

**IMPROVEMENT OF TROUT GROWTH USING A BY-PRODUCT  
FROM THE COMMERCIAL MANUFACTURE OF GH**

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**Abstract**

Trout were injected ( $1.5 \mu\text{g g BW}^{-1} \text{wk}^{-1}$ ), or fed on diets supplemented with (10 and  $100 \mu\text{g g BW}^{-1} \text{wk}^{-1}$ ), a by-product (GHby) from the industrial production of rbGH. The impact of treatments was evaluated using control (injected and fed) and rbGH injected ( $1.5 \mu\text{g g BW}^{-1} \text{wk}^{-1}$ ) groups. Fish receiving GHby parenterally returned higher growth rates ( $P < 0.05$ ) than control animals and matched the performance of animals receiving pure rbGH. Trout presented with the highest feed-based dose of GHby were heavier than control groups ( $P < 0.05$ ). FCEs were higher in all GH treated groups when compared to controls fish. Dietary supplementation with GHby did not effect skeletal form or fillet yields or dressout percentage ( $P > 0.05$ ).

**Introduction**

Adoption of growth factor (GF) technologies by the aquaculture industry would provide a variety of benefits for the producer, processor and consumer (review: McLean and Devlin, 2000). A major technological impediment to the use of growth-regulating peptides however, is the lack of appropriate delivery systems. Clearly, incorporation of GFs into feeds represents the ideal route of administration, since oral formulations eliminate the need for physical manipulations (capture, handling, injection and otherwise) and thus reduce stress. Moreover, oral delivery systems are labour and time efficient and, relative to other methods of drug administration, safe. Success in conveying GFs

to fish, using the oral approach, was initially inferred in studies during the first quarter of the last century (review: McLean *et al.*, 1999). Since that time, and specifically over the last decade, many advances have been made in oral drug delivery technologies and some of these new methods have been evaluated in the delivery of the growth hormone (GH) to cultured teleosts (see Schep *et al.*, 1999).

Commercial manufacture and agroindustry adoption of recombinant bovine GH (rbGH) as a lactogenic agent commenced during the 1980s. During industrial production, the level of purity of rbGH is determined using isoelectric focusing. When the purity of the product falls below 95 % it is considered unsuitable for distribution. Each year in excess of 20 tons of GH by-product (GHby) are produced. At present, even though rat and mouse tibia bioassays illustrate an  $\geq 80$  % retention of activity, GHby is bioremediated.

Clearly, methods for utilising industrial by-product gainfully deserve serious attention. Accordingly, the present study examined whether GHby could be employed as an ingredient for trout aquafeed. High concentrations of GHby were employed to counteract its possible degradation by the gastrointestinal tract. The effect of adding GHby to trout feeds was examined by evaluating growth rates, feed conversion efficiencies (FCE), and various processing impacts upon treated animals.

## **Materials & Methods**

### *Animals & Husbandry*

Rainbow trout ( $n = 300$ ; mean wt:  $32.8 \pm 0.15$  g and length:  $138.2 \pm 0.24$  mm) were anaesthetised (0.004 % benzocaine) and each implanted with a PIT tag. Fish were randomly placed into one of 12 tanks (1.0x1.0x0.6 m;  $n = 25$ /tank), and left to acclimate for 1 wk. Tanks were supplied with non-chlorinated municipal water ( $O_2$ :  $9.0 \pm 0.15$  mg/L; temp. range: 7.9-11.3°C; photoperiod 12L:12D).

### *Experimental Treatments*

Tanks were randomly assigned to one of six treatments (each in duplicate). These included: injected control (double distilled water); rbGH injected ( $1.5 \mu\text{g g BW}^{-1} \text{wk}^{-1}$ ); GHby injected ( $1.5 \mu\text{g g BW}^{-1} \text{wk}^{-1}$ ) and three diets, two of which

contained GHby calculated to deliver doses of 10 and 100  $\mu\text{g g BW}^{-1} \text{wk}^{-1}$ . Injected volumes were 200  $\mu\text{l}$ . The control diet was void of GH. Feed pellets were produced from a 3.2-mm non-extruded pellet normally used for the commercial production of ECOLife 23 (BioMar A/S, Denmark). GHby was dissolved in ion-exchanged water producing solutions of 0.01 (10  $\mu\text{g g BW}^{-1} \text{wk}^{-1}$ ) and 0.1 g GHby  $\text{mL}^{-1}$  (100  $\mu\text{g g BW}^{-1} \text{wk}^{-1}$ ). Pellets were sprayed with 14.28 mL GHby solution or ion-exchanged water per kg extruded pellet in a cement mixer (45 rpm, 8 min) before lipid addition, which encapsulated the GHby within the pellets. To obtain the identical lipid level in experimental diets to that of the commercial control, 196 g lipid was added per kg extruded pellets. Lipid was pre-warmed to 30-35°C to enhance absorption and added to the pellets using a vacuum-coater. All diets were stored at -18 °C until use. Throughout the study, all fish were hand-fed to satiation twice daily.

