The growth performance of F1 transgenic mutiara catfish

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Abstract. The growth of catfish (African or Sangkuriang strain) these days is tend to decreased. One of the solutions due to this problem is to improve the genetics of growth using transgenesis technology, toward more profitable. The specific objective of the research is to detect the transmission of exogenous GH (African catfish GH inserts) inside the F1 transgenic Mutiara catfish using PCR (Polymerase Chain Reaction) method and to evaluate the growth performance of transgenic Mutiara catfish made using the parameters of feed conversion (FCR = Feed Conversion Ratio). Transgenic catfish (strain mutiara) F0 and F1 carried African catfish GH (600 bp) can be produced. Superiority characters of transgenic catfish represented heritability ($h^2$) and heterosis (H), indicating that the offspring of hybrid F1 transgenic mutiara catfish had phenotypes rapid growth ($h^2 = 17.55\%$ and $H = 42.83\%$) compared to non-transgenic catfish ($h^2 = 10.07\%$ and $H = 18.56\%$). Evaluation of the efficiency of feed use parameters feed conversion ratio, shows that F1 transgenic mutiara catfish (FCR = 0.85) more efficient in converting feed into meat.

1. Introduction
Improving the growth of farmed fish can be achieved by applying genetic engineering methods, namely transgenesis or gene introduction which takes a relatively short time compared to selective breeding which requires a relatively long time. Gene transfer techniques (transgenesis technology) has advantages when compared to selective breeding, such as genetic improvement per generation that produces a higher, faster phenotype expression improvement and superior character that can be passed down to the next generation [1]. Research on fish transgenesis has been developing rapidly, especially studies of growth hormone gene transfer (growth hormone / GH) to improve the growth of a species of fish. Results of transgenes have shown that growth in fish such as Atlantic salmon [2] and tilapia [3] increased more than 2-fold compared to non-transgenic fish. While transgenic Pacific salmon has a 10-fold growth and the mud loach fish by 32 times compared to non-transgenic fish [4]. Foreign GH carrier transgenic fish in the second generation has the character of high growth compared to the growth of non-transgenic individuals.

Transgenesis technology is a method of gene transfer using sperm electroporation technique, in principle to modify the permeability of the cell membranes of sperm by using an electric field (electric shock). The electric current short that occurs will open the pores of the cell membrane, allowing the foreign DNA molecules (genes transferred) to penetrate the cell membrane and into the nucleus of sperm cells [5]. The degree of exogenous DNA integration with this sperm genome can reach 50%. The development of mass gene transfer methods is very important because it gives an advantage to the fish gene transfer system, resulting in a high number of fish eggs and external fertilization. Stable
transgenic production is necessary in the long run from generation to generation; therefore the inheritance of transgenes in the first offspring of transgenic fish (F1) only reaches 25%. Mass production of transgenic fish was obtained from F3 (the spawning results of F2 transgenic fish and non-transgenic fish) [6].

The B-actin promoter is a promoter capable of driven expression of the transgene in the transgenic fish [7]. In addition to the use of β-actin promoter, another promoter with potential to drive transgene expression is derived from the RSV promoter (Rous sarcoma virus) and CMV (Cytomegalovirus enhancer) and SV 40 (simian virus 40 enhancer). Promoter CMV has been used in the construct of the transgene pCMV-RGH-ires2-EGFP with the technique SMGT in the Rohu (Labeo rohita) carp in India, with an expression of GH which resulted in an increased 2-3 times growth compared to the control (non-transgenic) [8]. The use of CMV promoters has also been successful in driving transgene expression in the embryonic African catfish (Clarias gariepinus), zebrafish and rosy barb which ranged from 25-50 % [9]. The construction of the recombinant expression vector is pCMV/lac Z containing CMV IE promoter and SV 40 polyadenylation used at a concentration of 50 ug/mL.

The success of gene transfer in the larvae is detected using PCR to confirm the transmission of the transgene in the early generations (F0 = founder). Mating between F0 generation produces offspring F1 and the presence of transgenes in individuals F1 were detected to confirm transgene transmission from F0 generation to F1 generation. Research on transgenesis using SMGT in Indonesia have successfully been done on transgenic grouper duck (Cromileptes altivelis) containing the construction gene pkBP-ktGH with a concentration of 10 ug/mL on sperm electroporation with 50 Vcm⁻¹ pulse length, 30 milliseconds shock duration, with a number of 5 pulses and pulse interval of 0.1 seconds [10].

