Aquaculture Research, 2007, **38**, 1283–1292 doi: 10.1111/j.1365-2109.2007.01796.x

Effect of growth hormone transgenic *Synechocystis* on growth, feed efficiency, muscle composition, haematology and histology of turbot (*Scophthalmus maximus* L.)

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Abstract

The present study investigated the effect of supplementing feed with transgenic *Synechocystis* sp. PCC6803 containing the *Paralichthys olivaceus* growth hormone (GH) gene on growth, feed intake and feed efficiency ratio, muscle composition, haematology and histology of turbot (*Scophthalmus maximus* L.). At the end of the 40-day feeding trial, the specific growth rate of fish fed the supplemented feed with 1.0% transgenic *Synechocystis* sp. PCC6803 was 21.67% higher ($P < 0.05$) than that of control fish. Although body weight and feed efficiency ratio significantly increased ($P < 0.05$) in fish fed the diet supplemented with transgenic alga, feed intake and condition factor of the experimental fish were unaffected. Muscle composition analysis showed that the protein content was positively influenced by the transgenic alga, whereas the lipid content was unaffected. Haematological parameters, including red blood cell, white blood cell, haemoglobin, and serum biochemical indices, such as enzyme activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, concentrations of total protein, glucose, blood urea nitrogen, creatinine, triglyceride and cholesterol and ion levels of K, Na, Cl, P were not influenced by supplementing the transgenic *Synechocystis* sp. PCC6803. Furthermore, no histopathological alterations were induced by transgenic alga treatment in the stomach, intestine, liver, spleen and kidney of the experimental fish. The results of the present study indicated that transgenic *Synechocystis* sp. PCC6803 containing *P. olivaceus* GH gene is an efficient growth promoter and a safe feed additive for fish.

Keywords: growth hormone, transgenic, *Synechocystis*, feed efficiency ratio, muscle composition, haematology, histology, turbot (*Scophthalmus maximus* L.)

Introduction

To meet the increasing demand for fish production, in response to the depletion of natural fisheries stocks, global aquaculture production has increased steadily in the last two decades (Farmanfarmaian & Sun 1999). However, increasing population has made it difficult to meet the demand for fish protein by traditional aquaculture (FAO 2000).

With the development of modern biotechnology, genetic engineering has been playing an increasing role in enhancing commercially important production traits such as growth rate, feed efficiency and disease resistance. Among target traits for manipulation, growth has been improved through the use of various preparations of exogenous homologous or heterologous growth hormone (GH) delivered via injection (Farmanfarmaian & Sun 1999; Silverstein, Wolters, Shimizu & Dickhoff 2000; Rasmussen, Ronsholdt, Ostenfeld, McLean & Byatt 2001; Leedom, Uchida, Yada, Richman III, Byatt, Collier, Hirano & Grau 2002; Peterson, Small & Bosworth 2004), oral

feeding (McLean, Donaldson, Dye & Souza 1990; Moriyama, Yamamoto, Sugimoto, Abe, Hirano, Kawauchi 1993; Tsai, Hsih & Kuo 1997; Jeh, Kim, Lee & Han 1998) or by immersion (Schulte, Down, Donaldson & Souza 1989) and by the transfer of GH genes (Du, Gong, Fletcher, Shears, King, Idler & Hew 1992; Fu, Cui, Hung & Zhu 1998; Cook, McNiven, Richardson & Sutterlin 2000; Devlin, Swanson, Clarke, Plisetskaya, Dickhoff, Moriyama, Yesaki & Hew 2000; Nam, Noh, Cho, Cho, Cho, Kim & Kim 2001; Devlin, Biagi & Yesaki 2004). Because of food safety and environmental safety issues associated with transgenic fish, the use of recombinant GHs (rGHs) to increase yields in aquaculture has generated considerable interest.

Almost all the rGHs used to date in studies on the growth-promoting effect of rGHs were purified products from their expression hosts, and thus increase cost and reduce the competitive advantages in commercial production. Furthermore, as it seems that administration of rGHs to fish by means of injection or immersion is impracticable in mass-scale aquaculture, administering rGH together with its expression host as a feed additive appears to be the most practical method. We constructed transgenic *Synechocystis* sp. PCC6803 containing *Paralichthys olivaceus* (Temminck and Schlegel) GH gene to enhance fish growth by administering it as a feed additive and also avoid purification steps in making the production economically sound.

