HAYATI Journal of Biosciences 23 (2016) 150–154

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Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Original research article

Growth, Survival, and Body Composition of Transgenic Common Carp *Cyprinus carpio* 3rd Generation Expressing Tilapia Growth Hormone cDNA

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ARTICLE INFO

Article history:
Received 21 April 2015
Accepted 24 September 2016
Available online 19 December 2016

KEYWORDS:
body composition,
common carp,
growth performance,
transgenic

ABSTRACT

Transgenic has been known as one of the applicable methods to improve growth performance of cultured fish. This study was performed to evaluate the growth performance, survival, and body composition of the 3rd generation of growth hormone (GH) transgenic common carp (TG). Juveniles (BW: 1.53 ± 0.03 g) were reared for 60 days in 250-L glass aquarium with stocking density of 25 fishes/aquarium. Fishes were fed with commercial feed (protein content 36%), three times a day to satiation. Growth and survival were measured every 20 days. Our results showed that TG fish has 1.49 times higher in average weight growth ($p < 0.05$) compared with the non-transgenic common carp (NT). Higher total feed consumption, survival, body protein content, protein and lipid retention, hepatosomatic index, and lower feed conversion ratio were also shown on TG fish compared with NT fish ($p < 0.05$). However, body lipid content and blood glucose level of TG fish were lower ($p < 0.05$) compared with the NT fish. Total ammonium nitrogen level in rearing media of TG fish was 51.78% lower ($p < 0.05$) than that of the NT fish. In conclusion, culturing of GH-TG common carp showed potential to achieve high productivity, efficient, and environmental-friendly aquaculture.

1. Introduction

Common carp is one of the main freshwater fishes farmed in Indonesia. After 10 years (Ariyanto and Subagyo 2004), no research on growth improvement in Indonesian common carp has been reported. In aquaculture, growth improvement is the main key to increase the production (Raven *et al.* 2008). In addition, increasing fish growth would give a lot of benefits including to shorten production time, increase feed efficiency, and control product availability (Devlin *et al.* 2004).

Gene transfer is a technique, which is considerably fast and an effective way to increase somatic growth and aquaculture production (Devlin *et al.* 2004). The higher productivity, shorter time, and higher feed efficiency in transgenic fish also reported by Lu *et al.* (2009). Since generation of the first transgenic fish reported (Zhu *et al.* 1989), gene transfer has been used to produce various fast growing fish species such as Atlantic salmon (*Salmo salar*) (Du *et al.* 1992), channel catfish (Dunham *et al.* 1992), common carp (Chen *et al.* 1993), coho salmon (*Oncorhynchus kisutch*) (Devlin *et al.* 2004), mud loach (*Misgurnus mizolepis*) (Nam *et al.* 2002), and Nile tilapia (*Oreochromis niloticus*) (Kobayashi *et al.* 2007). In Indonesia, research on growth improvement in common carp using gene transfer technique has been initiated in 2011 (Alimuddin *et al.* 2012).

GH over-expression not only improves growth rate, but also affects metabolic rate (McKenzie *et al.* 2003), swimming ability (Lee *et al.* 2003), resistance against disease (Jhingan *et al.* 2003), feed consumption and feeding behavior (Stevens and Devlin 2005), and response against predator (Duan *et al.* 2010). Changes on nutrient metabolism affect ammonia (Kobayashi *et al.* 2007) and phosphorous excretion to the water (Lu *et al.* 2009), and this eco-friendly sounds. Furthermore, changes on feed consumption affect feed efficiency and production cost (Lu *et al.* 2009), and changes on resistance against disease affect survival and harvest biomass (Ling *et al.* 2009).