#### *Analytical Procedures*

Fish were weighed and measured every 2 wk for 10 wk. Specific growth rates (%/d), condition factor (*k*) dressout percentage, somatic indices for gut, liver, heart and gonads were recorded and calculated as described previously (Ronsholdt et al., 2000). Fillet yield, proximate analyses and feed conversion efficiencies (FCE) were assessed using the methods presented in Rasmussen et al. (2000). Bimonthly x-ray images (Siemens Polymobil III Rx; using 4 mAs and 52 kV on Mamoray MR5-2 AGFA film) were taken from rbGH injected, 100  $\mu\text{g g BW}^{-1} \text{wk}^{-1}$  GHby fed and control groups ( $n = 8/\text{treatment}$ ) to examine the effect of treatment upon bone growth. Every fifth vertebra of the column was examined with respect to rectangular and horizontal length using CorelDRAW (Version 6.00) software. The size of each vertebra was compared for identical positions along the backbone. Vertebrae were measured as depicted in Figure 1, and each value was compared to the length of the fish.

#### *Data Analyses*

Statistical analyses of growth performance, somatic indices, chemical composition, and morphology were performed using a two-factor mixed factorial nested design (Montgomery, 1997). Prior to using analyses of variance (ANOVA), each data set was tested for normality and for equal variance using Kolmogorov-Smirnov's and Bartlett's tests respectively. Treatment factors were considered fixed factors and fish tank a random factor. The factor, tank, was nested within the treatment factor. The experiment was conducted in duplicate for each treatment. Based upon these factors a model describing the experiment

was built, where  $y_{ijk}$  represented observed data (Montgomery, 1997). Significant differences between treatments identified by ANOVA were further subjected to Duncan's multiple range test to determine which means differed (Montgomery, 1997).

The model applied was:

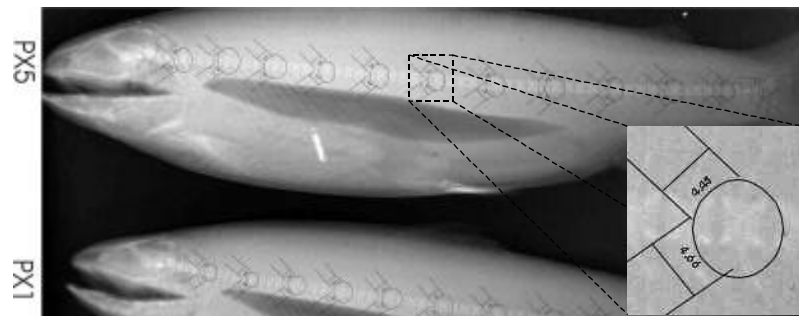
$$y_{ijk} = \mu + A_i + T(A)_{j(i)} + \varepsilon_{(ij)k}$$

Figure 1. X-ray of trout illustrating the method employed in measuring vertebrae of experimental animals.

where  $\mu$  was the true mean,  $A_i$  the treatment effect (fixed effect),  $T(A)_{k(i)}$  the tank effect nested within a fixed treatment effect (random effect), and  $\varepsilon_{(ij)k}$  the residual (random effect).

## Results

The weight and length growth response of experimental groups is summarized in Table 1. No differences were detected in start weights or lengths between groups. By trial termination however, all GH injected fish returned significantly



( $P < 0.05$ ) greater weight and length increases than either control groups or GHby fed trout (Table 1). Trout receiving high dose GHby aquafeed were significantly ( $P < 0.05$ ) heavier and longer than control fish at wk 10. Low-dose GHby fed fish however, while similar in weight and length to high dose GHby groups, did not differ to either control group ( $P > 0.05$ ; Table 1). Condition factors were identical for all groups at trial start and end (final range  $1.42 \pm 0.1$  to  $1.46 \pm 0.02$ ).

