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Promotion of growth in diploid and triploid coho salmon with parenteral delivery of a recombinant porcine somatotropin

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Abstract

The growth response of diploid ($2n$) and thermally-induced triploid ($3n$) coho salmon to injected recombinant porcine somatotropin was examined over a 10 week period. All treatments were undertaken using replicate groups of 25 animals per group. Control $2n$ and $3n$ replicate groups grew at identical rates throughout the period of study, although $3n$ fish exhibited lower condition factors. Recombinant porcine somatotropin treated $2n$ and $3n$ fish responded positively to therapy and, by week 4 of the trial, expressed significantly greater increases in both weight and length ($p<0.01$) when compared to controls. At the end of the study period, somatotropin treated animals had gained approximately twice the amount of weight of controls. Food conversion efficiencies were greater in treated salmon, regardless of ploidy state. This relationship also extended to weight and length specific growth rates. The economic benefits of commercial application of recombinant porcine somatotropin therapy to triploid Pacific salmon culture are discussed.

Keywords : Coho salmon, diploid, triploid, growth acceleration, recombinant porcine somatotropin, *Oncorhynchus kisutch.*

Augmentation de la croissance du saumon coho, diploïde et triploïde, par administration de somatotropine recombinée de porc à la génération parentale.

Résumé

L'effet de l'injection de somatotropine recombinée de porc sur la croissance de saumon coho diploïde ($2n$) et triploïde ($3n$), triploïdie obtenue par chocs thermiques, a été étudié sur une période de 10 semaines. Chaque traitement a été entrepris en utilisant un groupe réplicat formé de 25 animaux. Les réplicats des groupes de contrôle $2n$ et $3n$ ont des taux de croissance identiques tout au long de la période de l'étude, bien que les facteurs de condition des poissons triploïdes soient plus bas. La somatotroponine recombinée de porc a un effet positif sur les poissons $2n$ et $3n$ et, au cours de la 4e semaine de l'essai, les gains en poids et en longueur sont significativement plus importants ($p<0,001$) que ceux des groupes de contrôle. A la fin de période d'étude, les animaux traités à la somatotropine ont approximativement un gain de poids double de celui des poissons non traités. L'efficacité de la convertion de nourriture est plus grande chez les poissons traités, en regard de leur état de ploïdie. Cette relation s'étend également aux taux de croissance spécifiques en poids et en longueur. Les bénéfices économiques de l'application commerciale de cette thérapie à base de somatotropine recombinée de porc sont discutés à propos de l'élevage du saumon du Pacifique triploïde.

INTRODUCTION

The intensive culture of Pacific salmon (*Oncorhynchus* sp.) is currently considered a high risk business. While returns on investment (ROI) are comparatively rapid for egg/alevin and smolt producers, over 2 years must elapse before returns are realised from grow-out operations. ROI is influenced by consumer demand, the productivity of the traditional fisheries and by fluctuations in the price of fishmeal. Toxic algal blooms, serious outbreaks of disease, and human error may lead to total loss of investment capital in aquaculture. Methods of making the risks involved in intensive salmon culture more acceptable to potential investors thus deserve serious attention.

A reduction in the time required for the production of market-sized fish represents one method by which investment risk may be diminished and time to profit realisation decreased. While the potential for the genetic improvement of growth rates in teleosts is promising, heritability for body weight in salmonids is generally considered low (Gjerde, 1986). Thus genetic gain is gradual over a period of several generations. Somatotropin therapy represents an alternative or supplemental method by which growth may be maximized in salmonids.

Recombinant DNA technologies have provided a means by which large quantities of highly purified, potentially inexpensive somatotropins may be produced. To date, somatotropin therapy has been demonstrated to markedly accelerate growth and increase food conversion efficiencies in salmonids (e. g. Down *et al.*, 1988; McLean *et al.*, 1990). The application of this biotechnology to Pacific salmon culture would reduce the time-span required for fish to reach marketable size. Utilization of somatotropin therapy would also provide the option of growing larger and thus more valuable, salmon over a normal production cycle. Both scenarios would provide increased ROI and potentially attract greater capital investment in commercial culture operations.

An ability to sterilize cultured salmonids, by triploidization, represents another technology which offers an attractive proposition to the commercial producer. Sterilization is becoming increasingly important as a means of eliminating reproductive interaction between farmed and wild fish. As well, sterilization diverts metabolizable energy away from gamete production to somatic growth (Utter *et al.*, 1983). Sterile animals retain their "silver-bright" appearance; do not exhibit precocious maturation or decreased flesh quality associated with maturity; and achieve adult size (Small and Randall, 1989).