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Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2016.12.002

Growth improvement within relatively short time makes transgenic fish potential to be developed as food source for human (Zbikowska 2003). There is still limited information related to nutritional status and body composition of transgenic fish, although body composition of transgenic fish can be used as consideration in food safety assessment of genetically modified organism (Devlin *et al.* 2004). In this study, transgenic common carp (TG) expressing Nile tilapia GH (tiGH) complementary deoxyribonucleic acid (cDNA) was used to examine proximate composition (Alimuddin *et al.* 2012). In previous study, TG expressing rainbow trout GH gene was reported to contain higher protein level, lower lipid and moisture levels compared with control (Chatakondi *et al.* 1995). Moisture, protein, mineral, and energy contents of GH transgenic salmon were lower compared with control (Cook *et al.* 2000). The aim of this study was to evaluate growth performance, survival, and body composition of GH-TG expressing tiGH. The result of this study was expected to become a reference in transgenic fish development in Indonesia.

2. Materials and Methods

2.1. Fish source

One of the GH-TG male 2nd generation (G2) with the highest tiGH expression level was selected and crossbred with non-transgenic female to produce transgenic (TG) 3rd generation (G3). Those G2 transgenic fishes were produced from mating between tiGH-TG male G1 and non-transgenic (NT) female (Alimuddin *et al.* 2012). Using the same NT female, crossbred with NT male was also performed to produce control (NT).

Artificial spawning was performed by hormonal induction using ovaprim (Syndel Laboratories Ltd, Nanaimo, British Colombia, Canada). Induced ovulation on female broodstock was conducted by intramuscular injection of ovaprim at a dose of 0.5 mL/kg body weight (BW), whereas male was 0.2 mL/kg BW (Faridah 2012). Sperms and eggs were collected by stripping, mixed in a large plastic bowl, and then incubated in aquarium. Feeding for fish fry was performed by giving 4 days after hatching (dah) of artemia nauplii until 10-dah and continued with bloodworm until 20-dah. After grown to juvenile (body length [BL] 2–3 cm), fishes were reared in net cages ($2 \times 2 \times 1$ m^3) installed in a concrete pond until grown to BL of 3–5 cm.

Transgenic fishes carrying tiGH gene were identified individually using polymerase chain reaction (PCR) method with genomic deoxyribonucleic acid (DNA) template that have been extracted from caudal fin. PCR amplification program and primers used were as described by Kobayashi *et al.* (2007). Genomic DNA was extracted using DNA purification kit (Puregene, Minneapolis, MN, USA) according to the manufacturer's instruction.

2.2. Growth performance analysis

Common carp juveniles (BW 1.53 ± 0.03 g; BL 4.6 ± 0.45 cm) were reared for 60 days in $100 \times 50 \times 50$ cm^3 glass aquariums with stocking density of 25 fishes/aquarium. We conducted three repetitions for each group. A top filter and aeration were provided in each aquarium to keep good water quality. Water was changed 30% per day in afternoon. Fishes were fed with commercial feed (protein content 36%), three times a day (morning, afternoon, and evening) to apparent satiation.

2.3. Data collection and statistical analysis

Fish BL and BW were individually measured and number of survived fish was counted every 20 days. Hepatosomatic index (HSI), proximate composition, total ammonium nitrogen (TAN) in rearing water, and blood glucose level were measured at the end of the experiment. Specific growth rate (%) was calculated by the equation: specific growth rate = (lnWt − lnWo) × 100/t [Wt = average BW at the end of the rearing period (g); Wo = initial average BW (g); t = length of time maintenance (days)]. Feed conversion ratio (FCR) was calculated using the equation: FCR = [P/((Wt + Wm) − Wo))]; [P = amount of feed given during rearing (g); Wt = biomass of fish at the end of the rearing period (g); Wo = initial biomass of fish (g); Wm = weight of fish that died during rearing (g)].