Transgenesis has also been successfully conducted in catfish (Clarias sp.) with tilapia GH gene (tiGH) using sperm electroporation, with optimal conditions as follows: electric field strength of 125 Vcm⁻¹, pulse length of 30 milliseconds, with a number of 5 pulses and a pulse interval of 0.1 seconds. mBP-tiGH construction transfer efficiency on catfish was around 93 % for a DNA concentration of 80 ug/mL [11]. The results of the study by Dewi et al. [12] also successfully inserted stripped catfish GH gene contained in the construction PCeBA-PhGH on stripped catfish (Pangasionodon hypophthalmus) with a transfer efficiency of 85.71 % for a plasmid DNA concentration of 90 ug/mL. A shock duration of 30 milliseconds, shock interval of 0.1 seconds, a strong electric field (electric field strength) of 125 Vcm⁻¹ were the optimal conditions in the study.

The results of the research in 2015 showed the gene transfer of the African catfish (GH exogenous) growth hormone contained in pTarget-CMV-CgGH that was successfully inserted into mutiara catfish sperm through an electroporation of 125 Vcm⁻¹ with a number of 3 pulses. The African catfish GH inserts was detected in 1000 bp size fragment position (96 % identical to the GH C. gariepinus) with the bankgen accession number AF416488.1. The body size of F0 transgenic mutiara catfish at the age of four months was around three times larger than the size of the non-transgenic mutiara catfish, which shows the effects of transgenic catfish gigantism (over-expression of exogenous GH) [13].

Based on above description, it is necessary to evaluate the character of the growth of F1 transgenic mutiara catfish (containing GH exogenous) using parameters of heritability (h²) and heterosis (H) as a candidate of superior catfish.

2. Methodology
2.1. Design of the GH lele dumbo expression vector construction
The research method of the growth hormone gene transfer of the African catfish (CgGH) to the sperm of mutiara catfish to support the production transgenic mutiara catfish (carrier CgGH) consists of a stage of the CgGH transfer with the sperm electroporation technique and the detection of catfish transgenic F0 and F1 (offspring of the first generation) by using the PCR (Polymerase Chain Reaction). Based on the results of the research in 2015, the construction of a gene was successfully designed to produce a transgenic mutiara catfish pearl carrier of GH African catfish which was pTarget-CMV-CgGH containing CMV promoter sequences and fragments of African catfish (CgGH) GH as inserts
of GH exogenous to the construction of the gene (figure 1). The construction of genes (African catfish growth hormone gene expression vector) has been integrated in the genomic DNA of mutiara catfish sperm as detected in F0 transgenic catfish.

**Figure 1.** Construction pTarget-CMV-CgGH (6270 bp).

Before the transgenes (pTarget-CMV-CgGH) were transferred to the sperm, the sperm of mutiara catfish broodstock (aged 1 year, weight of 1.8 kg) was obtained by injecting ovaprim hormones (at a dose of 0.2 mL per kg weight of the broodstock) in order to induce the spermiation of the broodstock. As much as 0.5 mL sperm was collected in each petri dish and then diluted with a solution of Na-physiological (isotonic solution) to maintain sperm motility with the ratio of 0.5 mL sperm and 0.5 mL Na-physiological solution (1: 1). The female mutiara catfish broodstock were injected with ovaprim hormones at a dose of 0.5 mLkg⁻¹ body weight and stripped 12 hours after injection to ovulate. The eggs were collected in a plastic cup (dry conditions without water).

2.2. Transfer GH Lele Dumbo With Sperm Electroporation Technique

The volume of the DNA recombinant vector (pTarget-CMV-CgGH) solution that was used to transfer mutiara catfish sperm (*Clarias* sp.) was at about 60 ng / µl or 60 µg / mL. The electroporation process was done by mixing the sperm and DNA vector into the gene pulser II (Biorad) electroporator cuvette machine with the square wave shock type of 125 Vcm⁻¹; shock duration of 30 milliseconds; shock interval of 0.1 seconds, and a number of 3 pulses using a 0.4 cm cuvette. The African catfish GH transfer electroporation into mutiara catfish sperm technical flow exercise is presented in figure 2.

