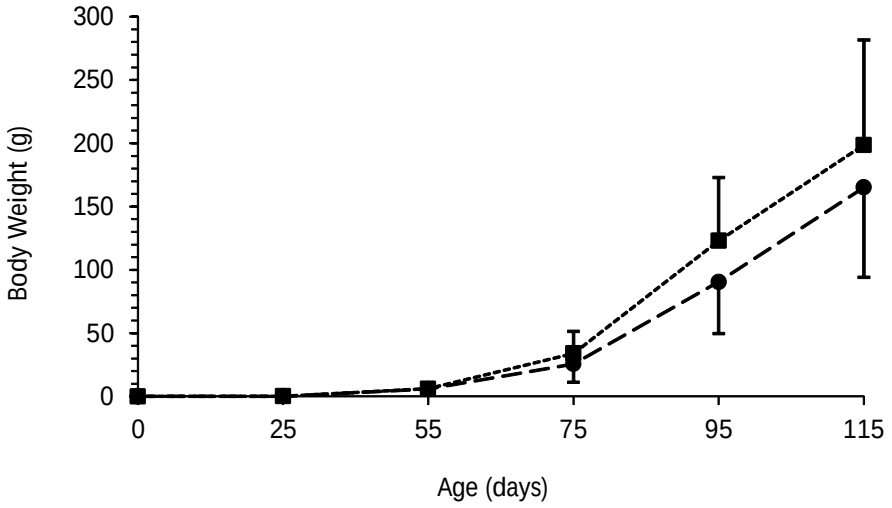

Figure 1. Growth performances based on body weight during 25 days of larval rearing phase, 30 days of nursery phase and 60 days of grow-out phase (based on samplings of 2% populations) of the third generation (■) and control population (●) of the African catfish (*Clarias gariepinus*) genetic improvement program held at Research Institute for Fish Breeding, Sukamandi. Vertical lines represent its each standard deviation

CONCLUSION: The conclusion describes the response of hypotheses and / or research purposes. Conclusions not contain looping of results and discussion, but rather to a summary of the research results.

ACKNOWLEDGEMENTS: thanks mainly devoted to research funders. Acknowledgements can also be delivered to the parties that support the implementation of the research and writing of the manuscript.

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