

# Growth Efficiency in Transgenic Tilapia (*Oreochromis* sp.) Carrying a Single Copy of an Homologous cDNA Growth Hormone

R. Martínez,\* J. Juncal,\* C. Zaldívar,† A. Arenal,‡ I. Guillén,\* V. Morera,§ O. Carrillo,† M. Estrada,¶ A. Morales,\* and M. P. Estrada\*

\*Division of Mammalian Cell Genetics, §Division of Physical Chemistry, Centro de Ingeniería Genética y Biotecnología, P.O. Box 6162, Havana, Cuba; †Faculty of Biology, University of Havana, 25th Street No. 455, Havana 10400, Cuba;

‡Centro de Ingeniería Genética y Biotecnología, P.O. Box 387, Camaguey 1, Cuba; and ¶Instituto de Hematología, San Francisco Avenue and Perla Street, Havana 10800, Cuba

Received October 26, 1999

**Growth hormone (GH) has been shown to have a profound impact on fish physiology and metabolism. However, detailed studies in transgenic fish have not been conducted. We have characterized the food conversion efficiency, protein profile, and biochemical correlates of growth rate in transgenic tilapia expressing the tilapia GH cDNA under the control of human cytomegalovirus regulatory sequences. Transgenic tilapia exhibited about 3.6-fold less food consumption than nontransgenic controls ( $P < 0.001$ ). The food conversion efficiency was significantly ( $P < 0.05$ ) higher (290%) in transgenic tilapia ( $2.3 \pm 0.4$ ) than in the control group ( $0.8 \pm 0.2$ ). Efficiency of growth, synthesis retention, anabolic stimulation, and average protein synthesis were higher in transgenic than in nontransgenic tilapia. Distinctive metabolic differences were found in transgenic juvenile tilapia. We had found differences in hepatic glucose, and in agreement with previous results we observed differences in the level of enzymatic activities in target organs. We conclude that GH-transgenic juvenile tilapia show altered physiological and metabolic conditions and are biologically more efficient.** © 2000 Academic Press

World fish and shellfish production must be increased through aquaculture with the application of biotechnology. Among target traits for manipulation, growth has been the most successfully improved, both through the use of various preparations of exogenous homologous or heterologous GH delivered via injection, orally or by gill penetration (1–10) and by the transfer of GH transgenes (11). However, the exogenous administration of GH to fish is not yet sufficiently practical for commercial aquaculture. Genetically manipulated or transgenic fish, on the other hand, are about to enter commercial exploitation for several species (12).

Endogenous and exogenous GH profoundly influence intestinal absorption and renal reabsorption of nutrients, food conversion efficiency, protein, lipid and amino acid metabolism, total N-retention and muscle amino acid profile in fish and mammals (8, 9). Therefore, the characterization of these processes in GH-treated or transgenic fish is of special relevance for understanding the effects produced by ectopic GH.

Previously we have reported the generation of transgenic tilapia with improved growth performance (13, 14). These tilapia express low ectopic levels of tilapia GH (tiGH) (13–17). Here we present the results of a series of correlated simultaneous experiments in which growth, food consumption, conversion efficiency and the activity of key metabolic enzymes in muscle, liver and plasma were monitored for transgenic tilapia. The aim of the study was to examine the relation between consumption and growth and between biochemical correlates of growth rate in transgenic and nontransgenic tilapia to help to understand the effects produced by the ectopically expressed GH.

## MATERIALS AND METHODS

### Animals

Male transgenic IG91-03/F70 (F70) and nontransgenic tilapia (*Oreochromis* sp.) were supplied by Mampostón (San José, Havana, Cuba) and maintained in the laboratory at 28°C in running aerated water in groups of 15 animals in 500 liter aquaria for 1 week before beginning the experiments. Tilapia were kept throughout in a 14 h light:10 h dark photoperiod and fed, unless otherwise indicated, to repletion twice daily with fish pellets (CENPALAB, Havana, Cuba). Juvenile and adult tilapia were considered when having around 100 and 300 g, respectively, grown under production conditions.

### Experiment 1: Food Conversion Efficiency

**Animals and husbandry.** Twenty size-selected tilapia (average wet weight  $107 \pm 14$  g) were acclimated in a 500 liter aquaria and fed with commercially prepared pellets (CENPALAB, Havana, Cuba).

Daily rations equivalent to 2% of the body weight were administered twice a day until they were used in the experiments.

**Preparation of food pellets.** Pellets labeled with glass beads were prepared by mixing the commercial food pellets with 3.7% vegetable oil, 1.8% carboxymethyl cellulose (CMC), 9.2% powder milk, distillate water and glass beads in a ratio of  $153 \pm 8$  beads (0.5 mm) per gram food pellets. Pellets were formed following compression of the mix through a silicon applier with a 2 mm diameter, and dried during 24 h at 26°C.