Synechocystis sp. PCC6803 is a model cyanobacterium adapted to a wide range of environmental conditions (Marsac & Gedamu 1975). The nucleotide sequence of its entire genome has been determined (Kaneko, Sato, Kotani, Tanaka, Asamizu, Nakamura, Miyajima, Hirosawa, Sugiura, Sasamoto, Kimura, Hosouchi, Matsuno, Muraki, Nakazaki, Naruo, Okumura, Shimpo, Takeuchi, Wada, Watanabe, Yamada, Yasuda & Tabata 1996). Taking into account its short growth cycle, ease of natural or artificial cultivation with low cost and an available vector system, it has been used as an expression host of recombinant gene constructs (Yan & Qiao 1997; Chen, Ren, Shao, Shi & Ru 1999; Wang, Li, Li, Shi, Ru & Zhang 2000; Song, Shi, Ning, Luo, Shao, Yu & Ru 2001). However, the feasibility of using *Synechocystis* sp. PCC6803 as a feed supplement is yet to be assessed.

The results of our previous study examining the effect of the transgenic *Synechocystis* sp. PCC6803 containing *P. olivaceus* GH gene on growth, feed efficiency, muscle composition and histology of flounder showed its effectiveness as a growth enhancer for fish and furthermore verified the safety of the transgenic alga as a feed additive (unpublished). With this background, the present study was designed to investigate the effect of this transgenic alga on the growth, feed efficiency, muscle composition, haematology and histology of turbot (*Scophthalmus maximus* L.). Our specific objectives were to further verify its effectiveness as a growth promoter and to evaluate its safety as a feed additive for a selected fish, turbot.

Materials and methods

Culture of transgenic *Synechocystis* sp. PCC6803

The blue-green alga transgenic *Synechocystis* sp. PCC6803, maintained in our laboratory, was cultured in a 5 L flask containing BG-11 medium supplemented with 50 $\mu g\,mL^{-1}$ kanamycin sulphate. Continuous air bubbling at 30 °C and illumination from fluorescent lamps at 60 $\mu mol\,m^{-2}\,s^{-1}$ were provided. In the late exponential growth phase, algal cells were harvested and inoculated into a medium without ferric ammonium citrate to trigger the expression of the GH gene of *P. olivaceus*. Final harvesting was performed by means of centrifugation at 12 000 *g* for 3 min at room temperature. Lyophilized alga was stored at − 20 °C until use.

Experimental protocol and fish husbandry

A preliminary investigation focused on the growth of flounder (20 ± 2.1 g) was conducted for 7 weeks. In that experiment, two doses of transgenic alga additive groups, 0.5% and 2.0% transgenic *Synechocystis* sp. PCC6803 groups were used and compared with two control groups: common commercial feed control and non-transgenic alga control. The results of the study revealed that both doses of transgenic alga significantly increased the body weight, specific growth rate (SGR) and feed efficiency in juvenile flounder, whereas no differences were observed between the two control groups for any parameters tested (data are shown in Tables 1 and 2). Consequently, it was decided to omit the non-transgenic alga control in the present study, and the supplement dose of transgenic alga was considered to be 1.0% (GH level: 2 $\mu g\,g^{-1}$ feed).

In the present study, the control group (C) consisted of fish fed with commercial pellets, and the experimental group (T) fed pellets supplemented with 1.0% transgenic alga. Pellet preparation was

Table 1 Effect of transgenic *Synechocystis* sp. PCC 6803 containing *Paralichthys olivaceus* GH gene on growth, feed intake and feed efficiency ratio of flounder over a 7-week growth period (means ± SD)

Group	Weight gain (g)	Length gain (cm)	SGR (% day^{-1})	Feed intake (g fish^{-1})	FER (g gain g^{-1} feed)
Control 1	7.26 ± 0.26^{a}	1.19 ± 0.10^{a}	0.58 ± 0.05^{a}	13.69 ± 0.98^{a}	0.53 ± 0.04^{a}
Control 2	7.74 ± 0.22^{a}	1.23 ± 0.10^{a}	0.59 ± 0.05^{a}	14.07 ± 0.45^{a}	0.55 ± 0.03^{a}
Low dose of transgenic alga	9.59 ± 0.34^{b}	1.53 ± 0.16^{b}	0.69 ± 0.04^{b}	14.99 ± 0.55^{a}	0.64 ± 0.02^{b}
High dose of transgenic alga	10.82 ± 0.61^{b}	1.57 ± 0.12^{b}	0.74 ± 0.07^{b}	15.03 ± 0.71^{a}	0.72 ± 0.02^{c}

Within columns, values with different letters are significantly different ($P < 0.05$).
Control 1, fish fed commercial diet; Control 2, fish fed commercial diet supplemented with 2.0% non-transgenic alga; low dose of transgenic alga, commercial diet supplemented with 0.5% transgenic alga; high dose of transgenic alga, commercial diet supplemented with 2.0% transgenic alga.
GH, growth hormone; SGR, specific growth rate; FER, feed efficiency ratio.