A total of four individuals whole body (about 100 g) of fish from each group were taken randomly and chopped until smooth, then homogenized for proximate analysis process. A complete proximate analysis was carried out on the first and last day of the experiment, according to the methods of Takeuchi (1988). The liver was removed from five fishes for each group and weighed for calculating HSI using the equation HSI = (liver weight/BW) × 100. Three fishes from each group were taken randomly before, 6, 12, and 24 hours after feeding. Blood glucose levels were measured by enzymatic colorimetric method (using a liquicolor GLUCOSE test (Human mbH diagnostics, Magdeburg, Germany). TAN level in the culture media was measured using a spectrophotometry method. Seven fishes from each group with four replications were used in this test. Tests were carried out at the aquarium with a volume of 70 L of water without top filter. Initial measurement was carried out after the fishes had been fasted for 24 hours and the aquariums were filled with new water (TAN 0.12 ± 0.01 mg/L). This was performed 24 hours after the end of the fishes were fed to satiation. TAN was calculated for each unit of feed consumed per fish biomass, as modified from Kobayashi *et al.* (2007).

Tilapia growth hormone (*ti*GH) messenger ribonucleic acid (mRNA) expression was analyzed by reverse transcription polymerase chain reaction (RT-PCR) method. Ribonucleic acid (RNA) total was extracted from liver from three fishes using Isogen reagent, and cDNA synthesis was performed using R-To-Go You-Prime First-Strand Beads (GE Healthcare, Little Chalfont, Great Britain). PCR amplification was conducted using a set primer of tiGH-1F forward (5′-AGA CAG CCA GCG TTT GTT CT-3′) and tiGH-1R reverse (5′-CCA GGA CTC AAC CAG TCC AT-3′) (Kobayashi *et al.* 2007). PCR products were separated by electrophoresis with 1% agarose gel. All data were analyzed by independent samples t-test using SPSS 16.0 software (Chicago, SPSS Inc., USA) at $p = 0.05$. Expression of *ti*GH gene was analyzed descriptively.

3. Results

In this study, tiGH-TG showed 1.49 times higher average growth rate ($p < 0.05$) compared with non-transgenic fish (Table 1). Weight increase, specific growth rate, total feed consumption, lipid retention, and protein retention of TG was also higher ($p < 0.05$; Table 1),

Table 1. Increase of body weight and length, specific growth rates, total feed consumption, feed conversion ratio, lipid and protein retentions, hepatosomatic index, and NH_4–N excretion of common carp reared for 60 days in aquarium

Parameters	Transgenic fish	Non-transgenic fish
Weight increase (g)	9.23 ± 1.40^a	6.21 ± 0.58^b
Length increase (cm)	2.39 ± 0.45^a	1.70 ± 0.17^a
Specific growth rate (%)	3.29 ± 0.22^a	2.73 ± 0.13^b
Total feed consumption (g)	417.05 ± 23.93^a	301.18 ± 32.01^b
Lipid retention (%)	111.90 ± 4.46^a	73.89 ± 6.43^b
Feed conversion ratio	1.97 ± 0.16^b	2.56 ± 0.31^a
Protein retention (%)	16.43 ± 0.64^a	8.73 ± 0.79^b
NH_4–N excretion (mg/g feed/g fish × 10^{-4})	5.69 ± 0.33^b	8.63 ± 1.39^a
Hepatosomatic index (%)	0.39 ± 0.08^a	0.25 ± 0.02^b

Different superscript letters in the same row showed significant differences based on independent samples t-test results at $p < 0.05$. Values followed by the letter "a" is higher than that of "b".

whereas length increase of transgenic fish was not different with non-transgenic fish ($p > 0.05$). Furthermore, over-expression of tiGH has significantly affected ($p < 0.05$) on body proximate content. Protein content of TG fish was 11.67% higher ($p < 0.05$), but the lipid content was 7.29% lower ($p < 0.05$) compared with NT common carp (Table 2). There was no significant difference in ash and carbohydrate contents between TG and NT fish ($p > 0.05$).

Expression of *ti*GH affected the survival of transgenic and non-transgenic common carp. Survival rate of transgenic fish was 36.73% higher compared with non-transgenic fish at the end of the experiment ($p < 0.05$; Figure 1). The died fish was found since 20 until 60 days experiment.

Expression of *ti*GH was detected in common carp transgenic fish, whereas *tiGH* gene expression was not detected in non-transgenic fish liver (Figure 2). This result showed that higher growth performance and other parameters in transgenic fish were caused by *tiGH* over-expression in transgenic fish.