It has been reported that triploidization may adversely affect early growth rates in salmonids (Utter *et al.*, 1983; Solar *et al.*, 1984). Somatotropin therapy may maintain performance in sterile fish and thus produce an ideal culture system. The ultimate salmonid for aquaculture purposes would be a fish that grew rapidly, retained its silver-bright appearance and never matured sexually. To date, however, no studies have examined the effect of combining somatotropin and triploidization technologies as a means of regulating growth in fish. The following study compared the growth performance of diploid ($2n$) and triploid ($3n$) coho salmon *Oncorhynchus kisutch*, and the potentiating effect of somatotropin therapy upon growth and feed conversion efficiencies.

MATERIALS AND METHODS

Experimental animals

Diploid and triploid coho salmon *Oncorhynchus kisutch*, of the 1989 Capilano Salmon Hatchery (North Vancouver, B.C., Canada) broodyear, were used throughout experimentation. Triploidy was induced by thermal shock using the methods described in Teskeredzic *et al.* (1991), and later confirmed by propodium iodide flow cytometry of erythrocytes from a sub-sample of experimental animals. 100 each of $2n$ and $3n$ individuals (4.10 ± 0.41 g, 71.65 ± 2.44 mm) were randomly placed into eight 20 *l* aquaria ($n = 25$/aquarium). Each aquarium was serviced with a diffusion stone and degassed well water (10.25 ± 0.45°C). Following sorting, the $2n$ and $3n$ groups were randomly paired. Each paired $2n/3n$ group received either 2.5 µg recombinant porcine somatotropin (rpSt; Amgen, Thousand Oaks, CA, USA)/g body weight, or 2.5 µg bovine serum albumen (BSA; Sigma Chemical Co. Ltd., St. Louis, MO, USA)/g body wt. Proteins were dissolved in 0.9% saline and injected intraperitoneally once weekly in a volume of 50 µl. The dose of rpSt and BSA received by each individual was adjusted weekly to take account of gains in body weight. Animals were fed to satiation three times daily with 1/32" Oregon moist pellets (Moore-Clark, Laconner, WA, USA).

Analytical procedures

All fish were weighed and measured to the nearest 0.1 g and mm on a weekly basis at the time of treatment. Weight and length specific growth rates (SGR, %/day), were determined using the formula: $(\ln V_1 - \ln V_0)/(t_1 - t_0) \times 100$, where $\ln V_1$ and $\ln V_0$ are the natural logarithms of weight or length at the end (t_1) or start (t_0) of the time interval respectively (Higgs *et al.*, 1975). Food conversion efficiencies (FCE %) were calculated by dividing the total dry weight gain of fish by the dry weight of food consumed ($\times 100$) for each treatment group. Condition factor was assessed using the formula: CF = (weight/lenth^3) $\times 100$ (Weatherly and Gill, 1987). At the termination of the experiment the chemical composition of treated and control fish ($n = 10$/group and ploidy

state) were evaluated. Moisture content was determined after sample drying for 16 hrs. at 110°C. Samples were transfered to a desiccator until constant weights were attained. The lipid extraction technique of Bligh and Dyer (1959) was used to determine lipid content. Protein determinations were accomplished using the micro-Kjeldahl procedure using N × 6.25 (Steyermark, 1961). Ash content was evaluated following tissue incineration in a muffle furnace for 2 hrs. at 600°C. Carcass weights ("gills on") were measured following evisceration of ten individuals from each treatment group. Weight and length data, CF, SGRs and chemical composition from paired treatment groups, were compared by one-way ANOVA followed by Duncan's multiple range test of significance. Weight and length data were log transformed to meet assumptions of normality and homogeneity of variance. FCE could only be calculated on the mean results recorded for each duplicate tank of fish and thus there was insufficient data for a meaningful statistical comparison of the effect of treatments.