Table 2 Effect of transgenic *Synechocystis* sp. PCC 6803 containing *Paralichthys olivaceus* GH gene on the proximate composition of body muscle and hepatosomatic index of flounder (means ± SD)

Group	Moisture (%)	Protein (%)	Lipid (%)	HSI
Control 1	75.96 ± 0.39	19.20 ± 0.54	1.33 ± 0.12	2.05 ± 0.51
Control 2	76.14 ± 0.19	19.44 ± 0.15	1.40 ± 0.13	1.75 ± 0.42
Low dose of transgenic alga	76.50 ± 0.37	19.00 ± 0.51	1.20 ± 0.15	2.01 ± 0.29
High dose of transgenic alga	75.90 ± 0.45	19.58 ± 0.38	1.26 ± 0.10	1.97 ± 0.38

Body composition data are presented on wet weight basis.
Within columns, values are not significantly different ($P > 0.05$).
Control 1, fish fed commercial diet; Control 2, fish fed commercial diet supplemented with 2.0% non-transgenic alga; low dose of transgenic alga, commercial diet supplemented with 0.5% transgenic alga; high dose of transgenic alga, commercial diet supplemented with 2.0% transgenic alga.
GH, growth hormone; HSI, hepatosomatic index.

performed using a mixture of commercial feed (approximately 50% crude protein and 11% crude lipid) and transgenic *Synechocystis* sp. PCC6803 powder (the protein content is 57%) with an appropriate amount of distilled water. The mixture was compressed using a laboratory-scale pellet mill equipped with a 4.0 mm die, dried at room temperature and stored at − 20 °C until use.

The feeding experiment was carried out for 40 days at an experimental station of Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, in Jiaonan City. Turbot with an average weight of 15 ± 2.1 g were randomly placed into two 2 × 2 × 1.6 m indoor tanks at 110 fish per tank. The tanks were under a natural photoperiod and supplied with flow-through sand-filtrated seawater with dissolved oxygen level > 6.5 mg L^{-1} and a salinity of 31 g L^{-1}. Water temperature was maintained between 17.0 and 19.0 °C. Before the start of the experiment, the fish were acclimated in the tanks for 2 weeks with commercial feed pellet.

Hand feeding was carried out twice a day (08:00 and 16:00 hours) for both groups. Adequate feed for all the fish was ensured by gradually introducing pellets until the feeding response ceased. Feed consumption was recorded daily for each tank. The weight gain and length of individual fish were recorded at 10-day intervals. Before the weight measurements were made, feeding was stopped for 24 h.

Chemical analysis of muscle tissue

At the end of the feeding trial, muscle tissue of the fish was taken using pooled sampling (10 fish per sample) in order to analyse the composition of muscle. Three such samples in each group were taken, homogenized and 5 g aliquot per each sample were oven dried at 105 °C until a constant weight to determine the moisture content. The remaining aliquot was lyophilized and stored at − 20 °C for chemical analysis. Protein content was determined according to the Kjeldahl method using a semi-automatic nitrogen/protein determinator (Huaye, KDN-04, Shanghai Qianjian Instrument Co. Ltd, China). Lipid quantification was performed according to a

standard method (National Standards of the People's Republic of China 2003). All measurements were performed in duplicate.

Haematological examination

Blood samples were collected from the caudal vein of selected fish into heparinized Eppendorf tubes held on ice using sterile syringes for haematological examination. Sampling was performed at the end of the feeding trial. Each group was represented by randomly selected 10 individuals. Immediately after the blood was drawn, red blood cell (RBC) and white blood cell (WBC) count, haemoglobin (Hb), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were tested using an automated haematology analyzer (Sysmex, KX-21, Sysmex Corporation, Kobe, Japan).

Another set of blood samples was collected from 10 specimens arbitrarily selected from each group. The Eppendorf tubes containing blood were kept at room temperature for 1 h, and the resulting clot was centrifuged for 3 min at 3000 *g*, serum was then collected and stored at $-80\,^{\circ}C$ for analysis of the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and metabolites, including total protein (TP), albumin (A), globulin (G), blood urea nitrogen (BUN), creatinine (CR), glucose (GLU), triglyceride (TG), cholesterol (CHO) and electrolytes, such as potassium (K), sodium (Na), chloride (Cl), phosphorus (P) and osmolality, using an automatic chemistry analyzer (Olympus, AU400,Tokyo, Japan).

