

# Study of growth and body composition of red snapper *Lutjanus erythropterus* fed diets containing *Escherichia coli* expressing recombinant tilapia insulin-like growth factor-I

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**ABSTRACT:** To examine the effects of insulin-like growth factor (IGF)-I on the growth performance of fish, juvenile red snapper *Lutjanus erythropterus* with an initial body weight of approximately 12 g were fed diets containing different amounts of IGF-I containing *Escherichia coli* BL21 powder (BL21-tIGF-I). The growth of fish was enhanced by lower doses (0.25, 0.5 and 1%), but deteriorated at higher amounts (2.5 and 5%). The best results were obtained with 0.5% BL21-tIGF-I supplementation, which resulted in 154 and 85% weight gain and feed efficiency, respectively. In addition, muscle proteins appeared to be elevated, but muscle lipids were reduced except for in the 1% BL21-tIGF-I group. In contrast, liver lipids were reduced at 0.25, 0.5 and 1%, but increased at 2.5 and 5% BL21-tIGF-I. Furthermore, to examine the effects of BL21-tIGF-I on later-stage red snapper, we selected fish of approximately 23–24 g weight and fed them a diet either with or without 0.5% BL21-tIGF-I for 12 weeks. Results showed that the growth performance of later-stage fish was better with BL21-tIGF-I than those without supplementation after 6 weeks of feeding. The average body weight of fish that did not receive BL21-tIGF-I during the first 6 weeks, but were fed BL21-tIGF-I in weeks 7–12, was significantly higher than those fed without BL21-tIGF-I for the entire experiment. These results suggest that a continuous supply of BL21-tIGF-I may be beneficial for the growth performance of red snapper.

**KEY WORDS:** growth, insulin-like growth factor-I, *Lutjanus erythropterus*, red snapper.

## INTRODUCTION

Red snapper *Lutjanus erythropterus* is also called redfish, red striped snapper or crimson snapper because of its bright red dorsal soft rays and anal fins. They are widely distributed in the Indo-West Pacific region, from the Gulf of Oman to South-east Asia, north to southern Japan and south to northern Australia. It is a commercially important food fish in Taiwan because of its bright reddish color and flesh tenderness. Although hatchery technologies were successfully developed for mass seed production in 1994, it takes nearly 2 years to grow

to a market size of 600–1000 g. This circumstance limits its popularity for culturing due to farmers' considerations of profitability. We therefore attempted to investigate the possibility of using insulin-like growth factors (IGFs) as a feed supplement to enhance the growth of red snapper.

IGFs are synthesized and released from the liver by stimulation of growth hormone (GH).<sup>1</sup> Although the liver is the primary organ for IGF synthesis, IGF mRNA localization and their syntheses have also been reported in other tissues as well.<sup>2</sup> IGFs have two isoforms: IGF-I and IGF-II. They are composed of four functional domains, B–C–A–D, which form a linear functional polypeptide. IGF-I and IGF-II have distinct differences in their amino acid sequences in domains C and D. IGF-I contains 70 amino acids and IGF-II has 67 amino acids and a

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structure similar to that of insulin.<sup>3</sup> Fish IGF-I amino acid sequences are highly homologous to their mammalian orthologs. For example, tilapia IGF-I shares 79.4% homology with human IGF-I and more than 80% homology with other fish like salmon and rainbow trout.<sup>4</sup> IGF-I is mainly involved in regulating cell proliferation, differentiation and metabolism. In addition, it stimulates the absorption of sulfur and protein synthesis in cartilage, and regulates osmotic homeostasis.<sup>5</sup> As a result of its diverse cellular functions, its effect on animal growth and potential use in aquaculture are of great interest.