**Experimental procedure.** Two experimental groups of 10 animals each, containing 7 males and 3 females ( $109 \pm 14$  g) and 6 males and 4 females ( $104 \pm 15$  g) of nontransgenic and transgenic F2 heterozygous tilapia respectively, were separated in aquariums of 500 L each. The experiment was conducted during five weeks with weekly measurements of individual fish weight and food consumption using radiography. Tilapia were fed three times daily with commercial food pellets (ratio = 4% total fish weight), except on the day where measurements were conducted. This day tilapia were fed once with the same ratio of food pellets labeled with glass beads to allow X-ray diffraction two h later. Food not consumed 1 h after feeding was removed from the tank.

## Experiment 2: Biochemical Correlates of Growth Rate

All the parameters, unless otherwise indicated, are expressed in fractional rates (percentage of the protein mass per day (%day<sup>-1</sup>)) (18).

**Growth rates.** Growth rates of whole animals were measured in groups of juvenile tilapia of similar weight. At the beginning of the experiment, 10 transgenic and non transgenic tilapia with an average weight of  $100 \pm 8$  g and  $102 \pm 7$  g, respectively, that had been kept as described above were killed and weighed, and the weight and protein content of the tissues were determined as described below. These animals were termed the weight control group. A further 10 transgenic and nontransgenic tilapia with a similar initial weight were weighed individually every 1 week. On each occasion the fish were not fed 1 day prior to weighing, and they were quickly rolled in a slightly damp cloth to remove surface moisture. After 42 days final weights were taken and the growth rate was calculated as the percent increase per day

$$\% \text{ growth rate} = \frac{W_2 - W_1}{W_2} \times \frac{100}{42},$$

where  $W_1$  and  $W_2$  are the live weights at the beginning and after the 6 week period, respectively.

Fish were then fed for a further day, denied food for 12 h, and injected with a flooding dose of phenylalanine in order to determine the rates of protein synthesis as described below. Immediately after the animals were killed, two portions of the white muscle of 0.1 g were taken. Protein content of tissue samples was determined as described below. The white muscle was removed on one side along the backbone. The entire fillet was then weighed and the weight was doubled to give the total white muscle weight. The total protein content of the tissues were estimated by multiplying the milligrams of protein per gram fresh weight for the samples taken for the analysis of protein content by the total fresh weight of the tissue. These estimates were carried out for each fish used. The rate of increase of tissue protein (mg protein day<sup>-1</sup>) was calculated by subtracting the mean protein content of white muscle tissue as determined from the initial weight control group. This estimate of the rate of growth at the time of death was compared with the rate of protein synthesis.

**Rate of protein synthesis.** The measurement of tissue fractional protein synthetic rate from the incorporation of radioactive phenylalanine was based on the method of Garlick *et al.* (19). On the day of injection food was withheld, and the fish was injected into the caudal vein. The injection solution contained 150 mM L-phenylalanine and

L-[2,6-<sup>3</sup>H]phenylalanine (Amersham, UK) at 167 μCi/ml in phosphate buffered saline, pH 7.4. The dose was 0.35 ml/100 g body weight. After the injection the fish were returned to aerated fresh water at 28°C, where they recovered immediately and rested quietly. Twenty and 40 min after the injection, five transgenic and nontransgenic tilapia were killed by a blow to the head and a sample of white muscle was taken as described above. Tissue samples were weighed and immediately homogenized in 2 ml of 2% perchloric acid. After centrifugation the supernatant was used to measure the specific radioactivity of homogenate free L-phenylalanine ( $S_A$ ).

The precipitate containing the protein was washed once in 2% perchloric acid, twice in 95% alcohol, and once in ether. It was resuspended in 2 M NaOH and incubated at 37°C overnight. Protein determinations were carried out on the resulting solutions using the method of Lowry *et al.* (20) with bovine serum albumin as a standard. Protein-bound phenylalanine was obtained by reprecipitating the protein in 2 M NaOH with 10% perchloric acid and hydrolyzing it in 5 ml of 6 N HCl for 24 h at 110°C. The HCl was removed by evaporation to dryness, and the amino acids were resuspended in 0.5 M sodium citrate, pH 6.3. Specific radioactivity of the free pools and the protein were then determined using a liquid scintillation counter 1214 RACKBETA (LKB, Sweden) using Aquasol (NEN, USA) and a 4151 Alpha Plus amino acid analyzer (LKB, Sweden) to determine the nanomolar amount of phenylalanine in the samples. The experimental results were obtained as  $S_A$ , the specific radioactivity of free L-phenylalanine (cpm/nmol) and  $S_B$ , the specific radioactivity of protein-bound phenylalanine (cpm/nmol). The fractional rate of protein synthesis,  $K_S$ , as a percentage of the protein mass synthesized per day was calculated as Pocrnjic *et al.* (21)

$$K_S = \frac{S_{B(t_2)} - S_{B(t_1)}}{S_{A(t_2-t_1)}} \times \frac{100}{t_2-t_1},$$

where  $S_{B(t_2)}$  is the protein-bound specific radioactivity at the experimental time  $t_2$ .  $S_{B(t_1)}$  is the average incorporation at an earlier time (20 min).  $S_{A(t_2-t_1)}$  is the average free-pool specific radioactivity over the period  $t_2-t_1$ . The value for  $K_S$  was calculated as an average of the results from five transgenic and nontransgenic tilapia. Finally, the average  $K_S$  value was multiplied by the total amount of protein in the tissue of individual fish per 1440 to give the milligrams of protein synthesized per day per fish. Data is given as mean  $\pm$  SD or average values as indicated.

