Journal of Aquaculture 12 (2) : 79～89 (1999)

Effect of Recombinant Bovine Somatotropin on Growth of Olive Flounder, *Paralichthys olivaceus*

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The recombinant bovine somatotropin (rBST) was administered to the oliver flounder, *Paralichthys olivaceus*, to know the effects and optimal administration frequency and dosage of the hormone. The experiment was conducted with three different treatment groups (A, B and C) designated based on the duration and administration frequency of rBST and one control (D) from April 14, 1996 to March 16, 1997. The fish of hormone treated groups grew 7.86 to 10.07% (47.45 to 60.75 g in weight) better than the control at the end of the experiment ($P<0.05$). No significant differences in their growth were detected among treatment groups. The distinct growth improvement was recognized four weeks after completion of the first four hormone administration. When considering water temperatures measured from the experimental tanks, the effect of rBST on the flounder was greater during the period showing relatively lower temperature. The survival rates were higher in treatment groups than in the control, revealing 98.3% (A), 98.4% (B), 97.7% (C) and 93.1% (D) during the first stage of culture; 92.7% (A), 91.3% (B), 86.7% (C) and 80.0% (D) during the second stage of culture.

Key words : Growth effect, Recombinant bovine somatotropin, Olive flounder, *Paralichthys olivaceus*

Introduction

As the purpose of finfish aquaculture is to grow fish to the marketable size with minimum cost and short period of time, many researchers have taken considerable efforts to achieve such aims by using several methods such as triploidy, selection, hormone treatment, gene transfer, etc. Of these, physiological manipulation using growth hormone became one of most effective way to promote animal growth since the classical work of Evans and Simpson (1931) on the somatotropic effects of growth hormone.

The growth of fish is controlled by growth hormone or somatotropin secreted from its pituitary grand as same as in other vertebrates. About he effects of the purified growth hormone on fish growth, Pickford and Thompson (1948) showed that purified mammalian growth hormone stimulates linear growth in intact teleost. Later, several more refined studies were attempted continuously and they demonstrated that purified bovine growth hormone also promote growth in hypophysectomized male *Fundulus heteroclitus* (Pickford, 1953, 1957, 1959); in the coho salmon, *Oncorhynchus kisutch* (Higgs et al., 1975, 1976, 1977, 1978; Market et al., 1977); in rainbow trout (Leatherland and Nuti, 1981).

However, natural growth hormone is both difficult and costly to accumulate in large quantities, it has not been used routinely as an augment agent by researchers or fish farm industry. The application of recombinant DNA techniques allowed the sequence of growth hormone to be deduced from the corresponding cDNA nucleotide sequence (Seeburg et al., 1977; Miller et al., 1980), and then the expression of cloned gene in *E. coli* was successfully accomplished (i.e. Goeddel et al., 1979; Keshet et al., 1981; Seikine et al., 1985). These technical development allowed us to solve the difficulties mentioned before.

In recent years, many studies has demonstrated the efficacy of recombinant growth hormone or its analogs on growth acceleration of various species of fish. For examples, Gill et al. (1985) have shown that recombinant bovine somatotropin (rBST), a single polypeptide chain of 161 amino acids, is effective in accelerating the growth of juvenile coho salmon. Schulte et al (1989) also reported that the ability of a recombinant bovine growth hormone analogue to accelerate growth in rainbow trout using a method of administering the growth hormone analogue by a repeated bath-immersion technique without the use of osmotic shock. Meanwhile, Agallon et al. (1988) have successfully produced recombinant salmon growth hormone and it can enhance growth relative to immersed groups when administered to rainbow trout by an immersion treatment after an osmotic shock. However, these studies have concentrated on the cold-water fishes such as salmons and trouts, but very few on the marine finfish.

More recently, some studies on the efficacy of growth hormone on the growth of marine finfish were attempted. Ishioka et al. (1992) have shown that injection of the recombinant bovine growth hormone of red seabream (*Pagrus major*) is effective in accelerating their juvenile growth. Cavari et al. (1993) reported that 15% growth acceleration was observed in only bovine growth hormone, when either human, bovine, piscine or chicken growth hormone was injected to gilthead seabream (*Sparus auratus*).