With its high sequence homology across species, it is possible that IGFs may have cross-species activity. Bovine recombinant IGF-I activates Na<sup>+</sup>/K<sup>+</sup>-ATPase in silver trout gills<sup>6</sup> and enhances somatic regulation in trout.<sup>7</sup> Human IGF-I can stimulate protein synthesis in Gulf killifish muscle.<sup>8</sup> Salmon growth can be enhanced by exogenous mammalian IGF-I.<sup>9</sup> It appears that IGF-I treatment may enhance fish growth, but the action of the growth-promoting activity by IGF-I has not been determined. Furthermore, utilization rates of carbohydrates are low in marine fishes, and carbohydrate metabolism may likely be related to IGF functions.<sup>10</sup> Generally, an exogenous supply of proteins and lipids in feeds is required to obtain sufficient energy for growth promotion. It is well documented that IGF promotes carbohydrate metabolism, elevating protein and lipid syntheses as well as inhibiting the breakdown of lipids.<sup>1</sup> Thus, IGFs may increase the utilization rate of carbohydrates to provide energy. On the other hand, the unnecessary consumption of proteins and lipids could be reduced for growth promotion.

It seems that the use of IGF-I may be beneficial to fish farming; however, excessive IGF-I has also been reported to cause low blood sugar and death in juvenile barramundi.<sup>11</sup> Therefore, determining adequate amounts of IGF-I for use in aquaculture is imperative. In addition, IGFs are often given by intramuscular or intraperitoneal injections because these methods are most direct and possibly most effective. However, injections are labor intensive and stressful to fish, so these approaches are not practical in the field. Oral administration seems to be the most convenient alternative for aquaculture, but digestion causes a reduction in potency when IGF-I is added as a feed supplement. However, if a proper IGF dosage can be determined, feeding is still the most plausible approach for application of IGF-I. In preliminary trials, we found that supplementing feed with IGF-I-containing bacteria powder can promote fish appetites, weight gain and feed efficiency in juvenile European eel, tilapia and red snapper of <5 g in

body weight (Liao, WL and Huang, SK, unpubl. data, 2001). However, the effects of IGF-I have been demonstrated to be developmental stage-dependent.<sup>12</sup> Therefore, we further tested the efficacy of IGF-I in stimulating growth of later-stage juvenile red snapper through oral administration. In addition, we also examined its effects on tissue protein and lipid levels.

## MATERIALS AND METHODS

### Fish and rearing methods

Red snapper were obtained from a local hatchery and raised in outdoor 50-t concrete ponds at the Mariculture Research Center, Fisheries Research Institute, Tainan, Taiwan. Fish were fed twice daily with a commercial fish feed for 10 days and then starved for 1 day before being transferred indoors to 100-L tanks for the experiments. Bricks were placed in each tank as shelter to prevent cannibalism. Each treatment was run in duplicate. Tank water was maintained at 27–29°C and approximately 27–30 salinity and circulated at a flow rate of 4 L/min. During the experiment, fish were fed with designated diets (Table 1) at 3.5% of the total fish body weight twice daily, but were starved 1 day before being weighed.

### Production and analysis of tIGF-I

The supplemented tilapia IGF-I containing *Escherichia coli* BL21 powder (BL21-tIGF-I) was produced as follows: the *tIGF-I* (GenBank accession no. AH006116) gene was subcloned into the *pET-14b* vector (Novagen, San Diego, CA, USA) and transformed into BL21 cells according to the manufacturer's instructions. The expression of *tIGF-I* was induced by 0.1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) and optimized for mass production by adapting the method for tilapia IGF-II production.<sup>15</sup> Cells were lysed and centrifuged to collect the pellets. The pellets were dissolved in 6 M urea in phosphate-buffered saline (PBS) (pH 12), incubated for 3 h, centrifuged to collect the supernatant, and diluted with an equal amount of PBS (pH 7.3), which was designated as the inclusion body fraction. The solution containing the inclusion bodies was loaded onto a Ni-NTA agarose gel column, and the flow-through portion was saved. The column was then washed with washing buffer (30 mM imidazole and 100 mM NaCl in PBS, pH 7.3), and the washed buffer was collected. Finally, the tIGF was eluted with eluting buffer (250 mM imidazole and 5 mM