Histological study

Samples of the stomach, intestine, pyloric caecum, liver, spleen and kidney from five individuals representing each group were collected for histological examination, and the liver weight of 10 fish from each group was recorded for the determination of hepatosomatic index (HSI). The intestines were divided into three segments: anterior intestine, posterior intestine and rectum. All samples were fixed in 10% buffered formalin for 24 h, dehydrated in a graded ethanol series (70%, 80%, 95% and 100%), embedded in paraffin and sectioned to 5 μm with a rotary microtome (YD-202A, Jinhua, China). Sections were stained with haematoxylin and eosin and observed under a light microscope (Olympus).

Calculations and statistical analysis

The following relationships: Specific growth rate (SGR) = $(\ln W_2 - \ln W_1) \times 100/\text{time}$ (d), where W_2 and W_1 represent the final and initial body weight (g), respectively, feed efficiency ratio (FER) = wet body weight gain (g)/dry feed offered (g), condition factor (CF) = body weight (g) × 100/body length $(\text{cm})^3$ and hepatosomatic index (HSI) = liver weight (g) × 100/body weight (g), were used to calculate values.

Student's t-test with a confidence level of $P < 0.05$ was used in determining the significance of the findings for the two experimental groups.

Results

Growth performance and feed efficiency

The growth performance and feed efficiency ratio of turbot are shown in Table 3. A significant ($P < 0.05$) increase in SGR of the treated group was seen at the end of the feeding trial where the increment was 21.67% higher than that of the control group. However, there was no influence on body length or CF

Table 3 Effect of transgenic *Synechocystis* sp. PCC6803 containing *Paralichthys olivaceus* GH gene on growth performance and feed utilization of turbot over a 40-day growth period

Group	Weight gain (g)	Length gain (cm)	SGR (% day^{-1})	Feed intake (g fish^{-1})	FER (g gain/g feed)	CF_{start} (g cm^{-3})	CF_{end} (g cm^{-3})
C	11.38^a	1.64^a	1.20^a	18.41^a	0.61^a	1.95 ± 0.31^a	1.92 ± 0.22^a
T	13.49^b	1.69^a	1.46^b	19.90^a	0.67^b	1.88 ± 0.19^a	2.01 ± 0.15^a

For body weight and length gain, SGR, feed intake and FER, comparisons between two groups were made on data of four measurements (once every 10 days).

Within columns, values with different letters are significantly different ($P < 0.05$).

C, control group; T, transgenic *Synechocystis* sp. PCC6803 group; SGR, specific growth rate (for body weight); FER, feed efficiency ratio; CF_{start}, condition factor at the start of the experiment; CF_{end}, condition factor at the end of the experiment; GH, growth hormone.

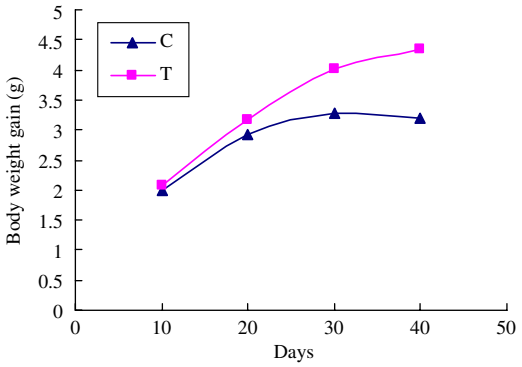

Figure 1 Effect of transgenic *Synechocystis* sp. PCC6803 containing *Paralichthys olivaceus* growth hormone gene on body weight gain of turbot. C, control group; T, 1.0% transgenic *Synechocystis* sp. PCC6803 group.

Table 4 Proximate composition of muscle tissue and HSI of turbot (means ± SD)

Group	Moisture (%)	Protein (%)	Lipid (%)	HSI
C	81.49 ± 1.07^{a}	16.11 ± 0.19^{a}	0.54 ± 0.06^{a}	1.25 ± 0.24^{a}
T	79.29 ± 0.25^{b}	18.16 ± 0.20^{b}	0.55 ± 0.13^{a}	1.19 ± 0.18^{a}

Body composition data are presented on wet weight basis; $n = 6$. For HSI, $n = 10$.
Within columns, values with different letters are significantly different ($P < 0.05$).
C, control group; T, transgenic *Synechocystis* sp. PCC6803 group; HSI, hepatosomatic index.

from feeding transgenic *Synechocystis* sp. PCC6803 (Table 3). Throughout the trial period, treated fish exhibited higher growth than the control group (Fig. 1) and significantly ($P < 0.05$) improved FER. However, feed consumption was not influenced by transgenic alga, although the treated fish consumed slightly more feed than that of the control (Table 3).

Muscle composition

With the exception of lipid content, feeding 1.0% transgenic *Synechocystis* sp. PCC6803 significantly ($P < 0.05$) decreased the moisture content, and significantly ($P < 0.05$) increased the protein content in muscle tissue of turbot (Table 4).