The sperm that has been electroporated was then mixed with mutiara catfish eggs and transferred to the aquarium for the fertilization of the egg with a water temperature of about 26-27 °C until the eggs hatch into catfish larvae. The detection of the success of gene transfer of the African catfish growth hormone (GH exogenous) that is integrated in the genome of F0 transgenic mutiara catfish and F1 is required to confirm the increased growth of transgenic fish due to over-expression of exogenous GH.
The mixture of sperm: Na physiological (1:1)
(500 µl : 500 µl)

10 µl 25 µl

Electroporator cuvette (0, 4 cm)
mixture with pipetting

Running electroporation sperm:
program shock type: Square Wave
pulse length: 30 milliseconds
pulse number: 3 x
pulse interval: 0,1 seconds
voltage: 125 Vcm⁻¹

Pasca electroporation sperm fitness increased with
add 265 µl Na-physiological solution

Sperm pasca electroporation
fertilization with eggs

The fertilized eggs by sperm pasca electroporation
dead embryo eggs
catfish larvae hatch grow out until adult

Figure 2. The exercise of transfer Target-CMV-CgGH to mutiara catfish sperm.

The existence of the insertions of African catfish growth hormone gene on F0 mutiara catfish broodstock (2015) and mutiara catfish first offspring (F1) (2016) needs to be detected using PCR to confirm the inheritance of the GH exogenous (GH African catfish) from F0 broodstock to offspring F1. Cg-F primer (5'-ATGGCTCGAGTTTTGGTGCTGCT-3') and Cg-R (5'-CTACAGAGTGCAGTTGGAATCCAGGG-3') was used to copy sequences of African catfish GH, while CgGH-F primer (5'-CAGTACATCAAGTGATCATC-3') and CgGH-R (5'-AGATAAGTCTCCACTTTGTA-3') was used to copy fragments of CMV-CgGH (transgene). The growth response of fish to the given feed is in correlation with the character of the fish growth which shows an improvement in the growth of fish and was evaluated using the parameters of feed conversion ratio (FCR). The inheritance of superior character (super growth) in the F0 and F1 generation was measured by heritability (h²) which is an indicator of inheritance evaluation of superior character of F0 broodstock to F1 offspring for a quantitative phenotype, while evaluating the performance superiority of fish in F1 offspring was measured using the heterosis (H) test.
3. Results and Discussion

3.1. Detection of GH lele dumbo in f0 mutiara catfish sperm

The results of electroporation in the sperm of mutiara catfish at 125 Vcm⁻¹ with a number of 3 pulses shows the success of the gene transfer of African catfish GH (CgGH) in the sperm of these catfish, which is indicated by the detection of 600 bp sized fragments which is a gene of African catfish GH on mutiara catfish sperm (figure 3).

![Figure 3. Detection CgGH (using primer Cg-F and Cg-R).](image)

The combination treatment of voltage and the number of pulses in sperm electroporation will increase the permeability of the cell, causing exogenous polynucleotide (foreign DNA) into the cell plasma membrane of sperm. More foreign DNA will be transferred by the sperm to the egg at fertilization which is effective in producing transgenic fish carrying a new phenotype (figure 4 and 5). The transgene (African catfish GH gene carrier) can be transmitted back through sexual reproduction of broodstock (F0) to the first offspring (F1) with the expression of a new phenotype.

![Figure 4. Broodstock mutiara transgenicF0 ♂ (A) and Broodstock mutiara non-transgenic F0 ♂(B) aged 11months.](image)
3.2. Fragment detection of CMV-CgGH OnF1 transgenic mutiara catfish

The detection of CMV-fragment transmission GgGH (transgen) in the F1 transgenic catfish was carried out to verify permanent inheritance inserts of African catfish GGH in the context of the mass production of transgenic catfish. The design of primers used to copy sequences of CMV-CgGH was designed online with Primer3 software version 4.0 and the size of 1434 bp fragment was obtained, called CgGH-F (5'-CAG TAC AAG TGT ATC ATC AT-3') and CgGH-R (5'-AGA TAA TTG GTC TCC ACT T-3'). Catfish genome amplification on the sample F1 transgenic mutiara with PCR program pre-denaturation at 95°C (5 minutes), denaturation at 94°C (45 seconds), annealing at 49.2°C (45 seconds), extension at 72°C (2 minutes) and a final extension at 72°C (7 minutes) and the number of cycles at 35 times to produce a product size of approximately 1500 bp (Figure 6).