Fractional protein growth rates ( $K_g$ % per day) expressed as a percentage of the protein mass were calculated for individual fish from the initial and final whole body protein content (22). The initial protein content for for each fish was estimated by using the mean whole-body protein content of the initial group. Transgenics animals  $5.1 \pm 1.22$ ,  $n = 10$  and nontransgenics animals  $7.2 \pm 1.09$ ,  $n = 10$ .

**Ration consumption.** Fraction of food protein ingested was estimated from data of food supplied (2.4 %day<sup>-1</sup> equivalent to 1.2 gday<sup>-1</sup> of pellets with 33.5% protein content) and food intake obtained from experiment 1.

**Biochemical correlates.** Houlihan *et al.* (18) have shown that biochemical correlates of growth rate could be represented by the equation  $y = ax + b$ , where for the correlates examined in our experiments:

$x$	$y$	$b$
Growth rate, $K_g$	Protein synthesis, $K_s$	Synthesis retention efficiency, $K_s/K_g$
Ration, $K_r$	Growth rate, $K_g$	Growth efficiency, $K_g/K_r$
Ration, $K_r$	Protein synthesis, $K_s$	Anabolic stimulation efficiency, $K_s/K_r$

## Biochemical Analyses

Ten juveniles and eight adults transgenic and nontransgenic tilapia were used per group. Mean weights for the groups were  $100 \pm 8$  g

**TABLE 1**  
Experiment 1: Growth Efficiency in Transgenic and Nontransgenic Juvenile Tilapia

Parameters	Transgenic (T)	Nontransgenic (NT)	(T/NT) × 100
Initial weight (W1, week 1) (g)	104.8 ± 5.9	109.7 ± 5.9	—
Final weight (W2, week 5) (g)	126.2 ± 8.6	137.5 ± 10.7	—
Total food intake (TFI) (g)	9.1 ± 1.8*	33.0 ± 5.2*	28%
Relative food consumption rate [TFI/(W1 × 35 days)]	0.002 ± 0.003*	0.009 ± 0.01*	22%
Food conversion efficiency (total fish weight gain/TFI)	2.3 ± 0.4**	0.8 ± 0.2**	290%

*Note.* Tilapias were weekly weighted and the food intake was determined to calculate the parameters of growth efficiency. Values are the average ± SE (*N* = 10). \**P* < 0.001, \*\**P* < 0.05 (Student *t*-test).

(juvenile transgenics), 101 ± 7 g (juvenile nontransgenics), 316 ± 28 g (adult transgenics) and 293 ± 19 g (adult nontransgenics). The activity of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase from liver, muscle and plasma was assayed enzymatically, glucose was determined by the glucose oxidase method and lactate was determined by a spectrophotometric method, all employing commercial kits (Sigma, USA or Boehringer Mannheim, Germany) and following manufacturer's recommendations. Hepatic glycogen was analyzed by the Pflugger method (23). Assay of pyruvate kinase activity was performed as described by Plisetskaya *et al.* (24). Protein was determined by the method of Lowry *et al.* (20). One gram of homogenized muscle from each fish was dried in an oven at 100°C for 24 h. The initial wet and dried weights were used to compute the moisture content.

#### Determination of RNA/DNA/Protein Indexes

Employing the same animals as for biochemical analyses, total RNA (25), DNA (13) and protein (26) content in 1 g of white muscle were determined. The RNA/DNA, RNA/protein and protein/DNA indexes were calculated as described by Sun and Farmanfarmaian (8).

#### Morphometric Analysis

Employing the same animals as for biochemical analyses, the hepatosomatic index was determined, for each fish, as the weight of the liver relative to total body weight × 100.

#### Statistical Analysis

The results between transgenic and nontransgenic tilapia, when possible, were compared employing a Student *t*-Test.

### RESULTS

We have previously obtained and characterized a fast-growing GH-transgenic tilapia line (13–17). The

enhanced growth produced by the ectopic expression of tiGH in this transgenic tilapia could be due to increased food consumption and/or improved food conversion. To differentiate these two possible mechanisms, the total food consumption was carefully recorded weekly. When relative food consumption rate was calculated, it was found that transgenic tilapia, when compared to nontransgenics, had a lower food consumption rate (Table 1). Furthermore, the food conversion efficiency was increased by 290% (Table 1).

Transgenic tilapia grow 60–80% faster than nontransgenic siblings (14). However, under the experimental conditions employed here, specific growth rates were similar in both experimental groups (0.6 ± 0.2 %day<sup>-1</sup>). This fact could be explained by rearing under laboratory conditions that are not optimal for growth. Nevertheless, the data obtained and the analyses conducted by us are valid as we compared experimental groups with similar specific growth rates but different biochemical and metabolic requirements due to the transgene expression.