In this study, we evaluated growth effects of rBST administration with different treatment frequency and dosage of hormone on the oliver flounder, *Paralichthys olivaceus*, which is the most important marine fish species for the mariculture in Korea.

Materials and Methods

Eight thousand individuals of fingerlings of oliver flounder, ranging 5 to 8 g in body weight, were obtained from a private fish farm located at the northern part of Cheju Island on March 29, 1996. These fingerlings were transferred to the indoor tanks, round-shaped and fiber reinforced plastic (FRP), facilitated at the Marine Research Institute of Cheju National University. Primary cultivation was conducted to let fingerlings adopt to a new tank system and then fingerlings were divided into three different treatment groups (A, B and C) based on dosage and administration frequency of the hormone as well as one control (D). Main cultivation for this experiment had been continued from April 14, 1996 to March 16, 1997. The amount of natural seawater for each tank were 4.25 tons and circulation rate was maintained to be 8 to 10 times per day.

Primary cultivation

Primary cultivation for adaptation of the fingerlings were performed from March 29, 1996 to April 14, 1996. During this period, some individuals showing deformed shape or extraordinary size were selected and removed from the tanks. Fish was fed with the extruded pellet (EWOS Ind.; 3 mm in grain size) at 10:00 AM, 02:00 and 06:00 PM everyday.

Experimental cultivation

After primary cultivation, "the first stage of culture" were performed with the fingerlings, 7.01±0.91 g in body weight, for 12 weeks (from April 14 to July 7 1996). Those were divided into three treatment groups and one control group, and each group had two replications. One thousand individuals were accommodated for each experimental group.

On July 8 1996, 300 individuals were randomly selected and reared for each experimental group until the end of this experiment in order to avoid growth retardation due to overcrowding in the tank. This period was called "the second stage of culture." The experimental groups were designated exactly same as mentioned before.

Fish was fed sufficiently with the extruded pellet (EP, EWOS Ind.) at 10 : 00 AM, 02:00 and 06:00 PM everyday and pellet size has been changed while fish was growing. From October 4, EP was replaced with semi-moist pellet due to feed shortage. The proximate feed composition is shown in Table 1.

Administration of rBST

The powder-type of drug used was formulated with three components such as recombinant bovine somatotropin, polyacrylate and D-mannitol (Table 2). Three different treatment groups (A, B and C) and one control (D) were designated based on the duration and frequency of hormone administration. The period of treatment was largely divided into three different sequential stages based on their body weight. As shown in Table 3, the first trial of rBST administration was attempted against the fish (about 5 g in body weight) belonging to the A, B and C treatment groups; the second trial against the fish (20-30 g in body weight) belonging to the B and C treatment groups; the third trial against the fish (about 100 g in body weight) belonging to only C treatment groups. The dosage of rBST was 20 mg per kg-body weight, which gave the greatest growth acceleration reported by Hoe et al. (1996). The feed containing rBST was prepared by the following procedure: 200 mg of the powder including rBST was dissolved into 2 ml of distilled water; the dissolved solution was soaked into 8 g of EP; and left it at room temperature for 10

Table 1. Proximate composition analyzed with the EWOS feed used in this

Components	Extruded pellet	Semi-moist pellet
Moist	6.3	10.0
Crude protein	40.4	35.0
Crude fat	19.9	7.0
Crude fiber		5.0
Ash	9.3	17.0
Ca		1.2
P		1.8
Carbohydrate	24.1	20.0

Table 2. The formulation of test drug including recombinant bovine somatotropin used in this study