Group length and weight SGRs and FCEs are presented in Table 2. GH injected groups expressed higher weight and length SGRs ( $P < 0.05$ ) than all other groups. At trial termination, differences ( $P < 0.05$ ) were also detected in weight SGR between the high dose GHby and control groups (Table 2). Irrespective of treatment, FCE increased throughout the trial (data not shown). Overall, GH injected fish returned lowest FCE < GHby < controls (Table 2).

Table 1. Weight and length data for control and treatment groups at trial start and termination, 10 weeks later. Different superscripts indicate significant differences ( $P < 0.05$ ), column-wise. All values are presented with 95% confidence intervals.

Treatment	Start wt.	Final wt.	Start length	Final length
Control	32.9±0.55 <sup>a</sup>	131.1±5.78 <sup>ac</sup>	137.7±0.72 <sup>a</sup>	209.1±2.83 <sup>ac</sup>
Control inj.	32.6±0.40 <sup>a</sup>	127.2±5.27 <sup>c</sup>	138.5±0.68 <sup>a</sup>	208.6±2.53 <sup>c</sup>
GHby10	32.9±0.47 <sup>a</sup>	137.0±6.19 <sup>ab</sup>	138.4±0.78 <sup>a</sup>	212.8±2.91 <sup>ab</sup>
GHby100	32.6±0.68 <sup>a</sup>	140.3±8.60 <sup>b</sup>	139.7±1.50 <sup>a</sup>	215.1±4.26 <sup>b</sup>
GHby inj.	32.9±0.49 <sup>a</sup>	151.5±5.95 <sup>d</sup>	137.8±0.75 <sup>a</sup>	220.5±2.35 <sup>d</sup>
rbGH inj.	33.1±0.43 <sup>a</sup>	157.8±7.35 <sup>d</sup>	138.1±0.67 <sup>a</sup>	222.1±3.12 <sup>d</sup>

Table 2. Specific growth rate (SGR, %/d) for weight and length and food conversion efficiencies (FCE) for control and treatment groups following a 10 week trial. Different superscripts indicate significant differences ( $P < 0.05$ ), column-wise. SGR values are presented with 95% confidence intervals.

Treatment	SGR <sub>wt</sub>	SGR <sub>l</sub>	FCE
Control	1.64±0.05 <sup>bc</sup>	0.50±0.01 <sup>a</sup>	0.822
Control inj.	1.60±0.04 <sup>c</sup>	0.49±0.01 <sup>a</sup>	0.833
GHby10	1.68±0.47 <sup>a</sup>	0.50±0.02 <sup>a</sup>	0.792
GHby100	1.71±0.06 <sup>a</sup>	0.51±0.02 <sup>a</sup>	0.801
GHby inj.	1.78±0.04 <sup>d</sup>	0.55±0.01 <sup>b</sup>	0.752
rbGH inj.	1.82±0.04 <sup>d</sup>	0.56±0.01 <sup>b</sup>	0.762

The impact of treatments upon various processing characteristics is presented in Table 3. No differences were seen in dressout percentages although distinctions ( $P < 0.05$ ) were observed in fillet yield and carcass percentage, with GH injected groups generally returning reduced fillet yields but higher carcass percentages (Table 3). Treatment effect upon fillet proximate composition of each group is

seen in Table 4. Reduced lipid levels ( $P < 0.05$ ) were identified in GH injected fish when compared against all other treatments. Between group differences ( $P < 0.05$ ) were also discerned for fillet moisture and ash content although no trends were apparent. Protein levels were identical for all groups ( $P > 0.05$ ; Table 4).

Table 3. Dressout percentage, fillet yields and carcass percentage of control and treatment groups at trial termination. Different superscripts indicate significant differences ( $P < 0.05$ ), column-wise. Values are presented with 95% confidence intervals.

Treatment	Dressout %	Fillet yield	Carcass %
Control	83.01±0.36 <sup>a</sup>	51.45±0.80 <sup>ab</sup>	26.60±0.59 <sup>a</sup>
Control inj.	83.86±0.43 <sup>a</sup>	52.44±0.85 <sup>a</sup>	25.95±0.89 <sup>a</sup>
GHby10	83.51±1.46 <sup>a</sup>	52.75±0.69 <sup>a</sup>	26.40±0.77 <sup>a</sup>
GHby100	84.60±4.32 <sup>a</sup>	51.98±1.64 <sup>ab</sup>	28.89±0.68 <sup>b</sup>
GHby inj.	83.16±0.42 <sup>a</sup>	50.95±1.13 <sup>b</sup>	28.41±0.92 <sup>b</sup>
rbGH inj.	82.81±0.43 <sup>a</sup>	51.51±0.88 <sup>ab</sup>	27.83±0.60 <sup>b</sup>

Table 4. Fillet proximate composition for control and treatment groups at trial termination. Different superscripts indicate significant differences ( $P < 0.05$ ), column-wise. All values are presented with 95% confidence intervals.

