

**GROWTH EFFICIENCY**  
**IN TRANSGENIC TILAPIA (*Oreochromis sp.*)**  
**CARRYING A SINGLE COPY**  
**OF AN HOMOLOGOUS cDNA GROWTH HORMONE**

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**Abstract**

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 non-transgenic controls ( $P<0.001$ ). The food conversion efficiency was significantly ( $P<0.05$ ) higher (290%) in transgenic tilapia ( $2.3\pm 0.4$ ) when compared to the control group ( $0.8\pm 0.2$ ). Efficiency of growth, synthesis retention and anabolic stimulation and average protein synthesis were higher in transgenic than in non-transgenic 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 the enzymatic activity in target organs. We conclude

that GH-transgenic juvenile tilapia show altered physiological and metabolic conditions and are biologically more efficient.

## Introduction

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) (13,14,15,16,17). 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 non-transgenic tilapia to help to understand the effects produced by the ectopically expressed GH.

## Results

We have previously obtained and characterized a fast-growing GH-transgenic tilapia line (13, 14, 15,16,17). 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 non-transgenics, had a lower food consumption rate (Table 1). Furthermore, the food conversion efficiency was increased by 290% (Table 1).

Table 1. Experiment 1: Growth efficiency in transgenic and non-transgenic juvenile tilapia.

Parameters	Transgenic	Non-Transgenic	T/NTx100
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 x 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 %

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

Transgenic tilapia grow 60%-80% faster than non-transgenic siblings (14). However, under the experimental conditions employed here, specific growth rates were similar in both experimental groups ( $0.6 \pm 0.2$  %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.

Table 2. Experiment 2: Biochemical correlates of growth rate in juvenile tilapia.

Coefficient	Transgenic	Non-Transgenic	(T/NT)
Protein synthesis, Ks (% day <sup>-1</sup> )	0.35	0.17	2
Protein synthesis per day (% mg day <sup>-1</sup> )	0.078 $\pm$ 0.02*	0.028 $\pm$ 0.004*	2.7
Protein growth, Kg (%day <sup>-1</sup> )	0.26 $\pm$ 0.06**	0.15 $\pm$ 0.03**	1.7
Ration consumption, Kr (% day <sup>-1</sup> )	0.7	2.4	3.6
Growth efficiency, Kg/Kr (%) $\times$ 100	37.4	6.45	5.7
Protein synthesis retention efficiency, Ks/Kg (%) $\times$ 100	133.5	64.5	2
Anabolic stimulation efficiency, Ks/Kr (%) $\times$ 100	50	4	12.5

Average  $\pm$  SD (N=10), \*P<0.01, \*\*P<0.05 (Student t-Test).

Average protein synthesis and protein growth (p<0.05) was higher in transgenic than in non-transgenic 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.

Table 3. Biochemical and morphometric analyses conducted in juvenile tilapia.

Parameters	Plasma		Liver		Muscle	
	Transgenic	Non Transgenic	Transgenic	Non Transgenic	Transgenic	Non Transgenic
GOT	284±54 μU/mg	246±66 μU/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 μU/mg	18.2±10.4 μU/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 μM	400±55 μM	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
Hepat. Index	ND	ND	2.4±0.1 %	2.4±0.2 %	ND	ND

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

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). 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 non-transgenics (Table 3). There were no differences regarding the levels of lactate and glycogen, neither in the hepatosomatic index between transgenic and non-transgenic tilapia (Table 3). In adult animals, no differences were found in the parameters measured (Table 4).

Table 4. Biochemical analyses conducted in adult tilapia.

Parameters	Plasma		Liver		Muscle	
	Transgenic	Non Transgenic	Transgenic	Non Transgenic	Transgenic	Non Transgenic
GOT	582±71 μU/mg	596±60 μU/mg	115±11 mU/mg	161±33 mU/mg	19.2±2.1 mU/mg	23.1±2.9 mU/mg
GPT	325±73 μU/mg	188±32 μU/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

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

The total contents of RNA, DNA and protein were measured in juvenile and adult muscle of transgenic and non-transgenic tilapia. No differences were found except in the d RNA/protein index in transgenic and adult muscle, respectively

### 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, non-protein 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 proved no differences in the digestibility test among transgenic, non-transgenic 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 carry 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 non-transgenic 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 aminoacids 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 non-transgenic tilapia. Juvenile transgenic tilapia have reduced free levels of alanine and aspartic acid in the muscle when compared to non-transgenic 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 aminoacids 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 non-transgenic 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.

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