Ingredients	Content (%)
Bovine somatotropin	10
Polyacrylate	10
D-mannitol	80

Table 3. Experimental schedule for the administration and dose of rBST

First trial				Second trial				Third trial			
No. of administration	Treatment date	Treatment group	Dose (Unit : g)	No. of administration	Treatment date	Treatment group	Dose (Unit : g)	No. of administration	Treatment date	Treatment group	Dose (Unit : g)
First	Apr. 19	A1	1.362	First	June 10			First	July 15		
		A2	1.398								
		B1	1.391			B1	5.524				
		B2	1.339			B2	5.254				
		C1	1.367			C1	5.335			C1	4.130
		C2	1.361			C2	5.854			C2	3.918
Second	Apr. 26	A1	1.778	Second	June 17			Second	July 22		
		A2	1.774								
		B1	1.766			B1	5.431				
		B2	1.848			B2	5.193				
		C1	1.942			C1	5.268			C1	5.263
		C2	1.848			C2	5.774			C2	4.900
Third	May 3	A1	2.201	Third	June 24			Third	July 29		
		A2	2.283								
		B1	2.307			B1	7.789				
		B2	2.267			B2	8.846				
		C1	2.295			C1	7.245			C1	5.840
		C2	2.232			C2	8.062			C2	5.651
Fourth	May 10	A1	2.741	Fourth	July 1			Fourth	August 5		
		A2	2.769								
		B1	2.693			B1	7.620				
		B2	2.699			B2	8.720				
		C1	2.735			C1	7.119			C1	6.467
		C2	2.770			C2	7.717			C2	6.134

minutes. It was fed at 10:00 AM prior to feeding time of the EP without rBST, and for each trial four times of rBST adminstration were performed with one-week intervals. The time schedule and amount of rBST administration are shown in Table 3.

Culture conditions and measurements for growth evaluation

Several environmental factors within culture tank such as water temperature, dissolved oxygen, pH and salinity were measured at 10 : 00 AM everyday. Total length and body weight were measured with 200 individuals per tank every four weeks until the fourth measurement (July 7, 1996) and later the number of individuals measured was reduced to 50 individuals per tank using top loading balance and measurement plate whose accuracies were 0.1 g and 1 mm, respectively.

Statistical analysis

The data obtained from each experimental group were analyzed statistically with regard to growth, daily growth rate, condition factor, feed coefficiency and survival rate. Condition factor was calculated using the following equation, CF = $(W/L^{3.038})$, where W indicates body weight (g); L, total length (cm); an exponent of 3.038 were from the slope of linear regression line between logarithmic value of total length and body weight (Down et al.,1988). The daily growth rate (DGR) by percentage was estimated by using the following method of Yoon (1994). $\%DGR = [(W_t/W_o)^{1/t} - 1] \times 100$, where t: duration of experiment (day); W_o: body weight at time t = 0; W_t: body weight at time t. Statistical analyses were performed by using the SAS software. ANOVA-test was performed to know whether the difference in data obtained from different experimental groups was statistically significant at 95% confidence level, whereas the Duncan test (Duncan, 1955) was used for multiple comparisons between all possible pairs of means.

Results

Culture conditions

Water temperatures of culture tanks ranged from 10.8 to 25.4℃, showing highest in August and lowest in December and February, whereas salinity measured was between 27.5 and 35.0‰ during the experiment, and it dropped to 27.5‰ in August and 29.1‰ in September because of the flood of the Yangtz River (Fig. 1). On the other hand, the range of DO and pH were 5.5-8.15 mg/ℓ, and 7.58-8.49, respectively (Fig. 2).

Growth

The growth of oliver flounder fingerlings treated with rBST is shown in Fig. 3 and Fig. 4. Growth acceleration was observed from all treatment groups when compared to the control (P<0.05). At the beginning of the experiment, there was no significant differences in both total

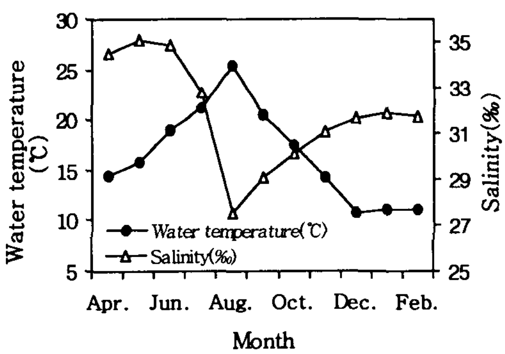

Fig. 1. Water temperature and salinity of rearing water during the study period.