Haematological parameters

Blood physiological indices are summarized in Table 5. The red blood cell count and Hb level of T group were slightly higher ($P > 0.05$) than that of the control group. However, the MCH was almost the same in two groups. No significant differences ($P > 0.05$) were found in the case of WBC count and MCV figures between the two groups.

Table 6 shows the serum biochemical parameters. There were no significant differences ($P > 0.05$) between the two groups in all tested indices. The activities of ALT, AST, ALP and LDH in fish fed with transgenic alga were almost similar to those of control fish. Plasma BUN and CR levels, TP, GLU, TG and CHO concentrations and plasma ions (K, Na, Cl, P) in the treated fish also exhibited levels similar to those of the control group.

Histological study

No appreciable histological alterations were observed in the digestive tract, liver, spleen and kidney in fish fed feed supplemented with 1.0% transgenic *Synechocystis* sp. PCC6803. The stomachs of fish from two groups were almost identical in terms of the appearances of the mucosa epithelium and gastric gland (Fig. 2), as was the case with the intestinal structure (Fig. 3). No changes in enterocytes occurred as a consequence of transgenic alga treatment, and the epithelial cells appeared to be normal. The structure of the pyloric caecum was similar to that of the intestine. The influence of the transgenic alga on the histology of pyloric caecum was neglectable (not shown in Fig. 3).

Hepatosomatic index of turbot was not changed by feeding transgenic *Synechocystis* sp. PCC6803 (Table 4). The livers of fish from the T group appeared to be normal with normal cellular architecture and regular cellular arrangement (Fig. 4). No pathological changes, such as hepatocyte necrosis or infiltration of inflammatory cells, were observed in fish fed with transgenic alga. Spleen and kidney tissue of fish from two groups presented normal appearances (Fig. 5). The renal glomerulus (arrow) and renal tubules (bold arrow) of fish treated with transgenic alga demonstrated regular structures.

Discussion

The results of the present study revealed that feeding a diet supplemented with transgenic *Synechocystis* sp. PCC6803 containing *P. olivaceus* GH gene significantly stimulated the growth of turbot. This was similar to the results obtained from our previous

Table 5 Effect of transgenic *Synechocystis* sp. PCC6803 containing *Paralichthys olivaceus* GH gene on blood physiological parameters of turbot (means ± SD, $n = 10$)

	Parameter				
Group	RBC (10^{12} L^{-1})	WBC (10^{10} L^{-1})	Hb (g L^{-1})	MCH (pg)	MCV (fL)
C	0.90 ± 0.12	1.82 ± 0.15	26.00 ± 4.28	28.99 ± 1.04	114.13 ± 1.29
T	1.09 ± 0.36	1.77 ± 0.21	31.57 ± 8.90	28.88 ± 1.77	109.49 ± 3.32

Within columns, values are not significantly different ($P > 0.05$).
C, control group; T, transgenic *Synechocystis* sp. PCC6803 group; RBC, red blood cell; WBC, white blood cell; Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; GH, growth hormone.

Table 6 Serum biochemical parameters of turbot (means ± SD, $n = 10$)

	Group	
Parameter	C	T
ALT (U L^{-1})	8.02 ± 2.07	6.99 ± 1.73
AST (U L^{-1})	25.88 ± 8.68	21.63 ± 5.86
ALP (U L^{-1})	17.54 ± 3.51	17.88 ± 4.02
LDH (U L^{-1})	203.42 ± 52.76	208.13 ± 61.23
TP (g L^{-1})	26.08 ± 3.38	24.86 ± 2.59
A (g L^{-1})	5.65 ± 1.15	4.91 ± 1.17
G (g L^{-1})	20.43 ± 2.23	19.95 ± 1.59
A/G	0.27 ± 0.03	0.25 ± 0.05
BUN (mmol L^{-1})	2.13 ± 0.32	2.01 ± 0.38
CR (μmol L^{-1})	45.50 ± 12.81	42.63 ± 11.22
GLU (mmol L^{-1})	2.94 ± 0.86	2.96 ± 0.54
TG (mmol L^{-1})	2.53 ± 0.48	2.15 ± 0.60
CHO (mmol L^{-1})	1.95 ± 0.25	1.82 ± 0.25
K (mmol L^{-1})	3.03 ± 0.43	3.13 ± 0.95
Na (mmol L^{-1})	162.63 ± 10.89	153.25 ± 10.57
Cl (mmol L^{-1})	141.75 ± 11.15	133.13 ± 9.54
P (mmol L^{-1})	4.11 ± 0.65	4.51 ± 0.88
OSM (mmol L^{-1})	336.38 ± 22.61	317.25 ± 22.49