**Table 1** Composition and proximate analysis of experimental diets

	Diet no.					
	1	2	3	4	5	6
<b>Ingredients (%)</b>						
White fish meal	54.0	54.0	54.0	54.0	54.0	54.0
Wheat flour	25.0	25.0	25.0	25.0	25.0	25.0
Vitamin mix <sup>†</sup>	1.5	1.5	1.5	1.5	1.5	1.5
Mineral mix <sup>‡</sup>	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1	0.1	0.1	0.1
Fish oil	6.0	6.0	6.0	6.0	6.0	6.0
Cellulose	7.9	7.6	7.4	6.9	5.4	2.9
<i>Escherichia coli</i> powder <sup>§</sup>	0.0	0.25	0.5	1.0	2.5	5.0
<b>Proximate analyses (%)</b>						
Moisture	9.0	9.1	9.2	9.2	9.1	9.1
Crude protein	40.9	41.0	41.3	41.6	42.7	43.4
Crude lipid	11.9	11.4	11.0	11.4	11.1	11.0
Ash	12.8	13.4	13.3	13.3	13.0	13.0
IGF-I content (mg)	0.0	0.1	0.2	0.4	1.0	2.0

<sup>†</sup>Ogino *et al.*<sup>13</sup>

<sup>‡</sup>Ogino and Yang.<sup>14</sup>

<sup>§</sup>Each gram of *Escherichia coli* powder contained 0.4 mg of tIGF-I.

DTT in PBS, pH 7.3). The above saved fractions were subjected to 15% Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel for analysis. For mass production, cells were killed by ozone perfusion, centrifuged and lyophilized. By analyzing the IGF-I bioactivity as described by Hu *et al.*<sup>15</sup> the final product was determined to have 0.4 mg tIGF-I/g of BL21 cells.

### Experimental diet preparation

The formulas for the experimental diets are described in Table 1. The experimental diets were supplemented with BL21-tIGF-I at approximately 0.25–5%. Ingredients were fully mixed, stirred with water at approximately 35–40% of the ingredient weight, made into 3–4-mm pellets and dried at 45°C for 18 h. Feed was stored at room temperature until used, except those for feed composition analysis, which were stored at –20°C.

### Experiment design and protocols

For the first set of experiments, red snapper with body weights approximately 12 g were selected for the experiments. Fish were weighed and randomly divided into six groups of 10 fish per tank and fed diets 1–6, which were supplemented with 0, 0.25, 0.5, 1.0, 2.5 and 5.0% BL21-tIGF-I, respectively. At

21 days after the start of the experiment, the fish were weighed, and feed efficiencies and survival rates were determined for all fish. The remaining fish continued to be fed the same diet until 42 days after the start of the experiment. Weight gains and feed efficiencies of surviving fish were determined. Five fish were randomly selected from each group fed the different diets, killed and stored at –20°C for proximate composition analysis. In the second trial, later-stage fish at 23–24 g were randomly divided into four groups of 30 fish each. The first two groups of fish were fed a diet without BL21-tIGF-I supplementation. The third and fourth groups of fish were fed a diet with 0.5% BL21-tIGF-I supplementation. After 6 weeks of feeding, the weight gain and feed efficiency of all fish were determined. The remaining fish were fed without BL21-tIGF-I supplementation in groups 1 and 3 or fed with 0.5% BL21-tIGF-I supplementation in groups 2 and 4. After an additional 6 weeks of feeding, the weight gain and feed efficiency of all fish were determined.

### Proximate composition analysis

Proximate composition analyses were carried out according to standard methods. The moisture and ash contents of the feed were determined by AOAC methods.<sup>16</sup> Crude protein contents of the feed and fish tissues were analyzed according to the

micro-Kjeldahl method, and the crude lipid contents of the feed and fish tissues were analyzed according to Folch *et al.*<sup>17</sup>

### Statistical analysis

All data from the experiments were analyzed using the GLM procedure of the SAS software<sup>18</sup> unless otherwise stated.