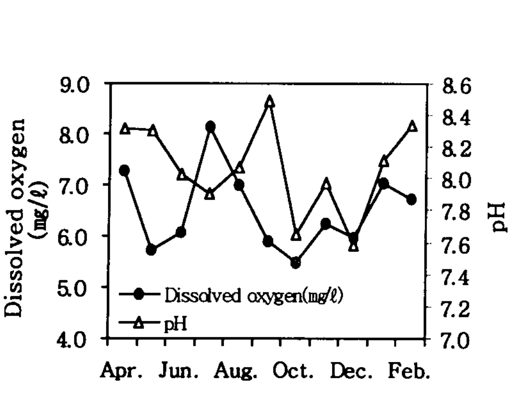

Fig. 2. Dissolved oxygen and pH of rearing water during the study period.

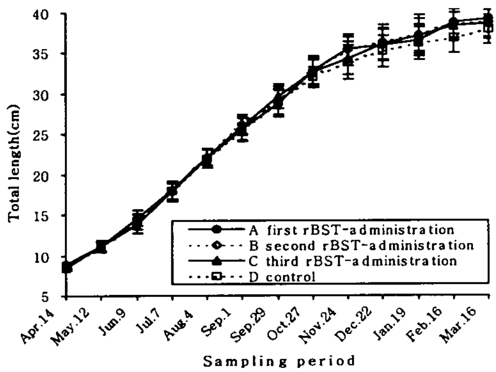

Fig. 3. Effect of rBST on the total length of flounders during the experimental period.

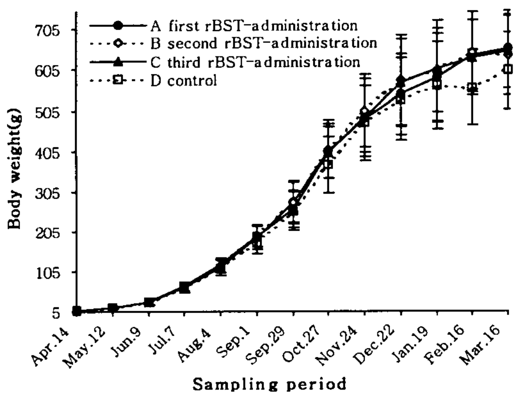

Fig. 4. Effect of rBST on the body weight of flounder during the experimental period.

length (F=0.91; df=7; P>0.05) and body weight (F=0.35; df=7; P>0.05) between experimental groups, showing 8.84±0.329 cm, 7.05±0.793 g in A; 8.66±0.421 cm, 6.96±0.847 g in B; 8.47±0.330 cm, 6.97±0.902 g in C; 8.74±0.442 cm, 7.05±1.067 g in D. However, when the experiment was finished, there was significant differences in both total length (F=12.22; df=7; P<0.05) and body weight (F=17.99; df=7; P<0.05) between experimental groups. Total length and body weight came to be 39.23±2.501 cm, 655.87±95.419 g in A treatment group, 38.79±1.763 cm, 664.292±98.724 cm in B, 38.72±1.653 cm, 650.97±88.951 g in C and 37.93±1.771 cm, 603.54±94.444 g in D (control group). Thus, the fish from treatment group grew 7.86-10.07% (47.4-60.8 g) more in their body weight than the control (P<0.05) and there was no significant difference in their body weight between treatment groups (see Table 4 for more detail). The growth difference between treatment and control group began to be recognized four weeks after completion of the first trial including four rBST administrations (from June 9, 1996) and became greater during the experiment (P<0.05). Meanwhile, group B showed the greatest growth acceleration among three treatment groups even if the differences among them were not statistically significant.