Average protein synthesis and protein growth (*P* < 0.05) was higher in transgenic than in nontransgenic tilapia (Table 2). Transgenic tilapia also showed a lower ration consumption (Tables 1 and 2). Therefore, the efficiency of growth, synthesis retention and anabolic stimulation were higher in transgenic tilapia.

The GH exerts its growth-promoting action through different metabolic pathways. Previous results had shown differences in free alanine and aspartic acid levels in the muscle of juvenile transgenic tilapia (14).

**TABLE 2**  
Experiment 2: Biochemical Correlates of Growth Rate in Juvenile Tilapia

Coefficient	Transgenic (T)	Nontransgenic (NT)	Increment (T/NT)
Protein synthesis, <i>K<sub>s</sub></i> (% day <sup>-1</sup> )	0.35	0.17	2
Protein synthesis per day (% mg day <sup>-1</sup> )	0.078 ± 0.02*	0.028 ± 0.004*	2.7
Protein growth, <i>K<sub>g</sub></i> (% day <sup>-1</sup> )	0.26 ± 0.06**	0.15 ± 0.03**	1.7
Ration consumption, <i>K<sub>r</sub></i> (% day <sup>-1</sup> )	0.7	2.4	3.6
Growth efficiency, <i>K<sub>g</sub>/K<sub>r</sub></i> (%) × 100	37.4	6.45	5.7
Protein synthesis retention efficiency, <i>K<sub>s</sub>/K<sub>g</sub></i> (%) × 100	133.5	64.5	2
Anabolic stimulation efficiency, <i>K<sub>s</sub>/K<sub>r</sub></i> (%) × 100	50	4	12.5

*Note.* Average ± SD (*N* = 10), \**P* < 0.01, \*\**P* < 0.05 (Student *t*-test).

**TABLE 3**  
**Biochemical and Morphometric Analyses Conducted in Juvenile Tilapia**

Parameters	Plasma		Liver		Muscle	
	Transgenics	Nontransgenics	Transgenics	Nontransgenics	Transgenics	Nontransgenics
GOT	284 ± 54 $\mu$ U/mg	246 ± 66 $\mu$ U/mg	21.4 ± 2.3 mU/mg*	49.3 ± 6.5 mU/mg*	99.2 ± 13.6 mU/mg*	57.5 ± 13.3 mU/mg*
GPT	26.3 ± 6.7 $\mu$ U/mg	18.2 ± 10.4 $\mu$ U/mg	6.0 ± 1.1 mU/mg*	28.4 ± 3.4 mU/mg*	6.8 ± 0.9 mU/mg*	3.3 ± 0.6 mU/mg*
LDH	ND	ND	2.1 ± 0.2 mU/mg	2.1 ± 0.4 mU/mg	7.5 ± 1.2 mU/mg	6.4 ± 0.6 mU/mg
Lactate	ND	ND	550 ± 83 $\mu$ M	400 ± 55 $\mu$ M	4.9 ± 0.2 mM	5.6 ± 0.3 mM
Pyruvate kinase	ND	ND	0.20 ± 0.02*	0.10 ± 0.01*	—	—
Glucose	1.2 ± 0.2 mM	1.5 ± 0.2 mM	40.8 ± 2.8 mM*	54.2 ± 3.9 mM*	0.34 ± 0.06 mM	0.38 ± 0.06 mM
Glycogen	ND	ND	0.28 ± 0.06 mmol/g	0.27 ± 0.03 mmol/g	ND	ND
Hepatosomatic Index	ND	ND	2.4 ± 0.1%	2.4 ± 0.2%	ND	ND

Note. Values are the average ± SD ( $N = 10$ ). \*  $P < 0.05$  (Student  $t$ -test). ND, not determined.

An increase in the GOT and GPT transaminases was found at this stage of life in transgenic fish, but not in lactate dehydrogenase enzyme activity, neither in the lactate nor glucose levels in muscle tissue (Table 3). Transgenic juvenile tilapia had lower hepatic glucose and a higher pyruvate kinase activity, showing an enhanced glycolysis when compared to nontransgenics (Table 3). There were no differences regarding the levels of lactate and glycogen, neither in the hepatosomatic index between transgenic and nontransgenic tilapia (Table 3). In adult animals, no differences were found in the parameters measured (Table 4).

The total contents of RNA, DNA and protein were measured in juvenile and adult muscle of transgenic and nontransgenic tilapia. No differences were found except in the d RNA/protein index in transgenic and adult muscle, respectively (Fig. 1).

## DISCUSSION

The transfer of GH transgenes has resulted in growth acceleration of economically important fish species (11). However, how much of this growth improvement is due to higher ration consumption or to better growth efficiency has not been determined. This is a fundamental question for biological studies and for cost-effective analysis.