RESULTS

Propidium iodide flow cytometric analyses of a sub-sample of animals ($n = 10$) from triploid groups revealed a triploidization incidence of 90%. Starting weights, lengths and CF of the experimental replicate groups are summarised in *table* 1. There were no significant differences ($p \geqq 0.158$) in each of these parameters for either of the $2n$ or $3n$ groups, but $3n$ animals expressed lower CF ($p \leqq 0.05$) than their respective $2n$ groups. Four weeks after the first injection, those animals in receipt of rpSt therapy exhibited significant increases in weight ($p < 0.01$) and length ($p < 0.001$) when compared to control salmon (*fig.* 1 and 2). There were no between-group differences in wt or length for either replicates of $2n$ or $3n$ rpSt treated salmon. Similar results were attained for control, BSA injected fish replicates. These relationships were maintained until the completion of the trial, at which time $2n$ and $3n$ rpSt treated groups had attained 136% and 99.4% greater gain in weight when compared to their respective control groups (*fig.* 1). This relationship extended to gains in length, with $2n$ salmon returning 26.4% greater gains in length when compared to identical ploidy controls (*fig.* 2). Throughout the treatment period, CF increased in $2n$ rpSt treated animals, but remained significantly lower ($p \leqq 0.05$) than $2n$ BSA treated fish (*table* 1). CF for both $3n$ groups increased throughout the period of study and, by the termination of the trial $3n$ fish expressed identical CF to that of $2n$ rpSt injected fish (*table* 1).

Similar protein to lipid ratios were observed in both $2n$ and $3n$ BSA injected groups, but $3n$ rpSt treated fish expressed decreased whole body lipid content, together with increased whole body moisture when compared to the other groups (*table* 2). Percentage ash was similar for each ploidy state, but $2n$ animals exhibited higher ash content than $3n$ individuals ($p \leqq 0.05$).

Table 3 summarises final weights and carcass mass for each of the treatment groups. Mean carcass mass, when expressed as a percentage of total wet body weight revealed the following sequence: $3n$ treated

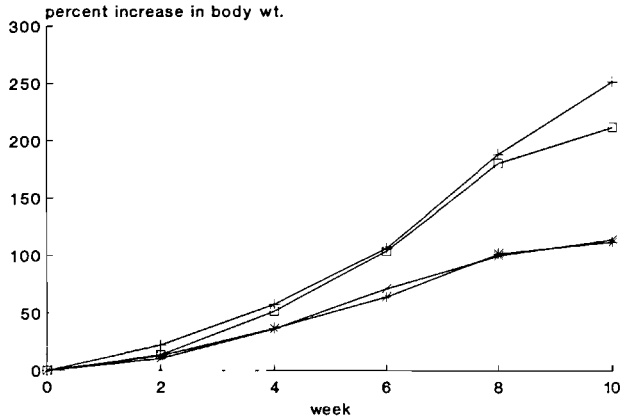

Figure 1. – Percent increase in body weight of coho salmon subjected to injections of 2.5 µg/g/wk rpSt therapy or 2.5 µg/g/wk BSA. Each data point represents the mean values of replicate tanks of 25 individuals (*i. e.* $n = 50$). + = $2n$, rpSt treated; $x = 2n$, BSA injected; □ = $3n$ rpSt therapy; ∗ = $3n$ BSA controls. For clarity, error bars are omitted. Significant ($p < 0.05$) differences in weight for rpSt treated $2n$ and $3n$ fish occurred by week 4 of the 10 week trial. No difference in weight was recorded between rpSt treated groups, or their respective controls.

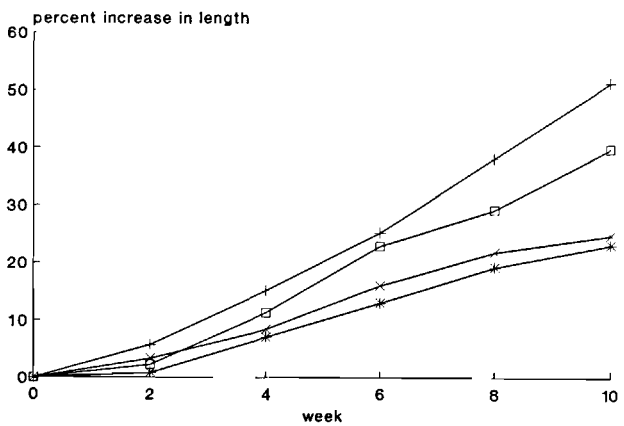

Figure 2. – Percent increase in body length of coho salmon subjected to injections of 0.5 µg/g/wk rpSt therapy or 2.5 µg/g/wk BSA. Each data point represents the mean values of replicate tanks of 25 individuals (*i. e.* $n = 50$). + = $2n$, rpSt treated; $x = 2n$, BSA injected; □ = $3n$ rpSt therapy; ∗ = $3n$ BSA controls. For clarity, error bars are omitted. Significant ($p < 0.05$) differences in length for rpSt treated $2n$ and $3n$ fish occurred by week 4 of the 10 week trial. No difference in length was recorded between rpSt treated groups, or their respective controls.