## RESULTS

### Analysis of tIGF-I expression in BL21 cells

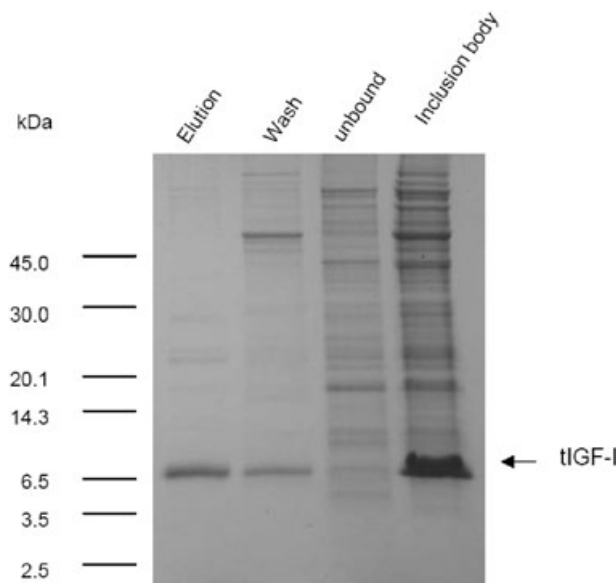
The protein tIGF-I has been shown to be successfully induced as a glutathione transferase (GST) fusion protein in BL21 cells.<sup>12</sup> To remove the GST moiety requires enzymatic digestion that causes the reduction of the yield and an increase in production costs. Therefore, we subcloned *tIGF-I* into the *pET-14b* vector, which produced a fusion protein with a small-molecular-weight histidine (His) tag, which does not need to be removed, and the fusion proteins can be purified using the Ni-NTA agarose gel column. SDS-PAGE analysis demonstrated that the expression of His-tagged tIGF-I at a molecular weight of approximately 6.5 kDa was successfully induced in BL21 cells (Fig. 1). Most of the His-tagged tIGF-I proteins were found in the inclusion body, which could be solubilized and purified as shown in Figure 1. For convenience and decreased production costs, we intended to test if the His-tagged tIGF-I was effective in promoting growth in the form of His-tagged tIGF-I-containing bacteria powder. Thus, we killed BL21 cells expressing the His-tagged *tIGF* by ozone perfusion, then centrifuged and lyophilized them. The lyophilized cell powder is referred to as BL21-tIGF-I.

### Experimental diet analysis

The ingredients of the six experimental diets supplemented with different amounts (–0–5%) of BL21-tIGF-I are shown in Table 1. The moisture, crude protein, crude lipids and ash contents were also analyzed as indicated in the proximate analysis of Table 1 and were in the ranges 9.0–9.2%, 40.9–43.4%, 11.0–11.9% and 12.8–13.4%, respectively.

### Survival and growth performance of *Lutjanus erythropterus* fed experimental diets with or without BL21-tIGF-I supplementation

Red snapper with approximate body weights of 12 g were selected for the experiments and fed



**Fig. 1** Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of tilapia insulin-like growth factor (tIGF)-I expressed in BL21 cells. Different fractions of a sample designated through purification were subjected to 15% gel SDS-PAGE analysis. A major tIGF-I band (arrow) at approximately 6.5 kDa was found in the inclusion body and appeared in the elution, and a lesser amount was found in the wash, but not in the unbound fraction. The standard molecular weights in kDa are shown at the left of the gel.

diets 1–6 supplemented with different percentages of BL21-tIGF-I as indicated in Table 2. We analyzed survival rates and growth performance, including body weight, weight gain and feed efficiency at 21 and 42 days after the start of the experiment. The initial body weights were between 11.5 and 12.4 g, and fish weights increased to 18.5–20.4 g at 21 days (Table 2). All experimental fish survived during this period. The weight gains and feed efficiencies were approximately 60–74% and 67–84%, respectively. The weight gain and feed efficiency were 74 and 84% at the optimal level of supplementation (diet 3 with 0.5% BL21-tIGF-I) compared to 63 and 70%, respectively, for diet 1 without BL21-tIGF-I. To compare the statistical difference of weight gain between treatments, we first analyzed the data by analysis of variance (ANOVA) using two factors including the BL21-tIGF-I supplementation percentage and the tank to take account of possible tank differences. Results showed that although the growth performance appeared to be enhanced in the BL21-tIGF-I-treated groups, no significant difference was observed at 21 days. At 42 days, the control fish (diet 1) had a survival rate of 75%. In contrast, the survival rates of the BL21-tIGF-I-treated groups were between 80 and 100%, except