Daily growth and feeding rates

The daily growth rates estimated were similar among all experimental groups although slightly higher rates were recognized at treatment groups rather than at the control after finishing all rBST treatments (Table 4). In detail, the mean daily growth rates were 1.37% in B; 1.36% in A and C; and 1.33% in D (control). When considering water temperature, there were not much

Table 4. Survival rate, condition factor and mean daily growth rate of flounder treated differently administration frequency of rBST at end of experiment*

Exp. No.	Initial growth (mean±SD)		Final growth (mean±SD)		Survival rate (%)		Condition[1] factor	Mean[2] daily growth rate(%)
	TL (cm)	BW (g)	TL (cm)	BW (g)	Apr. 14 ~Jul. 7	Jul. 8~ Mar. 16		
A	8.84±0.329[a]	7.05±0.793[a]	39.23±2.501[a]	655.87±95.419[a]	98.93	92.67	0.98±0.11[a]	1.36
B	8.66±0.421[a]	6.96±0.847[a]	38.79±1.763[a]	664.29±98.724[a]	98.42	81.33	0.99±0.11[a]	1.37
C	8.47±0.330[a]	6.97±0.902[a]	38.72±1.653[a]	650.97±88.951[a]	97.70	76.67	0.99±0.11[a]	1.36
D	8.74±0.442[a]	7.05±1.067[a]	37.93±1.771[b]	603.54±94.444[b]	93.11	70.00	0.97±0.112[b]	1.33

*Values (mean of two replications) in the same column not sharing a common superscript are significantly different (P<0.05).
[1]$CF = (W/L^{3.038})$, [2]$[(W_t/W_o)^{1/t} - 1] \times 100$

differences in the mean daily growth rate among experimental groups during the period maintaining the relatively higher water temperature, but those of treatment groups were higher than the control during the period showing the relatively lower water temperature (Table 5).

During and after the period of rBST administration, there were not much differences in mean daily feeding rates among experimental groups, whereas the mean daily feeding rates estimated were higher at the control (1.19%) than treatment groups (Table 5).

Feed coefficiency

The feed coefficiencies were estimated to be from 0.54 to 3.24 during this experiment. In general, the values were sightly lower at treatment group rather than the control (Table 5). Among treatment groups, group B showed lowest feed coefficiency, which meaned that growth was better in B rather than other treatment groups (A or C). While the feed coefficiencies observed from treatment groups were 0.07 to 0.10 lower than that from the control during the period of doing rBST administration, after completion of rBST administration, those differences were reduced and so the feed coefficiencies from different experimental groups were similar each other during the period showing relatively high water temperature (14.25-25.39℃); during the period showing relatively high water temperature (10.80-14.25℃), the feed coefficiency from the control was estimated to be 1.69, which were 0.25 to 0.45 lower than treatment groups.

Condition factor

Through the experiment, large variations in condition factor were not observed at all experimental groups (Table 4). The condition factors estimated at the end of the experiment were 0.98±0.11 in A, 0.99±0.11 in B and 0.99±0.12, and they were not statistically different each other (P>0.05). However, the difference between treatment groups and the control (D, 0.97 ±0.11) turned out to be significantly different (P<0.05).

Survival rate

At the beginning of the experiment, one thousand individuals of fish were accommodated into each tank and then reduced to be 300 individuals later to avoid possible problems

Table 5. Feed coefficient, mean daily feeding rate, mean daily growth rate during the treatment of rBST period and post-treatment period in different treatment groups

	Exp. No.	Treatment period	Post-treatment period	
		Apr.14-Aug.4 (14.52-25.39℃)	Aug.5-Nov.24 (14.25~25.39℃)	Nov.25~Mar.16 (10.80~14.25℃)
Mean[1] daily feeding rate (%)	A	1.06	1.05	0.56
	B	1.04	1.04	0.56
	C	1.08	1.10	0.59
	D	1.19	1.11	0.59
Feed[2] coefficiency	A	0.65	0.90	1.24
	B	0.63	0.89	1.39
	C	0.66	0.96	1.44
	D	0.73	0.94	1.69
Mean[3] daily growth rate (%)	A	2.53	1.29	0.28
	B	2.57	1.29	0.25
	C	2.58	1.28	0.25
	D	2.51	1.29	0.21

[1][Feed intake(dry weight)×100] / [(Initial fish weight + Final fish weight)×day fed /2]
[2]Feed intake(dry weight)/Fish weight gain
[3]$[(W_t/W_o)^{1/t} - 1]\times 100$

due to overcrowding. The survival rates measured from each experimental group were shown in Table 4. During the first stage of fish culture (from April 14 to July 7 in 1996), the survival rates were 98.3% (A), 98.4% (B), 97.7% (C) and 93.1% (D), whereas those were 92.7% (A), 91.3% (B), 86.7% (C) and 80.0% (D) during the second stage of culture.