Table 1. – Initial and final weights, lengths and condition factors (CF) for diploid and triploid coho salmon, subjected to control and somatotropin therapies over a 10 week period. The data presented represents the mean ± S.D. of combined groups for each treatment (*i. e.* $n = 50$ group). Figures accompanied by an identical superscript were not significantly different ($p > 0.05$).

Group	Initial weight (g) ± SD	Initial length (mm) ± SD	Final weight (g) ± SD	Final length (mm) ± SD	Initial CF	Final CF
Control 2*n*	4.07 ± 0.36[a]	70.2 ± 2.35[a]	8.7 ± 1.58[a]	87.6 ± 4.8[a]	1.17 ± 0.10[a]	1.30 ± 0.23[a]
Treated 2*n*	4.07 ± 0.44[a]	70.3 ± 3.10[a]	14.3 ± 2.50[b]	106.3 ± 5.9[b]	1.17 ± 0.12[a]	1.19 ± 0.20[b]
Control 3*n*	4.16 ± 0.46[a]	73.1 ± 1.77[b]	8.8 ± 1.16[a]	90.0 ± 3.8[a]	1.06 ± 0.11[b]	1.20 ± 0.15[b]
Treated 3*n*	4.11 ± 0.37[a]	73.0 ± 2.55[b]	12.8 ± 2.48[b]	102.0 ± 5.8[b]	1.05 ± 0.09[b]	1.20 ± 0.23[b]

Table 2. – Chemical composition of diploid (2*n*) and triploid (3*n*) coho salmon subjected to ten weeks rpSt (2.5 µg/g/wk by injection) therapy, or control injection with BSA (2.5 µg/g wk). Results represent the means of replicate analyses of five fish from each treatment group. Figures in the same column accompanied by the same superscript were not significantly different ($p > 0.05$).

Group	Moisture	Protein	Lipid	Ash
Control 2*n*	72.34 ± 1.52[a]	16.39 ± 0.81[a]	9.42 ± 1.84[a]	2.59 ± 0.14[a]
Treated 2*n*	72.44 ± 1.06[a]	16.05 ± 1.12[a]	9.20 ± 1.82[a]	2.60 ± 0.18[a]
Control 3*n*	71.88 ± 1.83[a]	15.93 ± 1.19[a]	9.18 ± 1.09[a]	2.33 ± 0.23[b]
Treated 3*n*	75.09 ± 1.22[b]	16.18 ± 0.97[a]	7.10 ± 0.94[b]	2.30 ± 0.17[b]

Table 3. Mean final and carcass weights SGR and FCE of coho salmon following 10 weeks rpSt therapy (2.5 µg/g/wk) or sham treatment with BSA (2.5 µg/g/wk). Figures exhibiting the same superscript in a column were not significantly different ($p > 0.05$). Carcass and body weight data were derived from 10 individuals treatment group.

Group	Mean body wt	Mean carcass wt	Weight SGR	Length SGR	FCE (%)
Control 3*n*	7.8 ± 1.15[a]	6.64 ± 0.99[a]	1.08	0.31	21.24
Treated 2*n*	15.8 ± 3.41[b]	13.35 ± 2.81[b]	1.80	0.60	24.56
Control 3*n*	8.8 ± 1.33[a]	7.48 ± 1.28[a]	1.07	0.30	20.83
Treated 3*n*	12.1 ± 2.31[b]	10.59 ± 2.08[b]	1.62	0.49	24.12

$>2n$ untreated $>2n$ treated $>3n$ untreated. Food conversion efficiencies were highest in 2*n* treated salmon $>3n$ rpSt treated $>2n$ control $>3n$ control coho. Weight and length SGRs (%/day) were greater in treated *versus* control animals (*table* 3).

DISCUSSION

The present study represents the first documentation of growth acceleration in a triploid teleost. These investigations also provide the primary account of the use of a rpSt to accelerate growth in fish. The results presented are consistent with those of others which employed recombinant avian and bovine (rbSt) somatotropins with 2*n* Pacific anadromous salmon (e. g. Gill *et al.*, 1985; McLean *et al.*, 1990). In these and the present studies, somatotropin therapy markedly increased SGRs and FCEs. However, significant growth acceleration, both in terms of weight and length in rpSt treated fish did not accrue until week 4 of the trial. This observation contrasts with many more recent studies on salmonids, wherein significant effects upon growth were recorded within 2 weeks of primary therapy (e. g. Gill *et al.*, 1985; Down *et al.*, 1988, 1989; McLean *et al.*, 1990). It is possible that this difference in responsiveness may have arisen due to changes in husbandry or reflect a decreasing stress during handling, over time.