Treatment	Protein	Ash	Moisture	Lipid
Control	18.40±0.15 <sup>a</sup>	1.33±0.03 <sup>a</sup>	71.30±0.30 <sup>b</sup>	7.94±0.41 <sup>a</sup>
Control inj.	18.5±0.23 <sup>a</sup>	1.34±0.55 <sup>a</sup>	72.41±0.69 <sup>a</sup>	8.26±0.52 <sup>c</sup>
GHby10	18.45±0.17 <sup>a</sup>	1.34±0.04 <sup>a</sup>	72.65±0.50 <sup>a</sup>	7.92±0.43 <sup>a</sup>
GHby100	18.64±0.29 <sup>a</sup>	1.40±0.05 <sup>b</sup>	71.70±0.57 <sup>b</sup>	7.56±0.76 <sup>a</sup>
GHby inj.	18.60±0.15 <sup>a</sup>	1.46±0.03 <sup>c</sup>	72.63±0.50 <sup>a</sup>	6.97±0.41 <sup>b</sup>
rbGH inj.	18.54±0.17 <sup>a</sup>	1.41±0.02 <sup>b</sup>	72.86±0.44 <sup>a</sup>	6.53±0.39 <sup>b</sup>

Irrespective of treatment or time of examination, no effect was observed when comparing every 5<sup>th</sup> vertebrae of fish randomly taken from control fed, rbGH injected or high dose GHby groups. Vertebra size changed for all groups with corresponding change in position, with largest vertebrae being observed between position 30-45.

## Discussion

The main objective of the present study was to establish whether a waste derivative, from the industrial production of recombinant bovine growth hormone, expressed sufficient biological activity to impart positive effects upon trout growth and feed conversion efficiency. Whether injected or incorporated into a diet, particularly at high levels, the by-product enhanced trout growth and provided greater efficiency of food utilization. These results were not unanticipated since mammalian tibia bioassays indicated retained bioactivity for the protein at an 80%<sup>+</sup> level. Such a level of bioactivity would correspond to by-product injected fish receiving a dose of 1.2  $\mu\text{g g BW}^{-1} \text{ week}^{-1}$  of uncorrupted rbGH. At the concentrations used in the current trial, the results from injected fish therefore, are of passing interest only; generally conforming to other studies with rainbow trout given purified natural and recombinant GHs parenterally (see: Weatherley and Gill, 1987; Agellon et al., 1988; Danzmann et al., 1990; Garber et al., 1995).

Of greater interest, both from an aquaculture and industrial downstream processing perspective were the observations made following the dietary incorporation of the GH by-product. Other studies with fish have reported growth and FCE enhancement with dietary GH supplementation (McLean et al., 1993; Tsai et al., 1994), and it is noteworthy that each record the simplicity of adding the bioactive material to pellets. In the present study this required nothing more than the availability of contemporary feedmill equipment. Furthermore, no special attention was paid to by-product handling such that a proportion of the protein may have been rendered inactive during processing. The latter prospect would appear of limited consequence since trout given the feed containing the highest dose of by-product expressed a 430% weight increase compared to 390% achieved by both control groups. Moreover, feed conversion was superior in the GHby fed animals and no negative impact was seen for dressout percentage, fillet yield or fillet composition. As well, no differences were observed between groups with regard to fish shape, as evidenced by condition factor and skeletal analyses. Comparison of results between the high dose GHby feed and GHby injected groups indicate that 99%<sup>+</sup> of the protein was degraded or lost to bulk following gut delivery.

The results of the present investigation thus indicate that the need to employ sophisticated, and usually expensive methods, to protect GH, or other bioactives, during their transit along the gastrointestinal tract might not be prerequisite to their commercial application. This would be especially so where protein

concentrations were high as used herein. Further work in identifying alternative and economic methods of supplementing feeds with GHby and in optimizing dosage will be required if this technology is to be applied in the commercial sense.

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