Discussion

About the discrepancy in response to growth hormone administration between large and small fish, Danzmann et al. (1990) reported that larger fish might have been more responsive to growth hormone stimulation. As a reason for it, they suggested that since growth rates were lower in larger fish, growth hormone administration might increase metabolic rates and subsequently accelerated growth rates. In this study, the daily mean growth rates of fish (5 g in mean body weight) prior to the second trial of rBST administration were 2.60% (A), 2.53% (B), 2.60% (C), and 2.48% (D), whereas those of fish (20-30 g in mean body weight) between the second and third trail of rBST adminstration were 2.88 % (A), 2.93% (B), 3.00% (C) and 2.85% (D). However, those of fish (100 g in mean body weight) reared for one month after the third trial of rBST administration were 1.89% (A), 2.01% (B), 1.89% (C) and 1.84% (D). These results indicated that fish of 20 to 30 g revealed largest growth promoting effect and which were not proportional to the size of fish. The mechanism causing such a result was not understood but might result from species-specific response to rBST stimulation.

The efficacy of rBST on the growth of fish were investigated by several researchers. Cavari et al. (1993) reported that 15% growth acceleration was observed when bovine growth hormone was injected to gilthead seabream

(*Sparus auratus*). Hoe et al. (1996) observed that rBST administration had promoting effect in the growth of oliver flounder. The results of this study showed that when compared with the control, the fish of treatment groups grew 7.86 to 10.07% (47.45 to 60.75 g in weight) better than the control group. However, no significant differences in their growth were not detected among treatment groups. With rainbow trout, eel, oliver flounder and Israeli carp, Hoe et al. (1996) also found that recognition of the first significant differences in body weight between treatment groups of rBST and the control took 1-2 weeks in both rainbow trout and eel, and 3-4 weeks in both Israeli carp and oliver flounder after first administration of rBST. This study showed that the significant growth improvement was revealed four weeks later after completion of the first four administration of the hormone.

Komourdjian et al. (1976) accelerated growth in hypophysectomised rainbow trout with porcine growth hormone but significant increase of condition factor (CF) was not detected, whereas a significant decrease in CFs had been recorded in coho salmon after bovine growth hormone administration (Higgs et al., 1975, 1976, 1977, 1978; Markert et al., 1977; Gill et al., 1985; Down et al., 1988, 1989). Significant reduction in CF also observed from rainbow trout treated with rBST (Danzmann et al., 1990). On the other hand, Agellon et al. (1988) reported that CFs increased in rainbow trout after the administration of rat growth hormone. Higgs et al. (1978) also reported that chinook salmon pituitary extracts enhanced CFs in coho salmon. This effect may have been due to other hormones or releasing factors present in the extract which could have had a stimulatory role on weigh increase (Danzmann et al., 1990). In this study, no significant increase or decrease in CFs were not detected throughout the course of the experiment, which was similar to the result reported by Komourdjian et al. (1976). As mentioned previously, there had been some contradictions about the effects of growth hormone on CFs so far, and thus more detail studies were needed in the future.

