SOMATOSTATIN MODULATES

THE GROWTH OF SALMONID FISH

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Somatostatins (SSs) are a diverse family of peptide hormones known to affect various aspects of the growth, development, and metabolism of vertebrates (Sheridan *et al.*, 1997). For example, SSs have been shown to influence organismal growth by inhibition of growth hormone (GH) release (*cf*. Holloway *et al.*, 1997). Extra-pituitary actions of SSs were suggested by previous studies that showed that growth retardation following premature transfer of salmon to seawater was accompanied by elevated plasma levels of SS (Sheridan *et al.* 1998). In addition, growth retardation of rainbow trout resulting from food deprivation was accompanied by altered plasma SS levels and altered expression of pancreatic mRNAs encoding SSs (Ehrman *et al.*, 1997). In this study, we examined further the modulation of the GH-Insulin-like growth factor (IGF-1) axis by SSs in rainbow trout.

Effects of SS on the GH-IGF-1 axis

Short-term (up to 6h) *in vivo* administration of SS to trout reduced plasma levels of GH, Insulin (INS), and IGF-1. Plasma GH levels were reduced from 4.1±0.3 ng/ml in control animals to 1.9±0.4 ng/ml in SS-14-treated animals 6h after injection. SS-14 injection caused plasma INS levels to decline from 3.1±0.3 ng/ml to 1.9±0.1 ng/ml 3h after injection; by 6h, plasma INS levels in SS-14-injected animals rebounded to near control levels. Plasma IGF-1 was reduced from 185±19 ng/ml in control animals to 104±19 ng/ml in SS-14-treated animals 6h after injection. Instantaneous growth, as assessed by ^{35}S-sulfate incorporation into gill cartilage was reduced from 35.5±2.8 cpm/µg dry weight in control animals to 16.4±1.6 cpm/µg dry weight in SS-14-injected animals.

Implantation of SS-14 into trout for 15 days resulted in significant growth retardation. Relative growth (length) was reduced from 6.31±0.34% in control animals to 3.43±0.57% in SS-14-treated animals. SS-14-induced growth retardation was associated with altered hepatic GH binding and with altered hepatic IGF-1 expression. Hepatic GH binding capacity was reduced from 1087±179 fmol/mg protein in control animals to 635±63 fmol/mg protein in SS-14-injected animals. Hepatic IGF-1 mRNA levels were reduced from 4.7±0.3 molecules of mRNA x 10^{-8}/µg total RNA in control animals to 2.4±0.5 molecules of mRNA x 10^{-8}/µg total RNA in SS-14-implanted animals. SSs also reduced the number of hepatic GH binding sites in hepatocytes *in vitro*.

Growth-reproduction interactions

SSs also interacted with the reproductive axis of trout. In general, plasma SS-14 levels declined as sexual maturation progressed. For example, in females plasma SS-14 levels declined from 0.43±.03 ng/ml in females with a Gonad Somatic Index (GSI) <5 to a low of 0.23±0.05 ng/ml in ovulated females in association with a decline in plasma estradiol (E2) levels. In males, SS-14 levels also declined during sexual maturation, reaching a low of 0.44±0.02 ng/ml in individuals with a GSI > 2.5; SS-14 levels increased in spermiated males to 0.55±0.03 ng/ml.

Implantation of trout with E2 elevated plasma GH levels, reduced plasma SS levels, inhibited the expression of mRNAs encoding SS, and inhibited the response of somatotropes to SS challenge. GH levels increased from 2.9±0.2

ng/ml in control animals to 4.3±0.7 ng/ml in E2-treated fish. Plasma levels of SS-14 fell from 0.56±0.02 ng/ml in control animals to 0.37±0.01 ng/ml in E2-treated animals. Pancreatic expression of mRNAs encoding the precursor of SS-14 was reduced from 4.1±0.1 molecules of mRNA x 10^{-8}/μg total RNA in control animals to 2.6±0.3 molecules of mRNA x 10^{-8}/μg total RNA in SS-14-injected animals. GH release from isolated trout pituitary fragments was reduced by nearly 40% in the presence of SS-14 for 20 min. However, SS-14 did not affect GH release from pituitary fragments obtained from fish treated with E2 for 2 weeks.