The faster growth of transgenic fish affected blood glucose level in the body. There was no significant difference of blood glucose level when fishes were in fasting condition (0 hour); afterward, the glucose level was increased and reached maximum level at the 6th hour after feeding (Figure 3). Blood glucose level was decreased 12 hours after feeding. Blood glucose level of TG went back to normal state before the 12th hour, whereas non-transgenic fish has not yet. After the 12th hour, glucose level increased until the 24th hour and blood glucose level of TG fish was 14.53% lower compared with NT fish at 24 hour after feeding ($p < 0.05$; Table 2)

Table 2. Body proximate composition of growth hormone transgenic common carp (3rd generation)

Parameters (% dry weight)	Transgenic fish	Non-transgenic fish
Protein	40.67 ± 0.73^{b}	36.42 ± 0.24^{a}
Lipid	46.06 ± 0.62^{b}	49.68 ± 0.47^{a}
Carbohydrate	4.21 ± 0.67^{b}	4.50 ± 1.31^{b}
Ash	5.88 ± 0.60^{b}	6.08 ± 1.32^{b}

Moisture of transgenic fish was 72.04%, and non-transgenic fish was 73.64%. Different superscript letters in the same row showed significant differences based on independent samples t-test results at $p < 0.05$. Values followed by the letter "a" is higher than that of "b".

4. Discussion

TG has higher total feed consumption compared with NT fish ($p < 0.05$; Table 1). Similar results also reported on transgenic Atlantic salmon (Cook *et al.* 2000), Nile tilapia (Rahman *et al.* 2001), mud loach (Nam *et al.* 2002), coho salmon (Devlin *et al.* 2004), rohu (Venugopal *et al.* 2004), and common carp (Fu *et al.* 2007). This increasing appetite assumed as an effect of increasing ghrelin hormone (Debnanth 2010) and neuropeptide activity of *agouti-related protein I* (Zhong *et al.* 2013), which is induced by GH. The increasing appetite was a consequence of the rapid growth of transgenic fish, which requires more energy sources. Physiologically, the mechanism of feed utilization in transgenic fish has not

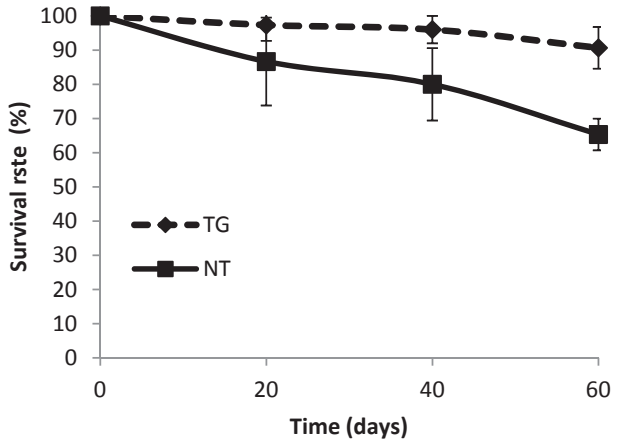

Figure 1. Survival rate of *ti*GH-transgenic (TG) and non-transgenic (NT) common carp.

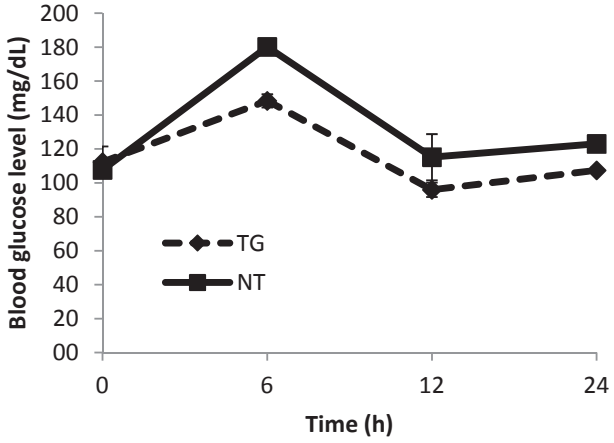

Figure 3. Blood glucose level of *ti*GH-transgenic (TG) and non-transgenic (NT) common carp in 0, 6, 12, and 24 hour after feeding.