Each species exhibited a range of optimal temperature for growth when sufficient food were provided and thus fish growth would be depressed at temperatures above or below such temperature regimes. Danzmann et al. (1990) demonstrated that bovine growth hormone and rat growth hormone had limited growth promoting ability in rainbow trout reared at elevated temperature, compared to results obtained at equivalent dosages of hormone when fish were reared in colder water. At the optimal temperature for maximum growth of fish, growth hormone administration might have little effect on growth rates but could alter other physiological processes in the fish because of a reallocation of mobilized energy reserves. It would appear that in rainbow trout reared at a high temperature, growth hormone administration influences gonadal and other metabolic functions to be greater than growth rates. Thus, largest growth promoting effect was obtained at the lower temperature than a range of temperature for maximum growth. This study showed that the daily growth rate, feed coefficiency and daily feeding rate were greater at all treatment groups than the control during the period of showing relatively lower temperature (10.80-14.25℃), but no significant differences in them were not detected during the period of showing relatively higher temperature (14.25-

25.39℃).

When the acute toxicity of rBST had been examined with oliver flounder, median lethal concentration (48-h LC_{50}) was greater than 2,000 ppm and no adult fish were dead with less than 1,000 ppm rBST (Heo et al., 1996). The survival rates tended to be decreased in this study, accompanied by increasing the number of rBST administration. This result might have nothing to do with the number of administration, but the accumulation of stress caused by handling for measurement or by re-allocation to avoid overcrowding problems was likely to be a reason.

Acknowledgements

This study was supported by the research grants from LG Chemical Co. We would like to thank all people who were connected with this research project.

References

Agellon, L. B., C. J. Emery, J. M. Jones, S. L. Davies, A. D. Dingle, and T. T. Chen, 1988. Promotion of rapid growth of rainbow trout (*Salmo gairdneri*) by a recombinant fish growth hormone. Can. J. Fish. Aquat. Sci., 45 : 146-151.

Cavari, B., B. Funkenstein, T. T. Chen, L. I. Gonzalez-Villasenor and M. Schartl, 1993. Effect of growth hormone on the growth rate of the gilthead seabream (*Sparus aurata*), and use of different constructs for the production of transgenic fish. Aquaculture, 111 : 189-197.

Danzmann, R. G., G. J. Van Der Kraak, T. T. Chen and D. A. Powers, 1990. Metabolic effects of bovine growth hormone and genetically engineered rainbow trout growth hormone in rainbow trout (*Oncorhynchus mykiss*) reared at a high temperature. Can. J. Fish. Aquat. Sci., 47 : 1292-1301.

Down, N. E., E. M. Donaldson, H. M. Dye, K. Langley and L. M. Souza, 1988. Recombinant bovine somatotropin more than doubles the growth rate of coho salmon (*Oncorhynchus kisutch*) acclimated to seawater and ambient winter conditions. Aquaculture, 68 : 141-155.

Down, N. E., E. M. Donaldson, H. M. Dye, T. C. Boon, K. E. Langley and L.M. Souza, 1989. A potent analoge of recombinant bovine somatotropin accelerates growth in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci., 46 : 178-183.

Duncan, D. B., 1955. Multiple range and multiple *F* tests. Biometrics, 11 : 1-42.

Evans, H. M. and M. E. Simpson, 1931. Hormones of the anterior hypophysis. Amer. J. Physiol, 98 : 511-546.

Gill, J. A., J. P. Sumpter, E. M. Donaldson, H. M. Dye, L. Souza, T. Berg, J. Wypych and K. Langley, 1985. Recombinant chicken and bovine growth hormones accelerate growth in aquacultured juvenile Pacific salmon (*Oncorhynchus kisutch*). Bio/technology, 3 : 643-646.

Goeddel, D. V., H. L. Heyneker, T. Hozumi, R. Arentzen, K. Itakura, D. G. Yansura, M. J. Ross, G. Miozzari, R. Crea and P. H. Seeberg, 1979. Direct expression in *Escherichia coli* of a DNA sequence coding for human growth hormone. Nature, 281 : 544-548.

Heo, G. J., B. S. Chang, H. S. Jeh, H. K. Lee and Y. T. JI, 1996. A study of efficacy and toxicity of recombinant bovine somatotropin (BST) in cultured fish. Kor. J. Vet. Publ. Hlth., 20 : 219-229 (in Korean).

Higgs, D.A., E. M. Donaldson and H. M. Dye, 1975. A preliminary investigation of the effects of bovine growth hormone on growth and muscle composition of coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol., 27 : 240-253.