Conclusions

These findings indicate that SSs modulate the GH-IGF-1 axis, perhaps underlying nutritional regulation of growth, and suggest that SSs may coordinate reproductive events with growth.

Acknowledgments

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References

Ehrman, M.M., Moore, C.A., Kittilson, J.D., Boser, J.E., and Sheridan, M.A. 1997. Nutritional state affects somatostatin gene expression in rainbow trout. 79th Meeting of the Endocrine Society, June 11-14, 1997. Minneapolis, MN.

Holloway, A.C., Sheridan, M.A. and Leatherland, J.F. 1997. Estradiol inhibits plasma somatostatin 14 (SRIF-14) levels and inhibits the response of somatotrophic cells to SRIF-14 challenge *in vitro* in rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 106:407-414.

Sheridan, M.A., Kittilson, J.D., Ehrman, M.M., and Moore, C.A. 1997. Polygenic expression of somatostatin in rainbow trout. In *Advances in*

Comparative Endocrinology (S. Kawashima and S. Kikuyama, eds), pp291-294. Monduzzi Editore, Bologna.

Sheridan, M.A. Eilertson, C.D., and Kerstetter, T.H. 1998. Changes in plasma somatostatin associated with seawater adaptation and stunting of coho salmon, *Oncorhynchus mykiss*. Aquaculture 168: 195-203.

EXTENSIVE MINISATELLITE POLYMORPHISM IN INTRONS OF THE GROWTH HORMONE GENE IN THE SPARIDAE FAMILY

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

GH plays a major role in stimulating somatic growth in vertebrates, including teleosts. Its potential implication in enhancing growth rate of fishes in aquaculture prompted the cloning and characterization of the GH cDNAs and genes from cultured fish species. Comparison of their structure demonstrates a higher variability compared to that found for mammalian GH genes. We are interested in the structure and regulation of expression of growth-related genes in the gilthead seabream, *Sparus aurata* - a member of the Sapridae family (common name, seabreams). Sparidae is an important family of marine fish, most of which are of notable commercial value and display worldwide distribution with many species common to the Mediterranean Sea and East Atlantic Ocean. Domestication of the gilthead seabream, the major aquacultured species to date in the Mediterranean region, started less than 30 years ago. Its successful performance has prompted the domestication of other sparid species and production of interspecific hybrids. Both domestication of new species and

hybrid production require extensive information on the phylogenetic relationships between the species, degree of genetic variation of the aquacultured stocks and the wild population, and geographical differences among cultured and wild stocks.

Results and Discussion

Cloning and structure characterization of the GH gene of the gilthead seabream (saGH) showed that it spans approximately 4.3 kb and consists of six exons and five introns, as found for all cloned teleost GH genes with the exception of carps and catfish. The first and third introns contain long stretches of repetitive tandem repeats. The second intron, which is unusually long compared with that in other teleosts (and other vertebrates) contains several inverted repeats. Intron-targeted polymerase chain reaction (PCR) analysis identified length polymorphism of the first and third introns. Sequence analysis of several variants of the first intron revealed that the variation in length is due to differences in the number of the repeat monomers as well as minor changes in their length (Almuly et al., 2000). Analyses of geographically different cultured stocks of gilthead seabream for GH intron I length revealed a considerably high degree of polymorphism and high level of heterozygosity. The fragment size ranged from about 400 bp to about 1450 bp with more than 12 alleles found.

Intron-targeted PCR was also used for analysis of GH intron I polymorphism in several species of the Sparidae family. High variation in the length of the first intron was found between several sparids studied (8 species) that ranged from about 250 nt to 1450 nt. Preliminary sequence analysis suggests that a consensus repeat unit is found in all sparid GH intron I studied sofar, with species-specific variations. In addition, it appears that sparid species with long GH intron I show length polymorphism, which results from different number of repeat units.

These studies will be useful for establishing the phylogenic relationship between members of Sparidae family based on GH intron sequences, for analysis of genetic variation of populations and for testing the potential application of GH intron polymorphism to serve as genetic marker for desirable traits, such as growth rate.

References

Almuly, R., B. Cavari, H. Ferstman, O. Kolodny, and B. Funkenstein. 2000. Structure and sequence of the gilthead sea bream (*Sparus aurata*) growth hormone-encoding gene: identification of minisatellite polymorphism in intron I. Genome, in press.