Higher growth rates have been shown to be the result of reduced maintenance costs and increased metabolic efficiency (18). In bovine GH (bGH)-injected striped bass hybrids an increase in the specific growth rate and food conversion efficiency without significant alteration of food consumption rate has been reported (8, 9). Due to the treatment with bGH, the relative nitrogen retention increases by 20% together with the intestinal nutrient absorption (27). Four weeks of GH treatment do not significantly alter water, nonprotein nitrogen, protein ash and fiber content when expressed as percent of fresh tissue weight. The mean DNA concentrations ( $\text{mg g}^{-1}$  tissue) do not show any appreciable change but the mean RNA/DNA and protein/DNA ratios are significantly higher for the treated fish (8, 9). Furthermore, GH treatment results in a significant variation in the level of some amino acids (27). Also, trout with higher protein growth efficiency are more efficient in their retention of synthesized protein (28). It has been reported food conversion efficiency in rainbow trout is stimulated after the application of ovine growth hormone (29).

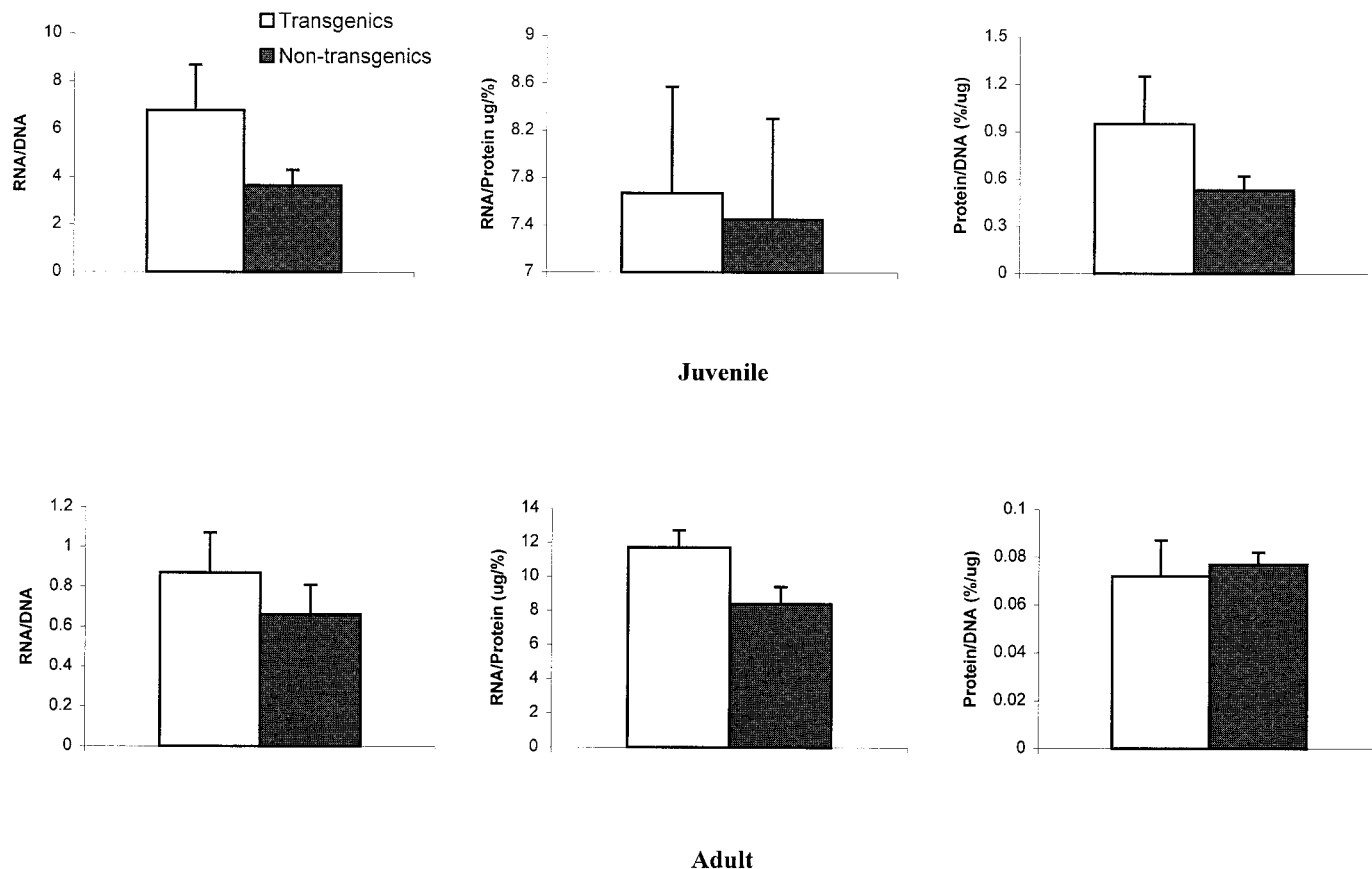
Transgenic F70 tilapia showed a higher protein synthesis rate and protein growth and lower ration consumption, resulting in higher efficiency of growth, synthesis retention and anabolic stimulation. It has been