Unlike the observations of Utter *et al.* (1983), who reported decreased growth in 3*n* coho salmon when compared to 2*n* animals, both control and treated 2*n* and 3*n* fish employed in the present study, grew at similar rates throughout the 10 weeks of investigation. One explanation for this apparent difference might be that in Utter *et al.*'s experiments, 2*n* and 3*n* salmon were reared in polyculture; and that 2*n* individuals were more agressive feeders than their 3*n* counterparts. Such observations have been recorded for 2*n* and 3*n* grasscarp *Ctenopharyngodon idella* (Cassani and Caton, 1986). In these experiments, grasscarp reared *en masse* as monocultures grew at similar rates; but when reared in polyculture, 3*n* fish grew less rapidly than 2*n* animals. Similar findings have recently been presented for coho salmon (Johnson *et al.*, 1986).

In the current study, CFs of 3*n* coho salmon were lower than 2*n* animals at the start of the experimental

period. While these observations contrast to those of Johnson *et al.* (1986), this parameter would be expected to differ, since the 50% increase in chromosome number of $3n$ individuals results in a proportionate increase in cell size to maintain the nucleocytoplasmic ratio (Small and Randall, 1989). Thus, proliferating chondrocytes would exhibit an approximate 30% increase in size, which might be expected to result in an overall longer fish. CFs increased steadily throughout the study, for all groups, until the termination of the trial. At this point, the CF of $3n$ groups matched that observed in $2n$ treated fish, but still remained significantly lower than $2n$ controls. In other trials rbSt therapy of coho salmon resulted in a decline in CF (Gill *et al.*, 1985). However, increased CFs have been observed in common carp *Cyprinus carpio*, following natural bSt therapy (Adelman, 1977); while administration of recombinant human somatotropin to brook trout *Salvelinus fontinalis* is reportedly without effect (Skyrud *et al.*, 1989). It is possible that increased CFs observed during the present study occurred as a result of decreased crowding, increased feeding and other alterations to husbandry rather than a direct effect of somatotropin therapy. However, it should be noted that, relative to control BSA treated fish, rpSt injected animals displayed lower CFs, an observation consistent with other studies (Gill *et al.*, 1985; Down *et al.*, 1989; McLean *et al.*, 1990).

Feed conversion efficiencies for rpSt treated animals were greater than observed in BSA treated controls. A positive correlation between FCE and weight and length SGRs was apparent. Both increased FCEs and SGRs have been recorded previously in somatotropin treated Pacific salmonids, and the array of potential physiological mechanisms responsible for improved performance are detailed in Markert *et al.* (1977). One such mechanism which might underlie improved growth performance is the mobilization of lipids, which may represent a partial explanation for the altered protein:lipid ratios observed in the $3n$ treated fish.

The present study, together with others which employed larger sized individuals (e. g. Down *et al.*, 1988, 1989; McLean *et al.*, 1990) demonstrate that parenteral administration of mammalian somatotropins to Pacific salmonids would clearly provide a significant advantage to the salmon culture industry. Foremost, food conversion efficiencies are increased and growth rates accelerated. These benefits may be translated into increased ROI since international fluctuations in the market value for fishmeal would be buffered to a certain extent by the increased efficiency of feed conversion. Second, market-sized fish may be produced more rapidly and be available out-of-season, which would result in higher market value and hence increased profitability of operation. Third, individual fresh fish of large size could be produced, which command higher prices per unit of weight, over one production cycle. Fourth, combined with the triploidization process, growth accelerated individuals could be retained on-site until market values were optimal, and hence maximization of ROI could be achieved. However, prior to applying rpSt therapy to the commercial culture of Pacific salmonids, more appropriate methods of administration must be developed and evaluated. It is evident that somatotropins gain access to the circulation in an immunologically (McLean *et al.*, 1988; McLean and Donaldson, 1990) and biologically (McLean *et al.*, 1990; Moriyama *et al.*, 1990) active form, following oral administration. While oral delivery techniques currently require that $\geq$10-fold more protein be delivered to each individual to attain a response similar to that observed in injected fish, the application of protection technologies, as used by McLean and Ash (1990) and Solar *et al.* (1990) demonstrate the feasibility of this route of somatotropin delivery. Future studies will address the long-term effects of sustained and intermittent somatotropin therapy upon salmon growth in an attempt to evaluate possible down regulation in tissue responsiveness to sustained therapy.

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