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PRODUCTION AND USE OF PLANT-DERIVED RECOMBINANT CARP SOMATOTROPIN AS FOOD SUPPLEMENT FOR INCREASING GROWTH RATE IN CULTURED FISH

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

While world demand for fresh water and marine fish products is growing rapidly, there is a decline in the catches of commercially important fish species. In order to increase fish production, many countries have turned to aquatic biotechnology. To increase growth rate of fish, a number of investigators have

examined two entirely different approaches including production of transgenic fish species that express elevated levels of growth hormone (GH) and treatment of cultured fish with recombinant GH. However, production of transgenic fish is considered by many regulatory agencies to pose environmental risks with respect to the possible escape of the genetically modified organisms into wild population. These risks may be ameliorated through controlled administration of fish growth hormone by feeding. In this context, fish growth hormone has no activity in mammals and can not be absorbed through human gut. Fish gut, however, has macro molecule absorption properties and can take up intact GH to allow enhanced growth rate. The overall objective of the present study was to develop a novel form of fish food based on canola seed protein containing an active form of fish somatotropin. The specific application of this technology would be to increase growth rate and food conversion efficiency in cultured fish using plant-produced fish somatotropin as food supplement. In this context, growth rate is a particularly important parameter in the economic equation, since it would significantly reduce the time to produce market-size fish and provide a major boost to the profitability of the industry.

Materials and Methods

A novel, plant-based system was used for the production and purification of recombinant carp GH (cGH) in oilseed species (Moloney and Holbrook, 1997). The system used the natural targeting of the native seed protein oleosin, for oil bodies, the subcellular storage organelles for triacylglycerides. This strategy provides for the rapid purification of the fusion protein through a simple process of flotation-separation (Moloney et al., 1996). We describe here the production of a functional carp GH (cGH) (Mahmoud et al., 1996) as an oleosin fusion protein in Brassica napus. A construct carrying an Arabidopsis oleosin sequence fused to that encoding cGH hormone was introduced into plants via Agrobacterium-mediated transformation. The oleosin-somatotropin fusion protein targeted specifically to oil bodies and accumulated in transgenic seeds to a level of approximately 0.4% of total seed protein.

Results

Common carp growth hormone was produced in Brassica napus as a translational fusion with the native seed oil body protein, oleosin. Natural targeting of the oleosin fusion protein to oil bodies provided for its rapid

purification through a simple process of flotation-separation. The purified somatotropin-oil body complex was demonstrated to be functional through an activity assay measuring induction of insulin-like growth factor-I (IGF-I; Kermouni et al., 1998. Experiments were carried out to test plant-derived cGH on rainbow trout fed a diet containing the recombinant product for eight weeks. Rainbow trout receiving a diet supplemented with oil body-coupled somatotropin equivalent to 0.29 μ g of carp growth hormone / g body weight / day exhibited significant increase in growth over controls during an eight-week feeding trial. Examination following completion of the study revealed no morphological abnormalities in animals receiving the experimental diet.

Conclusion

Plant-derived recombinant cGH can be used effectively to increase growth rate in cultured fish. The oleosin-based production system used in these studies is relatively inexpensive and can readily be scaled up to meet requirements for the commercial aquaculture industry.

References

Mahmoud SS, Moloney MM, and Habibi HR., (1996) Cloning and Sequencing of the goldfish growth hormone cDNA. General and Comparative Endocrinology. 101:139-144.

Kermouni A., Mahmoud S.S., Wang S., Moloney M., and Habibi H.R. (1998) Cloning of a full-length Insulin-like growth Factor-I complementary DNA in the goldfish liver and ovary and development of a quantitative PCR method for its measurement. General and Comparative Endocrinology, 111:51-60.

Moloney MM, Holbrooh LA 1997. Biotechnology and Genetic Engineering Reviews 14:321-336.

Moloney MM, van Rooijen GJH 1996. Recombinant proteins via oleosin partitioning INFORM 7:107-114.