**TABLE 4**  
**Biochemical Analyses Conducted in Adult Tilapia**

Parameters	Plasma		Liver		Muscle	
	Transgenics	Nontransgenics	Transgenics	Nontransgenics	Transgenics	Nontransgenics
GOT	582 ± 71 $\mu$ U/mg	596 ± 60 $\mu$ U/mg	115 ± 11 mU/mg	161 ± 33 mU/mg	19.2 ± 2.1 mU/mg	23.1 ± 2.9 mU/mg
GPT	325 ± 73 $\mu$ U/mg	188 ± 32 $\mu$ U/mg	67 ± 12 mU/mg	80 ± 10 mU/mg	6.2 ± 0.4 mU/mg	7.7 ± 1.2 mU/mg
LDH	ND	ND	ND	ND	10.1 ± 1.8 mU/mg	14.0 ± 3.7 mU/mg
Glucose	7.5 ± 0.7 mM	7.0 ± 0.4 mM	21.2 ± 2.5 mM	18.6 ± 1.3 mM	3.5 ± 0.1 mM	3.4 ± 0.1 mM
Glycogen	ND	ND	0.35 ± 0.03 mmol/g	0.34 ± 0.08 mmol/g	ND	ND

Note. Values are the average ± SD ( $N = 8$ ).  $P > 0.05$  (Student  $t$ -test). ND, not determined.





**FIG. 1.** RNA/DNA, RNA/protein, and protein/DNA indexes in juvenile and adult transgenic and control tilapia. Values are the average  $\pm$  SD ( $N = 10$ ). \* $P < 0.05$  (Student  $t$ -test).

proven no different in the digestibility test among transgenic, nontransgenic and wild type tilapia (17). Therefore, these transgenic tilapia are metabolically more efficient, capable of supporting growth with better food conversion efficiency. Similar results have been reported by Krasnov *et al.* (30) in rapidly growing transgenic Arctic char. They found specific growth rate and muscle protein content equal with respect to non-transgenic siblings. However, the rate of  $\text{NH}_4$  excretion appeared equal in control and transgenic fish, therefore indicating that the rapid growth correlates with higher efficiency of protein retention. In transgenic carps expressing the trout GH transgene, an increase in muscle protein content of about 7.5% and variation in some aminoacid levels were also reported (31). Studies carried out by Zongbin *et al.* (32) in the MThGH-transgenic  $F_2$  red carp (*Cyprinus carpio* L. red var.) showed feeding rates of transgenic significantly lower than of the non transgenic control, however the specific growth rates of the transgenic in wet weight, dry weight, energy and protein and the conversion efficiencies of the transgenic  $F_2$  in all these parameters were also higher than nontransgenic fish.

The results obtained in wild type or GH-injected fish are essentially in accordance with the results obtained

in transgenic tilapia expressing ectopic tiGH. Differences in the magnitude of the effect may respond to the levels of GH present in each case.

Transgenic tilapia will need to partition a lower proportion of ingested energy into basal metabolism and the replacement of existing body tissue, making more available for growth. How are transgenic tilapia obtaining the energy required to support a better and more efficient growth rate? It looks like GH-transgenic fish utilize the energy released by oxidation of amino-acids more efficiently.

Transgenic tilapia F70 express ectopic tiGH in various tissues including the liver, muscle, gonads and brain (13, 15). For biochemical analyses we selected the muscle, liver and plasma. Studies in the muscle correlate well with estimates for the whole body (33) and are the portion of the animal used for commercialization and human consumption. The liver is an important organ for biochemical studies and is the target of GH action to induce the expression of insulin-like growth factors (IGF) which, together with GH, provoke the growth-promoting action (10). The plasma connects all organs of the body and reflects the nutritional status of the organism, affecting among other factors, the synthesis of GH and IGF (34, 35).

Biochemical studies were conducted in juvenile and adult tilapia. We have shown that the effect of ectopic tiGH on growth performance is more pronounced in juvenile transgenic tilapia (13, 36), therefore reflecting better the biochemical processes induced by ectopic GH. Adult tilapia, on the other hand, will be used for human consumption and it is of special interest to compare in these animals the biochemical profile of transgenic and nontransgenic tilapia.

Juvenile transgenic tilapia have reduced free levels of alanine and aspartic acid in the muscle when compared to nontransgenic controls (14). It is probably these gluconeogenic amino acids are used to produce energy (37). The increase in the GOT and GPT transaminases in the muscle correlated well with the decrease in alanine and aspartic acid levels as these enzymes are involved in the production of energy from these amino acids (38). Although it is not common the oxidation of amino acids by muscle cells, this reaction could be favored in GH-transgenic tilapia. Gluconeogenesis from alanine has been reported in rainbow trout (39) and coho salmon (*Oncorhynchus kisutch*) (40) hepatocytes and in the eel *Anguilla japonica* (41).

In the liver, the opposite effect was recorded. In this tissue the activity of GOT and GPT was lower in juvenile transgenic tilapia, thus suggesting that in the liver gluconeogenic amino acids are not used for energy production. Increase activity in hepatic GOT and GPT has been described for Red Sea bream (*Chrysophrys major*) that conserve the glycogen and metabolize proteins during starvation at low temperature (42).