**Table 2** Growth performance of *Lutjanus erythropterus* fed the experimental diets for 3 and 6 weeks

Diet No.	<i>E. coli</i> (%)	Body weight (g)			Weight gain (%)		Feed efficiency (%)	
		Day 0 ( <i>n</i> ) <sup>†</sup>	Day 21 ( <i>n</i> )	Day 42 ( <i>n</i> )	Day 21	Day 42	Day 21	Day 42
1	0.00	11.6 ± 1.7 (20)	18.5 ± 2.3 (20)	23.6 ± 3.1 (15) <sup>c</sup>	63	104	70	71
2	0.25	12.4 ± 1.6 (20)	20.1 ± 2.3 (20)	27.6 ± 3.9 (19) <sup>a,b</sup>	60	119	67	76
3	0.50	11.7 ± 1.4 (20)	20.4 ± 2.6 (20)	29.8 ± 4.7 (18) <sup>a</sup>	74	154	84	85
4	1.00	11.5 ± 1.3 (20)	19.2 ± 2.9 (20)	25.8 ± 3.9 (16) <sup>b,c</sup>	67	123	77	77
5	2.50	12.2 ± 1.6 (20)	20.0 ± 3.1 (20)	23.8 ± 3.9 (20) <sup>b,c</sup>	64	96	72	58
6	5.00	12.2 ± 1.4 (20)	20.0 ± 2.6 (20)	24.5 ± 4.2 (14) <sup>b,c</sup>	64	101	73	54

<sup>†</sup>Data are presented as the mean ± standard deviation with the number of replicates (*n*) in parentheses.

Different superscript letters in the same column indicate a significant difference between treatments at  $P < 0.05$ .

**Table 3** Proximate compositions of experimental fish the first set of experiments

Diet No.	<i>E. coli</i> (%)	Muscle		Liver
		Protein (%) <sup>†</sup>	Lipid (%)	Lipid (%)
1	0.00	18.37 ± 0.58 <sup>a</sup>	2.45 ± 0.53 <sup>a</sup>	33.34 ± 3.30 <sup>a</sup>
2	0.25	19.91 ± 0.42 <sup>b</sup>	1.63 ± 0.20 <sup>b</sup>	26.34 ± 1.96 <sup>b</sup>
3	0.50	19.91 ± 0.67 <sup>b</sup>	1.60 ± 0.18 <sup>b</sup>	20.15 ± 0.02 <sup>c</sup>
4	1.00	19.86 ± 0.56 <sup>b</sup>	2.29 ± 0.34 <sup>a</sup>	31.97 ± 4.20 <sup>a</sup>
5	2.50	20.42 ± 0.31 <sup>b</sup>	1.56 ± 0.36 <sup>b</sup>	34.08 ± 2.82 <sup>a</sup>
6	5.00	20.31 ± 0.42 <sup>b</sup>	1.45 ± 0.04 <sup>b</sup>	35.63 ± 5.14 <sup>a</sup>

<sup>†</sup>Data are presented as the mean ± standard deviation ( $n = 5$ ).

Different superscript letters in a column indicate a significant difference between treatments at  $P < 0.05$ .

for fish fed the highest amount of BL21-tIGF-I (diet 6), which had a survival rate of 70%. Although the survival rates differed, they were not statistically significant according to  $\chi^2$ -analysis ( $P > 0.05$ ). According to ANOVA of 10 randomly selected fish from each group, BL21-tIGF-I significantly ( $P = 0.0036$ ) enhanced the increase in body weight. The tanks also appeared to affect the fish body weights, but the significance of the difference was lower ( $P = 0.024$ ). Duncan's multiple comparison analysis showed that red snapper in the 0.25% (diet 2) and 0.5% (diet 3) BL21-tIGF-I-treated groups showed significantly increased body weight ( $P < 0.05$ ) compared to the control (diet 1). In contrast, no statistical significance was observed at the higher doses of BL21-tIGF-I (diets 4, 5 and 6). The percentage of weight gain and feed efficiency of fish with 0.5% BL21-tIGF-I (diet 3) were 154 and 85% compared to 104 and 71% for the control (diet 1), respectively. However, higher amounts of BL21-tIGF-I (diets 5 and 6) had lower feed efficiencies of 58 and 54%, respectively, while their weight gains were comparable to the control group.