NEUROPEPTIDE Y AND ITS EFFECT ON FEEDING AND GROWTH

IN CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Feeding and the behavioral control of feeding and growth by fishes is critical to survival and fitness. While food intake and subsequent growth is controlled by an interacting group of endocrine factors (Silverstein and Plisetskaya, 2000), neuropeptide Y (NPY) has been implicated in these processes in many animals, including some fish. In teleosts, NPY is expressed in response to restricted food availability (Silverstein *et al*., 1998) and treatment with NPY stimulates feeding (Lopez-Patino *et al*., 1999). Additionally, NPY is involved in the control of growth hormone action (Peng *et al*., 1993). Although NPY is ubiquitous in the brain, its involvement in food intake regulation is localized to the hypothalamic areas. We are investigating how NPY mRNA responds to variation in food availability as well as how it differs between different strains of catfish that are known to grow differently. We have detected NPY mRNA in channel catfish brain using both Northern blotting and RT-PCR (fig 1). RT-PCR has detected NPY mRNA in hypothalamus, myelencephalon, optic tectum and telencephalon, but not in the cerebellum or the pituitary. We have now developed a competitive RT-PCR assay to quantify the differences in mRNA levels in catfish brain in large numbers of samples. Using this technique we are able to quantitate differences in expression of this hormone in fish starved for varying periods. Our studies indicate that NPY mRNA expression is affected by food availability and that our methodology will allow us to further examine the affects of other hormones on the NPY system.

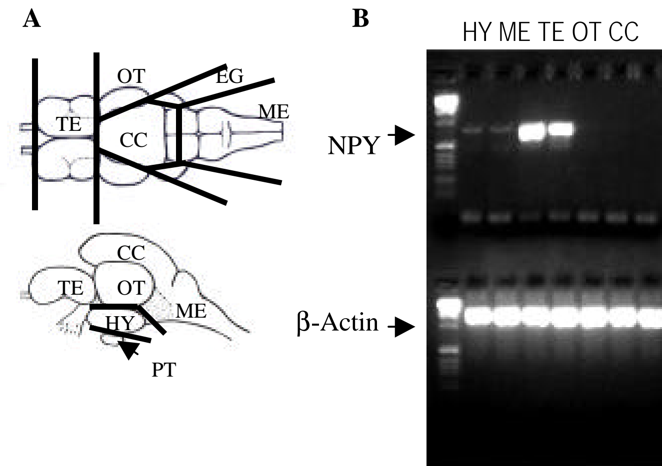

Figure 1. A. Drawing showing brain morphology of channel catfish, Ictalurus punctatus as sampled for brain distribution of NPY mRNA. Upper frame is dorsal aspect, lower is lateral aspect. Dark lines indicate lines of dissection. TE (telencephalon), OT (optic tectum), CC (corpus cerebellum), EG (eminentia granularis of the cerebellum), ME (myelencephalon), HY (hypothalamus), PT (pituitary). B. Agarose gel (2% with ethidium bromide) showing UV detection of RT-PCR products from several catfish tissues as indicated. Upper lanes show amplification with NPY primers, lower lanes with β-actin primers.

References

Lopez-Patino, M.A., Guijarro, A.I., Isorna, E., Delgado, M.J., Alonso-Bedate, M. and de Pedro, N. 1999. Neuropeptide Y has a stimulatory action on feeding behavior in goldfish (*Carassius auratus*). Eur. J. Pharm. 377:147-153.

Peng, C., Humphries, S., Peter, R.E., Rivier, J.E., Blomqvist, A.G. and Larhammar, D. 1993. Actions of goldfish neuropeptide Y on the

secretion of growth hormone and gonadotropin-II in female goldfish. Gen. Comp. Endocrinol. 90:306-317.

Silverstein, J.T., Breininger, J.,Baskin, D.G. and Plisetskaya, E.M. 1998. Neuropeptide Y-like gene expression in the salmon brain increases with fasting. Gen. Comp. Endocrinol. 110:157-165.

Silverstein, J.T. and Plisetskaya, E.M. 2000. Study of the effects of NPY and insulin in food intake regulation in fish. Amer. Zool. 40: in press.

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GROWTH HORMONE-INDUCED INCREASE IN AGGRESSION IN JUVENILE RAINBOW TROUT

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Growth hormone promotes mammalian growth (Steele and Evock-Clover, 1993) as well as growth of teleosts (McLean and Donaldsson, 1993). By stimulating growth, GH increases the metabolic demands, which should elevate the hunger level experienced by an animal. Consequently, studies have shown that GH increases dominant feeding-behaviour and reduces anti-predator behaviour of salmonids (see Björnsson 1997).