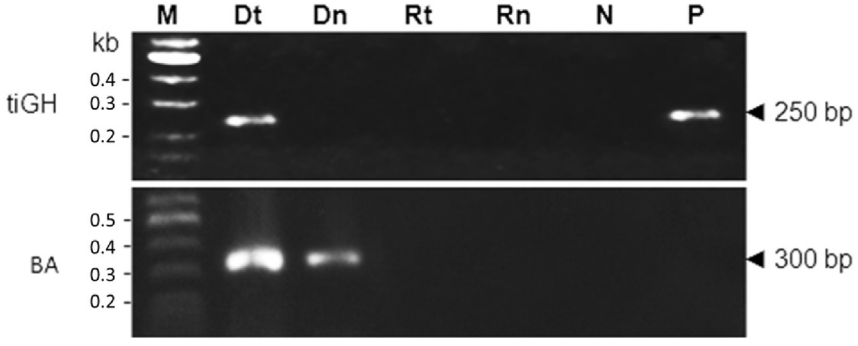

Figure 2. Tilapia growth hormone (*ti*GH) and β-actin (BA) genes expression analyzed by reverse transcriptase polymerase chain reaction (PCR) method. M: deoxyribonucleic acid (DNA) marker (KAPA Universal DNA Ladder KL6302, Kapa Biosystems), Dt: PCR product of transgenic complementary ribonucleic acid (cDNA) template, Dn: PCR product of non-transgenic cDNA template, Rt: transgenic RNA template, Rn: non-transgenic RNA template, N: PCR product without cDNA template and P: PCR product with *pm*BA-*ti*GH plasmid as template. The expected size of the amplified fragment was 250 bp (*ti*GH) and 300 bp (β-actin).

been clearly known yet; however, transgenic fish expected to possess better ability to digest, absorb, and distribute nutrition (Fu *et al.* 1998; Stevens *et al.* 1999; Cook *et al.* 2000). Furthermore, transgenic coho salmon has wider, bigger, and longer digestive tract compared with control, which enables better food absorption and faster growth (Stevens *et al.* 1999).

The high total feed consumption also positively correlated to protein and lipid retention in TG fish compared with NT ($p < 0.05$; Table 1). Higher protein and lipid retentions in TG fish showed that transgenic fish had higher capability to deposit protein and lipid intake from feed. Fu *et al.* (1998) has also reported that *h*GH TG has higher protein retention compared with control. Higher protein and lipid retention in TG also correlated to FCR (Table 1). The role of GH in decreasing FCR of transgenic fish has also been reported in mud loach (Nam *et al.* 2002), salmon (Devlin *et al.* 2004), and Nile tilapia (Kobayashi *et al.* 2007).

TG common carp has lower blood glucose level compared with NT at 24 hours after feeding ($p < 0.05$; Figure 1). Low blood glucose level during observation suggests that most likely tiGH fish have higher blood glucose utilization and mobilization as energy for metabolism and growth. The same pattern was showed in transgenic salmon (6 hour after glucose injection), whereas at the 24th hour after injection salmon has lower glucose level ($p < 0.05$). Transgenic salmon was also able to go to glucose basal level faster (Panserat *et al.* 2014). Transgenic salmon also has better ability in using feed with high carbohydrate content (Higgs *et al.* 2009) and also has better ability to use carbohydrate as energy source and lipid synthesis compared with control (Leggatt *et al.* 2009). However, in rat, GH can increase glucose utilization and reduce glucose level in blood as energy source fort growth (Boparai *et al.* 2010).