Higgs, D. A., E. M. Donaldson and H. M. Dye, 1976. Influence of bovine growth hormone and L-thyroxine on growth, muscle composition and histological structure of gonads, thyroid, pancreas and pituitary of coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Can., 33 : 1585-1603.

Higgs, D. A., U. H. M. Fagerlund and J. R. McBride, 1977. Influence of combination of bovine growth hormone, 17-methyltestosterone and L-thyroxine on growth of yearling coho salmon (*Oncorhynchus kisutch*). Can. J. Zool., 55 : 1048-1056.

Higgs, D. A., E. M., Donaldson and J. R. McBribe, 1978. Evaluation of the potential for using a chinook salmon (*Oncorhynchu tshaurytscha*) pituitary extract versus bovine growth hormone to enhance the growth of coho salmon (*Oncorhynchus kisutch*). Can. J. Zool., 56 : 1226-1231.

Ishioka, H., R. Kosugi, K. Ouchi, A. Hara, T. Nagamatsu, S. Mihara and H. Ogai, 1992. Effects of recombination red sea bream growth hormone on growth of young red sea bream. Nippon Suisan Gakkaishi, 58 : 2335-2340.

Keshet, E., A. Rosner, Y. Bernstein, M. Gorecki and H. Aviv, 1981. Cloning of bovine growth hormone gene and its expression in bacteria. Nucleic Acid Res., 9 : 19-30.

Komourdjian, M. P., R. L. Saunders and J. C. Fenwick, 1976. The effect of porcine somatotropin on growth and survival in seawater of Atlantic salmon (*Salmo salar*) parr. Can. J. Zool., 54 : 531-535.

Leatherland, J. F. and R. N. Nuti, 1981. Effects of bovine growth hormone on plasma FFA concentration and liver, muscle and carcass lipid content in rainbow trout. J. Fish. Biol., 19 : 487-498.

Market, J. R., D. A. Higgs and H. M. Dye, 1977. Influence of bovine growth hormone on growth rate, appetite and food conversion of yearling coho salmon (*Oncorhynchus kisutch*) fed two diets of different composition. Can. J. Zool., 55 : 74-83.

Miller, W. L., J. A. Martial and J. D. Baxter, 1980. Molecular cloning of DNA complementaryto bovine growth hormone mRNA. J. Biol. Chem., 255 : 7521-7524.

Pickford, G. E., 1953. The response of hypophysectomized male *Fundulus* to injection of purified beef growth hormone. Bull. Bingham. Ocenog. Coll., 14 : 46-68.

Pickford, G. E., 1957. The growth hormone. In The Physiology of the Pituitary Gland (ed by G.E. Pickford and J. W. Atz). N.Y. Zool. Soc., New York. pp.84-99.

Pickford, G. E., 1959. The nature and physiology of the pituitary hormones of fishes. In Comparative Endocrinology (ed by A. Gorbman). Wiley, New York. pp. 404-420.

Pickford, G. E. and E. F. Thomson, 1948. The effect of purified mammalian growth hormone on the killifish *Fundulus heteroclitus* (Linn.). J. Exp. Zool., 109 : 367-383.

Schulte, N. E., P. M. Down, E. M. Donaldson and L. M. Souza, 1989. Experimental administration of recombinant bovine growth hormone to juvenile rainbow trout (*Salmo gairdneri*) by injection or by immersion. Aquaculture, 76 : 145-156.

Seeburg, P. H., J. Shine, J. A. Martial, J. D. Baxter and H.M. Goodman, 1977. Nucleotide sequence and amplification in bacteria of structural gene for rat growth hormone. Nature, 270 : 486-494.

Sekine, S., T. Mizukami, T. Nishi, Y. Kuwana, A. Saito, M. Sato, S. Itoh and H. Kawauchi, 1985. Cloning and expression of cDNA for salmon growth hormone in *Escherichia coli*. Proc. Natl. Acad. Sci. USA, 82 : 4306-4310.