In freshwater streams, juvenile rainbow trout (*Oncorhynchus mykiss*) either defend territories or form dominance-based hierarchies. In these structures, agonistic behaviour is an important component since dominance is established in pairwise encounters, and the outcome of these mainly depend on the relative fighting ability of the opponents (Huntingford and Turner, 1987).

The aim of the present study was to clarify the role of growth hormone in social interactions in juvenile salmonids. The hypotheses that GH increases aggression levels and/or the fighting ability were tested.

Material and methods

Agonistic behaviour in pairs of juvenile rainbow trout consisting of two control fish (C/C pairs), two growth hormone treated fish (GH/GH pairs), or one growth hormone treated and one control fish (C/GH pairs) was observed. After implantation with either a cholesterol pellet containing 25 µg GH/g fish, or a placebo, each pair was placed in an aquarium. Each individual was separated by a removable longitudinal wall, and fed for four days to allow acclimation to the new environment and to allow the GH treatment to take effect. The following four days the experimental observations were carried out.

Each morning, the PVC longitudinal dividers were removed, allowing the fish to interact. Each pair of fish was then observed during three sessions each. The agonistic behaviour observed were: 1) frontal/lateral display, 2) circling, 3) replace, 4) attack, 5) chasing, and 6) bite. The initiator and the winner of each aggressive act were registered. After the last observation on each day, the dividers were inserted, separating the two fish of each pair. The fish were fed, and kept isolated until the next morning when the fish were observed again. For each pair, the individual that won most of the interactions was considered to be dominant.

Statistics

The behavioural data were not normally distributed and were therefore $\log_e$ transformed, whereupon they conformed to normal distribution and could be analyzed using ANOVA. Multiple comparisons were made with a Tukey test.

Results and discussion

Aggression was lowest in the C/C pairs, intermediate in the C/GH pairs, and highest in the GH/GH pairs, the difference between the C/C pairs and the GH/GH pairs being significant (Figure 1). This supports the hypothesis that GH increases aggression levels. However, in the C/GH pairs, the number of conflicts won by GH-treated and control fish did not differ significantly. In nine of these pairs, the control fish became dominant and in the remaining seven fish the GH-treated fish became dominant. Thus, because social status was not increased, GH did not appear to affect fighting ability. A possible explanation for these results

is that GH increases swimming activity, thereby elevating the encounter rate between the two individuals. It may also be speculated that the GH-treated fish increased their foraging activity, to due a GH-induced increase in appetite.

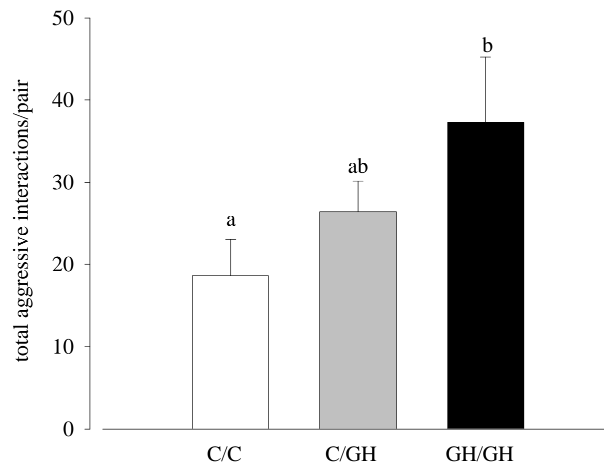

Figure 1. Total number of aggressive interactions in the three treatment groups of juvenile trout, see text for further description. Data are presented as means±se. Bars with the same letter are not significantly different ($p<0.05$).

In wild mice, GH increased aggression without affecting non-aggressive motor activity when two identically treated individuals were confronted (Matte, 1981). Thus, GH may affect agonistic behaviour of mammals and teleosts differently. In juvenile Atlantic salmon, elevated aggression in food-deprived fish was only partly an effect of increased locomotion (Symons, 1968), indicating that hunger also has a direct effect on aggression. Thus, hunger and GH may influence agonistic behaviour by partly different routes.

It has previously been shown that GH injected salmonids have an improved ability to compete for food (Björnsson, 1997). In the present study, by feeding the competing fish separately, the stimulatory effect of GH per se was removed. Hence, it appears that GH increases feeding motivation rather than social status.