Within rows, values are not significantly different ($P > 0.05$).
C, control group; T, transgenic *Synechocystis* sp. PCC6803 group; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase. TP, total protein; A, albumin; G, globulin; BUN, blood urea nitrogen; CR, creatinine; GLU, glucose; TG, triglyceride; CHO, cholesterol; K, potassium; Na, sodium; Cl, chloride; P, phosphorus; OSM, osmolality.

study on flounder, where the increase in body weight and SGR of fish treated with 0.5% and 2.0% transgenic *Synechocystis* sp. PCC6803 was significantly higher than those of control fish (Table 1). Many studies have demonstrated the efficiency of oral administrated exogenous GHs in promoting the growth in teleost fish. Moriyama *et al.* (1993) reported that feeding rainbow trout *Oncorhynchus mykiss* (Walbaum) with GH-EPM resulted in significantly increased body weight and length. McLean, Donaldson, Teskeredzic and Souza (1993) observed an increase in the weight of coho salmon *O. kisutch* (Walbaum) treated with a dietary delivery of recombinant porcine somatotropin. Supplementation of feed with recombinant fish GH resulted in a 1.6-fold increase in weight in black seabream *Sparus macrocephalus* (Basilewsky) over control fish (Tsai *et al.* 1997). The oral delivery of recombinant flounder *P. olivaceus* (Temminck and Schlegel) GH once a week for 4 weeks at a dosage of 40 μg g^{-1} BW induced a 24% increase in the average weight of juvenile flounder (Jeh *et al.* 1998). All these results clearly demonstrated that the dietary delivery of rGH is an effective method in promoting the growth of fish.

Increased feed intake and/or improved feed efficiency could be the possible reason for the enhanced growth of fish by exogenous GH treatment. Silverstein *et al.* (2000) found that rbGH-injected (2.5 μg g^{-1} BW) channel catfish *Ictalurus punctatus* (Rafinesque) exhibited significant weight gain and a 16% increase in feed consumption. However, feed efficiency was improved only when fish were maintained at 21.7 °C (at 26.0 °C, feed efficiency was not improved). Peterson *et al.* (2004) did not observe increased feed intake or FCR in rbGH-injected channel catfish, although the treated fish showed accelerated growth. When striped bass hybrids [female *Morone saxatilis* (Walbaum) × male *M. chrysops*] were injected with bGH (10 μg g^{-1} BW), a 50% increase in SGR was accompanied by a 51% improvement in FCR; feed consumption was not affected by rbGH injection (Farmanfarmaian & Sun 1999). Garber, DeYonge, Byatt, Lellis, Honeyfield, Bull, Schelling and Roeder (1995) also noted similar results in rainbow trout injected with recombinant bovine somatotropin where the treated fish had increased growth rate and feed efficiency, although feed intake decreased. In the present study, fish weight and FER were significantly increased by feeding transgenic *Synechocystis* sp. PCC6803; however, in the case of feed intake, no difference was found between the T and C groups.

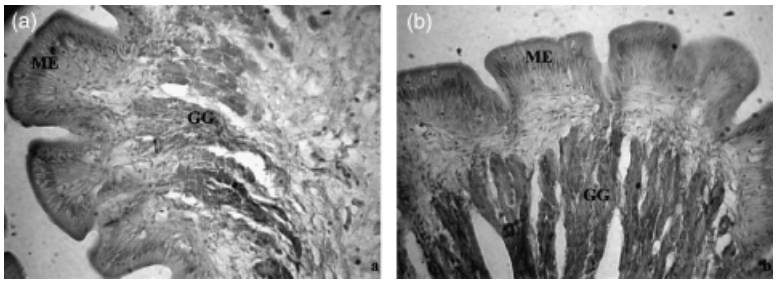

Figure 2 Stomachs from fish fed with control pellet (a) and fed with transgenic alga pellet (b) were almost identical in appearance (haematoxylin and eosin, × 400). GG, gastric gland; ME, mucous epithelium.