The potential for gluconeogenesis could be assessed indirectly by measuring kinetic parameters of liver pyruvate kinase (43). The lower hepatic glucose and higher pyruvate kinase activity could reflected that, in juvenile transgenic tilapia, glucose was used in the liver to produce energy. However, since the levels of glycogen remained unchanged, the glucose used for oxidation and energy production was not obtained from hepatic glycogen. Although these results reflect a metabolic disbalance in the liver of juvenile transgenic tilapia, the maintenance of the hepatosomatic index denotes that this disbalance is probably within physiological levels. The injection of high supraphysiological concentrations of recombinant tiGH in juvenile *O. aureus* tilapia results in the increase of the hepatosomatic index (10).

An increase in the RNA/DNA ratio was found in the muscle of juvenile transgenic tilapia. This result reflected an increase in the protein synthesis in these tilapia. Similar results have been reported for GH-treated fish (8, 9). In adult transgenic tilapia, an increase in the RNA/protein ratio reflected an effect of ectopic tiGH on ribosomal capacity.

Biochemical analyses in adult tilapia showed no differences between transgenic and nontransgenic animals. This result is important for the evaluation of the

possible effects of consuming transgenic tilapia as it further documents that transgenic tilapia F70 are safe as food (44).

In conclusion, the results reported by us support that (a) transgenic tilapia have a better food conversion efficiency, protein synthesis and growth efficiency adding more value to this transgenic line and supporting that differences in protein turnover are important determinants of growth efficiency in fish (18), and (b) we have found differences in the hepatic glucose values and the muscle GOT and GPT activity to compare transgenic and non transgenic fish. The energy required for the accelerated growth in juvenile transgenic tilapia could be produced from hepatic glucose and the gluconeogenic amino acids alanine and aspartic acid oxidation in muscle.

## ACKNOWLEDGMENTS

The authors thank R. Pimentel, B. Alvarez, R. Gonzalez, I. Mendoza, Z. Abad, and E. Cabrera for sample collection from adult tilapia and E. Cabrera (Manuel Asuncion's Hospital, Camaguey) for excellent technical assistance.

## REFERENCES

1. Donaldson, E. M., Fagerlund, U. H. M., Higgs, D. A., and McBride, J. R. (1979) in *Fish Physiology VIII* (Hoar, W. S., Randall, D. J., and Brett, J. R., Eds.), pp. 456–598, Academic Press, New York.
2. Gill, J. A., Sumpter, J. P., Donaldson, E. M., Dye, H. M., Souza, L., Berg, T., Wypych, J., and Langley, K. (1985) *Bio/Technology* **3**, 643–646.
3. Down, N. E., Donaldson, E. M., Dye, H. M., Langley, K., and Souza, L. M. (1988) *Aquaculture* **68**, 141–155.
4. Agellon, L. B., Emery, C. J., Jones, J. M., Davies, S. L., Dingle, A. D., and Chen, T. T. (1988) *Can. J. Fish Aquat. Sci.* **45**, 146–151.
5. LeBail, P. Y., Sire, M. F., and Vernier, J. M. (1989) *The J. Exp. Zoo.* **251**, 101–107.
6. McLean, E., Donaldson, E. M., Dye, H. M., and Souza, M. (1990) *Aquaculture* **91**, 197–203.
7. Moriyama, S., Takahashi, A., Hirano, T., and Kight, K. (1990) *J. Comp. Phys. B* **160**, 251–257.
8. Sun, L. Z., and Farmanfarmaian, A. (1992) *Comp. Biochem. Physiol.* **101A**, 237–248.
9. Sun, L. Z., and Farmanfarmaian, A. (1992) *Comp. Biochem. Physiol.* **103A**, 381–390.
10. Guillén, I., Lleonart, R., Agramonte, A., Morales, R., Morales, A., Hernández, C. A., Vázquez, M. M., Díaz, M., Herrera, M. T., Alvarez-Lajonchere, L., Hernández, O., and de la Fuente, J. (1998) *J. Mar. Biotechnol.* **6**, 142–151.
11. de la Fuente, J. (1998) in *Gene Transfer in Aquatic Organisms* (de la Fuente, J., and Castro, F. O., Eds.), pp. 1–15, Springer-Verlag, Heidelberg, and Landes Bioscience, Austin.
12. Aleström, P., and de la Fuente, J. (1999) *Biotechnología Aplicada* **16**, 127–130.
13. Martínez, R., Estrada, M. P., Berlanga, J., Guillén, I., Hernández, O., Cabrera, E., Pimentel, R., Morales, R., Herrera, F., Morales, A., Piña, J. C., Abad, Z., Sánchez, V., Melamed, P.,