Transgenic catfish F0 and F1 was detected using this pair of primers, and the insertion transmission of African catfish GH (CgGH) inherited from the broodstock (F0 transgenic mutiara catfish) to the offspring (F1 transgenic mutiara catfish). Based on the designed primer using Primer3 version 4.0 software on-line, a fragment size of 1434 bp was obtained, hence the primer CgGH-F and CgGH-R is a primer suitable for detecting transgenic catfish containing a portion of the insertion fragment promoter CMV connected with partial fragment inserts of African catfish GH (CMV-CgGH fragment size of about 1500 bp) (figure 6). Similar results were obtained from the studies of Dewi et al. [14], that the size of the transgene (fragment pCcBA-PhGH) was detected in F3 transgenic African catfish of 1500 bp. Transgene expression (CMV-CgGH) in F1 transgenic mutiara catfish is driven by the CMV promoter as a transgene expression regulator and demonstrated that the transgene was stably transmitted from parent F0 to F1 generation.
3.3. Performance growth F1 transgenic mutiara catfish
to prove the inheritance of the growth rate characterization of F0 transgenic mutiara catfish which had properties that grew threefold (474 g/133.9 g) compared to non-transgenic catfish (normal mutiara catfish), the calculation of the value of heritability in the first offspring (F1) transgenic mutiara catfish was required. Tsai [5] reported that the growth of F2 transgenic fish loach (Misgurnus anguilicaudatus) was twice the growth of the non-transgenic. Over expression of growth also occurred in F2 transgenic salmon (Salmo salar) of 2.62 to 2.85 times the growth of non-transgenic salmon. Results with a cross of F0 male transgenic mutiara catfish with a female Sangkuriang catfish produced a hybrid offspring F1 mutiara transgenic showing rapid growth as the broodstock when the fish was aged 4.5 months. The average growth of F1 transgenic mutiara catfish hybrid (age 4.5 months) at 460.74 g is close to the average growth of F0 transgenic mutiara catfish (age 4 months) which amount to 474 g (figure 7).

![Figure 6. Inheritance character catfish growth of transgenic. F0 (A) to F1 (B).](image)

Rapid growth properties that is possessed by the offspring of the hybrid of F1 transgenic mutiara catfish in particular is the average weight of fish F1 97.19% (460.74 g / 474 g) was identical to the average weight of the parent (F0 transgenic mutiara catfish) when the fish was aged 4.5 months. This is an indication that the additive gene effects on the parent is fully inheritable on to the offspring (F1) [15]. The level of inheritance contained in the F1 generation (offspring of hybrid transgenic mutiara catfish) could explain that the additive effect is inheritable from the parent (F0 transgenic mutiara catfish), especially the character of high growth (super growth) that is ia trait inherited by its broodstock. The heritability weight growth of transgenic mutiara catfish offspring F1 hybrid aged 4.5 months is presented in table 1 below.

| Table 1. Heritability ($h^2$) of transgenic mutiara catfish offspring F1 hybrid age 4.5 months. |
|-------------------------------------|-----------------|-----------------|-----------------|
| Growth character                   | Purebreed of transgenic hybrid | Control         |
| Average weight (g)                 | F1 ($♂ MT x ♂ S$) | F1 ($♂ MNT x ♂ S$) | F1 ($♂ S x ♂ S$) |
| Deviation Standard                 | 460.74          | 268.52          | 184.44          |
| Varians                            | 6.32            | 2.31            | 2.22            |
| Heritability ($h^2$)               | 17.55 %         | 10.07 %         | -               |
Note:
♂ MT = Male of Mutiara Transgenic
♀ S = Female of Sangkuriang
♂ MNT = Male of Mutiara Non-Transgenic