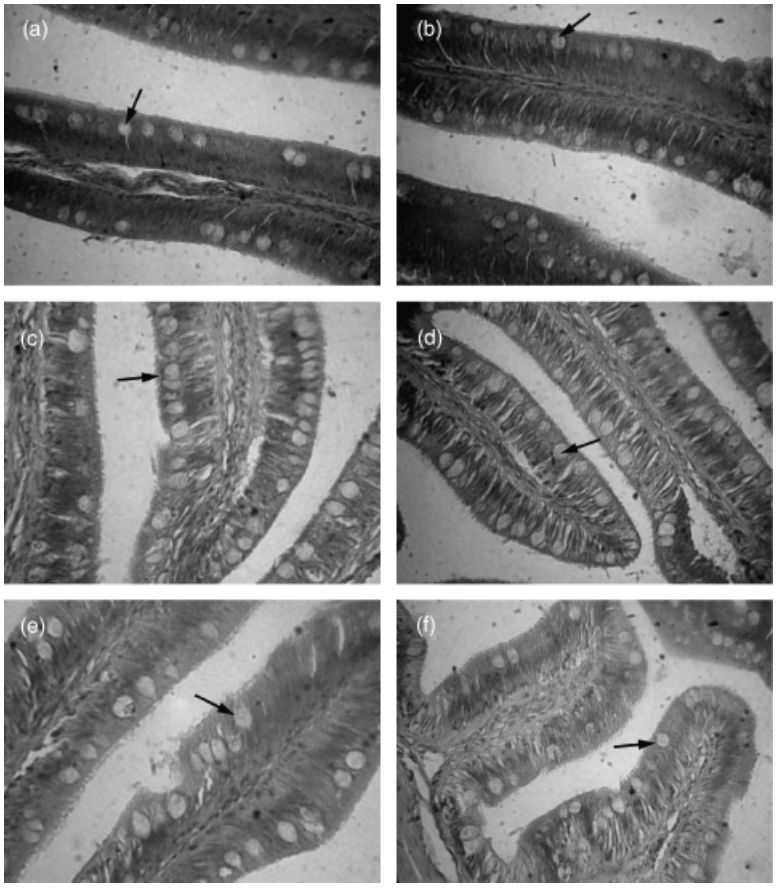

Figure 3 Anterior, posterior intestine and rectum from fish fed with control pellet (a, c, e), and from fish fed with transgenic alga pellet (b, d, f) (haematoxylin and eosin, × 400). Arrow, goblet cell.

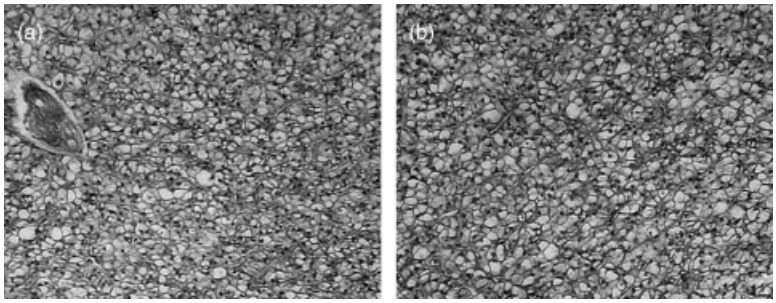

Figure 4 Livers from fish fed with control pellet (a) and fed with pellet supplemented with 1.0% transgenic alga (b) showed regular-shaped hepatocytes (haematoxylin and eosin, × 100).

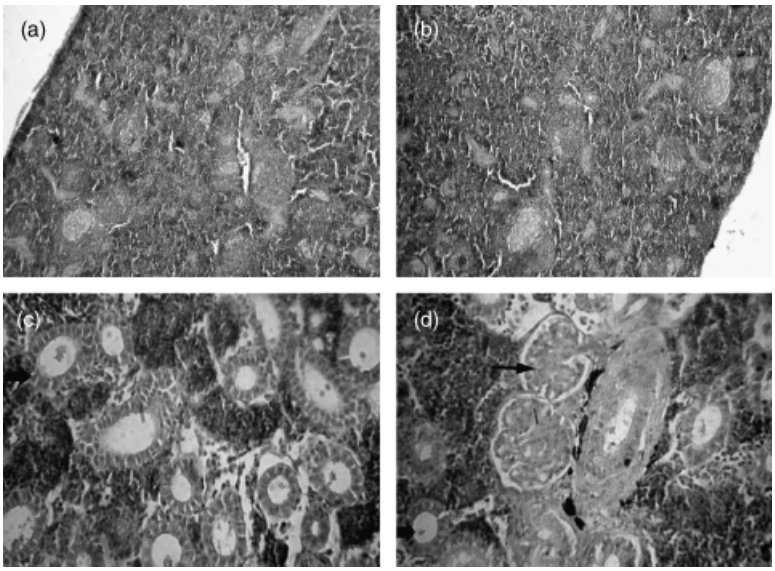

Figure 5 Spleens [haematoxylin and eosin (H&E), × 100] of fish from control group (a) and from transgenic alga group (b) showed similar appearances. Kidney (H&E, × 400) from the control group (c) and from the transgenic alga group (d) exhibited a normal structure. Arrow, renal glomerulus; bold arrow, renal tubule.

This was found to be similar to the results of our preliminary investigation conducted with flounder (Table 1). Therefore, further investigations are needed to verify whether the growth-promoting effect of rGH is a consequence of increased feed consumption or improved feed efficiency.