### Proximate composition analysis

After completing the 6-week trials, five fish randomly selected from each treatment were killed to

collect muscle and liver tissues for analyses of crude protein and lipids. The initial muscle protein contents were 20.3%. After 6 weeks, the muscle protein contents ranged 18.4–20.3% (Table 3). All BL21-tIGF-I-treated groups had significantly ( $P < 0.05$ ) higher protein contents compared to the control group at 18.4%. In contrast, the lipid contents were significantly ( $P < 0.05$ ) lower in BL21-tIGF-I-treated groups (1.4–1.6%) compared to the control group (2.4%) except for diet 4 (2.3%). The effects of BL21-tIGF-I on liver lipids varied with the amounts of BL21-tIGF-I supplemented. The hepatic lipids decreased in the low-supplement groups (diets 2–4, 20.2–32%) and increased in the high-supplement groups (diets 5 and 6, 34.1 and 35.6%, respectively) compared to the control group (diet 1, 33.3%).

### Growth performance of later-stage *Lutjanus erythropterus* fed experimental diets with or without BL21-tIGF-I supplementation

It appeared that BL21-tIGF-I significantly improved the growth performance of red snapper at the later stage (Table 2, day 42). To further confirm the effects of BL21-tIGF-I on the growth performance of later-stage red snapper, we selected

**Table 4** Growth performance of later-stage *Lutjanus erythropterus* with or without BL21 powder supplementation containing tIGF-I for 6 weeks, after which supplementation was continued or discontinued for an additional 6 weeks

Diet No.	Percent (%) of supplementation		Body weight (g)			Weight gain (%)		Feed efficiency (%)	
	W0–W6	W7–W12	Initial <sup>†</sup>	W6	W12	W6	W12	W6	W12
1	0	0	23.7 ± 2.5	–	79.0 ± 11.2 <sup>c</sup>	125	239	64	55
2	0	0.5	22.8 ± 2.8	51.6 ± 8.0 <sup>b†</sup>	88.4 ± 11.9 <sup>b</sup>	119	279	63	66
3	0.5	0	24.3 ± 2.8	–	93.3 ± 13.0 <sup>a,b</sup>	137	290	79	61
4	0.5	0.5	23.5 ± 2.6	56.9 ± 7.9 <sup>a†</sup>	96.4 ± 13.7 <sup>a</sup>	141	303	80	67

<sup>†</sup>Data are presented as the mean ± standard deviation ( $n = 30$ ), except for mean ± standard deviation of all fish fed diets 1 and 2 ( $n = 60$ ) or diets 3 and 4 ( $n = 60$ ).

Different superscript letters in the same column indicate a significant difference between treatments at  $P < 0.05$ .

Wx, week, where x is the number of weeks into the feeding trial.

bigger fish of approximately 23–24 g, with or without feeding the optimal diet (supplemented with 0.5% BL21-tIGF-I) for 6 weeks. Then, BL21-tIGF-I supplementation was either continued or discontinued for an additional 6 weeks of feeding. Results showed that with BL21-tIGF-I supplementation (diets 3 and 4) in the first 6 weeks, the body weights ( $56.9 \pm 7.9$  g) of red snapper were significantly ( $P < 0.05$ ) higher than those without BL21-tIGF-I supplementation (diets 1 and 2,  $51.6 \pm 8.0$  g), and the weight gain and feed efficiency were also better with diets 3 and 4 (Table 4). These results confirmed the effects of BL21-tIGF-I supplementation on red snapper growth performance as described above (Table 2). Once the BL21-tIGF-I was withdrawn from the diet (diet 3), the average body weight of the red snapper dropped but did not significantly differ from those with a continuing supply of BL21-tIGF-I (diet 4) at 12 weeks after the start of the experiment. On the contrary, the average body weight of fish that did not receive BL21-tIGF-I during the first 6 weeks but were fed BL21-tIGF-I from weeks 7 to 12 (diet 2) was significantly ( $P < 0.05$ ) higher than those fed without BL21-tIGF-I for the entire experiment, and were comparable to those fish fed diet 3. These results suggest that a continuous supply of BL21-tIGF-I may be beneficial for the growth performance of red snapper.