The results presented in table 1 above show that the transgenic mutiara catfish offspring F1, especially transgenic mutiara catfish hybrid (♂ MT x ♀ S) has a value of h² of 17.55%, which means that the growth rate of phenotype inheritance (the size of the individual weight) of parent (F0) was inherited in the offspring (F1), with a potential inheritance that is quite high, therefore the value of h² was above 15% [16]. Heritability measurement results of the other catfish group, namely the stripped catfish (Pangasianodon hypophthalmus) from the cross three strains by diallel crossing 3 x 3, yielding a value h² of 17% in the offspring hybrid third generation (G3) [17]. The heritability value of the hybrid offspring of F1 transgenic mutiara catfish (h² = 17.55%) is slightly higher than stripped catfish of the third-generation hybrid offspring with a heritability amount of 17%. The calculation of the value of heterosis (H) weight of fish obtained from F1 transgenic mutiara catfish (♂ MT x ♀ S) amount to 42.83% higher compared to F1 non-transgenic mutiara catfish hybrid (♂ MNT x ♀ S) which is only 18.56% of the average weight of pure strains of catfish (♂ S x ♀ S) (table 2).

Table 2. Heterosis (H) value of hybrid F1 transgenic mutiara catfish.

| Growth fenotipe | Purebreed of transgenic hybrid | Purebreed |
|-----------------|-------------------------------|-----------|
| Average weight (g) | F1 (♂ MT x ♀ S) | F1 (♂ MNT x ♀ S) | F1 (♂ S x ♀ S) |
| Heterosis (H) | 460.74 | 268.52 | 184.44 |

Note:
♂ MT = Male of Mutiara Transgenic
♀ S = Female of Sangkuriang
♂ MNT = Male of Mutiara Non-Transgenic

This evidence shows that the growth of the highest weight obtained from the cross male transgenic mutiara catfish (♂ MT) and female Sangkuriang (♀ S) than the cross pure strains (♂ S x ♀ S) or weight growth of hybrid transgenic mutiara is 42.83% more than the weight of the pure strain. The heterosis value (42.83%) was obtained from the results of this study (cross ♂ MT x ♀ S) by 2-fold (42.83%: 18.56%) of heterosis in hybrid cross ♂ MNT x ♀ S) (table 2).

Feed conversion ratios for growth show that F1 transgenic mutiara catfish are more feed efficient (FCR = 0.85) compared to non-transgenic mutiara catfish (FCR = 1.38) and sangkuriang catfish (FCR = 1.5) (table 3).

Table 3. Feed conversion ratio of F1 mutiara catfish hybrid offspring.

| F1 offspring hybridization | Weight fenotipe : | Feed amount | FCR |
|---------------------------|-------------------|-------------|-----|
|                           | Wt₁              | Wt₃₀        |     |
| (pure strain)             | 3500 g           | 4980 g      | 1.5 |
| (non-transgenic)          | 4860 g           | 7250 g      | 1.38|
| (transgenic)              | 5400 g           | 12420 g     | 0.85|

Note:
Wt₁ = biomass weight of early treatment (1st days)
Wt₃₀ = biomass weight of end treatment (30th days)
These results confirm the fact that the offspring of transgenic mutiara catfish F1 by consuming feed (feed protein content hi-provide 30%) of 850 g can grow up to 1000 g (1 kg), thus economizing the use of 150 g of feed. This was considered efficient for the fish farming business where 60% of production costs are for the fish farming feed requirements [18]. This efficiency is associated with the over-expression of the two genes contained in the GH transgenic fish so that the synthesis of growth hormone levels causes an excessive and rapid growth rate of fish. The research of Marnis et al. [19] showed that the F2 transgenic fish of African catfish had a high feed efficiency (FCR 0.86) compared to non-transgenic catfish (FCR 1.79). Kobayashi et al. [3] also showed that transgenic tilapia had a 35% higher fish feed efficiency than non-transgenic tilapia which led to increased growth. The high feed efficiency of transgenic fish was due to the exogenous GH (GH inserted) which stimulated the production of IGF-I (Insulin-Like Growth Factors) in the liver in response to GH accelerating their metabolic rate to transform feed protein into the growth of fish. Plasma GH and transgenic IGF-I catfish increases higher than the non-transgenic fish, showing that the synthesis of GH transgenic fish was much higher than non-transgenic fish, causing the fish to be very responsive to feed due to increased levels of GH plasma and tend to be satiated, thereby reducing the consumption of feed [18, 20].

5. References

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