- Lleonart, R., and de la Fuente, J. (1996) *Mol. Mar. Biol. Biotechnol.* **5**, 62–70.
14. Martínez, R., Arenal, A., Estrada, M. P., Herrera, F., Huerta, V., Vázquez, J., Sánchez, T., and de la Fuente, J. (1999) *Aquaculture* **174**, 271–283.
15. Hernández, O., Guillén, I., Estrada, M. P., Cabrera, E., Pimentel, R., Piña, J. C., Abad, Z., Sánchez, V., Hidalgo, Y., Martínez, R., Lleonart, R., and de la Fuente, J. (1997) *Mol. Mar. Biol. Biotechnol.* **6**, 364–375.
16. de la Fuente, J., Guillén, I., and Estrada, M. P. (1998) in *New Developments in Marine Biotechnology* (Gal, L., and Halvorsen, H. O., Eds.), pp. 7–10, Plenum Press, New York, NY.
17. de la Fuente, J., Martínez, R., Guillén, I., Estrada, M. P., and Lleonart, R. (1998) in *Gene Transfer in Aquatic Organisms* (de la Fuente, J., and Castro, F. O., Eds.), pp. 83–105, Springer-Verlag, Heidelberg, and Landes Bioscience, Austin.
18. Houlihan, D. F., Mathers, E. M., and Foster, A. (1993) in *Fish Ecophysiology* (Rankin, J. C., and Jensen, F. B., Eds.), pp. 45–71. Chapman & Hall, London.
19. Garlick, P. J., McNurlan, M. A., and Preedy, V. R. (1980) *J. Biochem.* **192**, 719–723.
20. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
21. Pocrnjic, Z., Mathews, W. R., Rappaport, S., and Haschemeyer, A. E. V. (1983) *Comp. Biochem. Physiol. B* **74**, 735–738.
22. McCarthy, I. D., Houlihan, D. F., Carter, C. G. (1994) *Proc. R. Soc. London B* **257**, 141–147.
23. Adcid, W. Z., and Abraham, S. (1957) in *Methods of Enzymology* (Colowick, P., and Kaplan, N. O., Eds.), pp. 34–35, Academic Press, New York.
24. Plisetskaya, E. M., Ottolenghi, C., Sheridan, M. A., Mommsen, T. P., and Gorbman, A. (1989) *Gen. Comp. Endocrinol.* **73**, 205–216.
25. Chomchynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
26. Association of Official Analytical Chemists, (1984) *Official Methods of Analysis*, 14th ed., p. 1141, Arlington, VA.
27. Farmanfarmaian, A., and Sun, L. Z. (1999) *Biomol. Eng.*, in press.
28. Houlihan, D. F., McMillan, D. N., and Laurent, P. (1986) *Physiol. Zool.* **59**, 482–493.
29. Foster, A. R., Houlihan, D. F., Gray, C., Medale, F., Fauconneau, B., Kauskik, S. J., and Le Bail, P. Y. (1991) *Gen. Comp. Endocrinol.* **82**, 111–120.
30. Krasnov, A., Pitkänen, T., and Mölsä, H. (1999) *Biomol. Eng.*, in press.
31. Chatakondi, N., Lovell, R. T., Duncan, P. L., Hayat, M., Chen, T. T., Powers, D. A., Weete, J. D., Cummins, K., and Dunham, R. A. (1995) *Aquaculture* **138**, 99–109.
32. Zongbin, C., Zuoyan, Z., Yibo, C., Guohua, L., and Kesheng, X. (1996) *Chinese Sci. Bull.* **41**, 591–596.
33. Cowey, C. B., and Tacon, A. G. (1983) in *Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition* (Pruder, G. D., Lagoon, C. J., and Conklin, D. E., Eds.), pp. 13–30. Louisiana State University Press, Division of Continuing Education, Baton Rouge, LA.
34. Daucey, M. J., Burton, K. A., White, P., Harison, A. P., Gilmour, R. S., Duchamp, C., and Cattaneo, D. (1994) *FASEB* **8**, 81–88.
35. Straus, D. S. (1994) *FASEB* **8**, 6–12.
36. Cabezas, L., Herrera, F., Martínez, R., Arenal, A., Estrada, M. P., and de la Fuente, J. (1997) in *Tilapia Aquaculture* (Proceedings from the Fourth International Symposium on Tilapia in Aquaculture, Orlando, Florida, November 9–12, 1997), (Fitzsimmons, K., Ed.), 1, 109–115, University of Arizona.
37. Sheridan, M. A., and Mommsen, T. P. (1991) *Gen. Comp. Endocrinol.* **81**, 473–483.
38. Wood, C. M. (1993) in *The Physiology of Fishes* (Evans, D. H., Eds.), Vol. 2, pp. 469–502, CRC Press, Boca Raton, FL.
39. Mommsen, T. P., and Suárez, R. K. (1984) *Mol. Physiol.* **6**, 9–18.
40. Mommsen, T. P. (1986) *Can. J. Zool.* **64**, 1110–1115.
41. Hayashi, S., and Ooshiro, Z. (1979) *J. Comp. Physiol.* **132**, 343–350.
42. Woo, N. Y. S., and Fung, A. C. Y. (1981) *Comp. Biochem. Physiol.* **69A**, 461–465.
43. Sheridan, M. A., Eilertson, C. D., and Plisetskaya (1991) *Gen. Comp. Endocrinol.* **81**, 365–372.
44. Guillén, I., Berlanga, J., Valenzuela, C., Morales, A., Toledo, J., Estrada, M. P., Puentes, P., Hayes, O., and de la Fuente, J. (1999) *Mar. Biotechnol.* **1**, 2–14.