Presence of non-protein energy source (lipid and carbohydrate) reduces protein utilization as energy for metabolism process (protein sparring effect), so that protein retention increases and ammonia excretion reduces (Suprayudi *et al.* 1994). In this study, TG fish was able to increase protein retention of 88.30% and reduce ammonia excretion of 51.78% (Table 1). Similar result also showed in transgenic Nile tilapia where TAN excretion reduced up to 69% (Kobayashi *et al.* 2007). It shows that TG common carp is more efficient in using protein from the feed. Furthermore, a number of studies have also reported that transgenic fishes were able to use lipid (Pérez-Sanchez 2000) and carbohydrate (Leggatt *et al.* 2009) as energy source (protein sparring effect). Nitrogenous waste, such as ammonia and nitrite, can be toxic to fish (Randall and Tsui 2002). Moreover, the nitrogenous compounds that are excreted by farmed fish can enhance the process of eutrophication, which is associated with plankton blooms (Wu 1995). This can potentially result in low-oxygen conditions that are known as “dead zones” (United Nations Environment Programme 2005). This fish, therefore, has great potential for promoting more sustainable and eco-friendly for fish farming.

The result of this study showed that TG common carp has 1.49 times higher weight growth compared with NT fish ($p < 0.05$; Table 1). This result was in line with the research conducted by Fu *et al.* (2007) with increased growth 67%–77% in TG “all-fish” G2, and 19%–25% in TG *h*GH F4 (Fu *et al.* 1998). The different growth increase might be caused by different kinds of gene used (Devlin *et al.* 2004), integration site in chromosome, transgene copy number, and the activity of promoter used (Moav *et al.* 1992). The differences found might also be caused by different rearing time and methods.

The HSI in TG fish was higher compared with NT fish ($p < 0.05$; Table 1). It was also assumed that the higher liver glycogen (data not shown) in TG fish has increased the liver mass. Increasing HSI has also been reported in transgenic salmon 30% higher compared with control (Leggatt *et al.* 2009). Increasing HSI also showed that the size of the liver has to be larger, and this may be as a compensation of the faster growth in transgenic fish. In addition, liver is the center of nutrient metabolism in the body and HSI has been known generally as an indicator of growth (Ighwela *et al.* 2014).

Survival rate of TG fish was also 36.73% higher compared to NT fish ($p < 0.05$; Figure 2). It was assumed as effect of the better non-specific immune in transgenic fish (Wang *et al.* 2006). Furthermore, Ling *et al.* (2009) reported that TG has better resistance against *Ichthyophthirius multifiliis* infection. This study also found that TG has better hematological parameters (hematocrit, hemoglobin, and phagocytic activity) compared with the non-transgenic (data not shown). Wang *et al.* (2006) added that TG has higher lysozyme activity, serum bactericide, leukocyte, and phagocytic activity. The better health status of transgenic fish also assumed has significant effect on adaptability of the fish to the environment.

The tiGH TG has higher body protein content, with lower lipid content compared with the non-transgenic fish. This result was in line with the research conducted by Chatakondi *et al.* (1995) and Fu *et al.* (1998) in transgenic common carp. Different with transgenic Atlantic salmon with lower lipid and protein content compared with the non-transgenic salmon (Cook *et al.* 2000). The different body composition might be caused by pre-treatment feed composition and nutritional history (feed quality and feeding rate; Raven *et al.* 2006). Higher protein level and lower lipid level in fish can be good for health and economy viewpoint. Low lipid content might reduce the risk that the fish will absorb off-flavor molecule which destroys flesh aroma (Robin *et al.* 2006). The lower lipid content in TG fish may be due to higher energy requirement and higher metabolism compared with control (Cook *et al.* 2000; Guan *et al.* 2008). Over-expression of tiGH in TG improved growth, survival, and nutrient utilization. TG 3rd generation has 1.49-fold and 1.40-fold higher growth and survival compared with non-transgenic common carp, respectively. The body protein content of transgenic fish was higher, whereas the lipid content was lower compared with the non-transgenic fish. Therefore, TG is potential to achieve higher productivity, more efficient, and sustainable aquaculture.

Acknowledgment

This research was partially supported by PSU Research Grant No.: 53/IT3.11/LT/2014 from Ministry of Research and Higher Education, Indonesia.

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