In contrast to our previous findings, where moisture, protein and lipid levels in *P. olivaceus* were unaffected by feeding transgenic *Synechocystis* sp. PCC6803 (Table 2), the present results of significantly increased protein content, decreased moisture content and unaffected lipid content were highly impressive. Interestingly, the previously reported effects of rGH treatment on body composition have not been consistent. Silverstein *et al.* (2000) found that rbGH injection induced fatter fillets in USDA-103 and Norris strain of channel catfish, whereas rainbow trout injected with rbGH demonstrated a higher protein content than control fish (Rasmussen *et al.* 2001). Garber *et al.* (1995) discovered that recombinant bovine somatotropin treatment increased carcass moisture and protein content and decreased fat content in rainbow trout. On the contrary, Rønsholdt and McLean (2004) failed to identify changes in body proximate composition in rainbow trout treated with bGH injection. Similarly, Peterson *et al.* (2004) observed no effect of rbGH on the body composition of channel catfish. It has been documented that fish size and age (Rønsholdt 1995), and diet (Rasmussen, Ostenfeld, Rønsholdt & McLean 2000) can affect body composition. Therefore, differences among the two groups may be due to different experimental variables such as sample time (after GH treatment), diet composition or different fish size. Nevertheless, the cause may more likely be the different responses to GH treatment from different species of fish and the hormone dose used in the experiments.

Measurement of blood physiological indices and serum chemistry parameters are commonly used diagnostic tools in fish toxicology and biomonitorings (Folmar, Gardner, Hickey, Bonomelli & Moody 1993; Adams, Ham, Greeley, LeHew, Hinton & Saylor 1996). Changes in the metabolite concentrations and enzyme activities in blood often directly reflect cell damage in specific organs (Casillas, Meyers & Ames 1983). Blood urea nitrogen and CR are indicators of kidney function; increasing concentration may reflect kidney dysfunction. Lactate dehydrogenase is an indicator of cellular damage in a range of organs, principally the liver, kidney and muscle (Bernet, Schmidt, Wahli & Burkhardt-Holm 2001). High values of ALT and AST are indicative of liver impairment. In the present study, feeding transgenic alga had no effect on fish blood cell counts, Hb level, serum enzyme activities of ALT, AST, ALP and LDH, and other blood biochemical parameters such as BUN, CR, TP, GLU and so on. It is a fact that little information is available on the effect of rGH on fish haematology. Our results suggest that exogenous heterologous recombinant fish GH has no negative effects on fish haematology, and thus transgenic *Synechocystis* sp. PCC6803 seems to be a safe feed additive for fish, at least in short-term application.

The results of the present study and our previous flounder experiment showed that HSI of turbot and flounder were not altered by feeding transgenic *Syne-*

chocystis sp. PCC6803 containing *P. olivaceus* GH gene. This was consistent with the findings in coho salmon fed with recombinant bovine somatotropin (McLean, Donaldson *et al.* 1990) and in bGH-injected channel catfish (Peterson *et al.* 2004). Histological examination on the liver of turbot revealed that there was no histopathological alteration induced by transgenic *Synechocystis* sp. PCC6803. The stomach, intestine, spleen and kidney of fish fed with transgenic alga exhibited similar histological structures as those of control fish. It has been known that the receptor of GH of fish mainly distributed in liver cells, and the posterior intestine of teleosts is the spot to absorb protein hormones in a immunologically and biologically active form (Moriyama, Takahashi, Hirano & Kawauchi 1990; McLean, Donaldson *et al.* 1990; McLean, Von Der Meden & Donaldson 1990; Hertz, Tchelet, Madar & Gerder 1991). Thus, the liver and intestine should be important organs for evaluation of the safety of transgenic *Synechocystis* sp. PCC6803 containing *P. olivaceus* GH gene. Our results of histological examination in the present trial and the previous flounder study support the possible assumption that transgenic *Synechocystis* sp. PCC6803 containing *P. olivaceus* GH gene is biologically safe feed additive for fish.

In conclusion, the results of the present study indicated that transgenic *Synechocystis* sp. PCC6803 containing the *P. olivaceus* GH gene is an efficient growth promoter and a safe feed additive for fish. It could therefore be suggested that the transgenic alga could be of great benefit to fish farmers. Nevertheless, as our conclusions were based on a 40-day trial, investigations on long-term feeding of transgenic *Synechocystis* sp. PCC6803 would be needed to further confirm its effects and safety as a feed additive to fish.

Acknowledgments

The authors wish to thank Mr Xuezhou Liu, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, for sharing his experience in designing. This study was authorized by Management Office of GMO Biosafty, Ministry of Agriculture of the People's Republic of China (2005-T104).

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