## DISCUSSION

IGF-I has been demonstrated to promote fish growth experimentally by implantation<sup>9</sup> or intraperitoneal injection.<sup>11</sup> We further showed that oral administration of BL21-tIGF-I at moderate doses could also enhance the growth of the red snapper without significant effects on fish survival. This suggests that BL21-tIGF-I-supplemented feeds

may potentially be used in aquaculture for the growth promotion of cultivars.

The induction of tIGF-I expression in BL21 cells was previously shown by Chen *et al.*,<sup>12</sup> in the form of a GST-fusion protein. However, GST is a much bigger protein (21 kDa) than tIGF-I (6.5 kDa). It was demonstrated that after thrombin digestion to remove the GST moiety, tIGF-I was effective in stimulating tilapia ovary cell proliferation and promoting juvenile tilapia growth by injection.<sup>12</sup> However, enzymatic digestion of the recombinant fusion protein is inefficient and costly. Therefore in this study, we generated a His-tagged tIGF-I protein and showed that this protein was effective without removal of the His tags in promoting growth of red snapper in a form of a bacteria cell powder (Tables 2,4). To rule out the possibility that the effects of BL21-tIGF-I supplementation may have been caused by the BL21 cell powder only, we also fed the red snapper BL21 cell powder without tIGF-I and found no significant difference in the growth performance compared to the non-supplemented control fish over a 42-day feeding period (Liao, WL and Huang, SK, unpubl. data, 2001). This suggests that the growth promotion effects were primarily a result of the presence of tIGF-I.

Supplementation with 0.25–5% BL21-tIGF-I in this study boosted the feed crude protein to approximately 41–43% and crude fat to approximately 11–12%, which are suitable for reported feed protein at approximately 41–43% and fat at approximately 9–12% for silver snapper.<sup>19</sup> After 6 weeks of culture of red snapper with an initial weight of approximately 12 g, the 0.5% BL21-tIGF-I-supplemented group (diet 3) had the best growth performance of 154% weight gain and 85% feed efficiency. Similar effects of BL21-tIGF-I supplementation on fish weight gain and feed efficiency were observed in European juvenile eel *Anguilla*

*anguilla* (Liao, WL and Chien, TH, unpubl. data, 2000). With an increase in BL21-tIGF-I supplementation, the weight gain, feed efficiency and survival rate were all reduced (Table 2). Degger *et al.*<sup>11</sup> also reported a deteriorating effect of excess IGF on fish health. This indicates that the dosage of IGF-I used for growth promotion should be carefully determined to avoid affecting fish health. With proper amounts of BL21-tIGF-I, oral administration appears to be a practical way of enhancing fish growth performance in aquaculture. However, more-intensive field studies are required to resolve health issues, especially public health and safety.

IGF-I can stimulate carbohydrate metabolism and subsequent protein synthesis.<sup>5,20</sup> BL21-tIGF-I-supplemented feed enhanced the protein content in fish muscle, which might reflect a specific effect of IGF-I (Table 3). Similarly, IGF-I was also demonstrated to inhibit degradation and increase protein retention in the hindlimb muscle of lambs.<sup>21</sup> However, we did not previously observe a significant effect of IGF-I on protein retention in red snapper fry (Liao, WL and Huang, SK, unpubl. data, 2001). This suggests that the effects of IGF-I may be developmental stage-dependent as discussed by Chen *et al.*<sup>12</sup>

BL21-tIGF-I also appeared still to be effective in later-stage fish as indicated in Table 4. More importantly, we demonstrated that a continuous supply of BL21-tIGF-I is essential for growth promotion of fish without affecting the survival rate. Therefore, it may be possible to feed BL21-tIGF-I to fish for a longer period in order to shorten the time required for harvesting.

## CONCLUSION

Despite the well-known effects of IGF-I on cell proliferation and fish growth, it has been difficult to use it in the field. Here, for the first time, we demonstrate that tIGF-I in BL21 cells retains its potency at least partially by oral administration, and this is important for its application in aquaculture.

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