In conclusion, the results of the present study indicate that GH elevates aggression indirectly, without affecting fighting ability, in juvenile rainbow trout. This further strengthens the view that GH may induce numerous behavioural changes in salmonids. Since these behavioural changes may incur energetic and mortality costs, this may limit selection for high GH levels in natural populations.

References

Björnsson, B. Th. (1997). The biology of salmon growth hormone: from daylight to dominance. Fish Physiol Biochem. **17**. 9-24.

Huntingford, F. A., and Turner, A. K. (1987). *Animal Conflict*, 480 pp. Chapman and Hall, London.

Matte, A. C. (1981). Growth hormone and isolation-induced aggression. *Pharmac. Biochem. Behav*. **14**, 85-87.

McLean, E., and Donaldson E.M. (1993). The role of growth hormone in the growth of poikilotherms. *In* M. P. Schreibman, C. G. Scanes and P. K. T. Pang (Eds.), *The endocrinology of growth, development, and metabolism in vertebrates*, pp. 43-71. Academic Press, San Diego.

Steele, N. C. and Evock-Clover C. M. (1993). Role of growth hormone in growth of homeotherms. *In* M. P. Schreibman, C. G. Scanes and P. K. T. Pang, (Eds.), *The endocrinology of growth, development, and metabolism in vertebrates*, pp. 73-90. Academic Press, San Diego.

Symons, P. E. K. (1968). Increase in aggression and in strength of the social hierarchy among juvenile Atlantic salmon deprived of food. *J. Fish. Res. Bd. Canada* **25**, 2387-2401.

THE EFFECTS OF GROWTH RATE AND EXERCISE ON BASAL METABOLIC RATE AND MAXIMUM AEROBIC METABOLIC RATE IN JUVENILE NORTHERN PIKE

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Basal metabolic rate (BMR), the cost of maintaining cells and organs for higher levels of biological activity, is the primary energy drain on the aerobic energy budgets of ectothermic vertebrates (Calow 1985). In ectotherms, BMR is known to vary between individuals within a species (Garland & Else 1987; Metcalfe *et al.* 1995; Yamamoto *et al.* 1998). Individual ectotherms are generally assumed to have a constant BMR at any given temperature. A strategy of flexibility in BMR might have evolved to cope with changing environmental conditions. The effects of exercise and ration on individual variations in BMR, MMR, enzyme activity and body composition of juvenile northern pike *Esox lucius* were studied.

Initial measurements of BMR and MMR (following exhaustive exercise) were made and factorial metabolic scope calculated (MMR/BMR), all fish having been held at a low food ration prior to experiments (c. 1.2% w.b.w d^{-1}). Fish were then split into a high ration (c. 2.5% w.b.w d^{-1}) no-exercise group (n = 10), low ration (c. 1.2% w.b.w d^{-1}) no-exercise control group (n = 10) and sustained exercise group (n = 13) that swam continuously at approximately 0.5 body lengths second. The exercise fish were initially held at the low ration, between

times 1 and 2, and at an intermediate ration between times 2 and 3 (c. 2% w.b.w d^{-1}). No-exercise fish were kept individually in static water tanks. Initial measurements were termed time 1, with subsequent measurements made after approximately 3 weeks (time 2) and 11 weeks (time 3).

On comparison of the log residual values for individual fish from a graph of log BMR vs. log body mass at each time point (1-way ANOVA with Bonferroni correction), there were found to be statistically significant differences in the BMR of individual fish between the different experimental times. Group mean BMR (standardised to a 35 g pike) at time 2 was significantly lower for non-exercised low ration fish compared to time 1, with a statistically significant increase in BMR for exercised fish between times 2 and 3. A comparison of the group mean mass adjusted MMR between treatment groups indicated a significant decrease for non-exercised low ration fish at time 3 and for non-exercised high ration fish at times 2 and 3 (Wilcoxon's signed rank test). Exercised fish had a significantly larger MMR and factorial metabolic scope following 3 weeks of sustained swimming at time 2, with a similar MMR at time 3 to that measured at time 2. There was a significant decrease in metabolic scope for both high and low ration fish at time 3 only (1-way ANOVA with Bonferroni correction). For all fish there were significant correlations between individual BMR and MMR values at time 1 ($r^2 = 0.26$, $p<0.01$) and time 3 ($r^2 = 0.20$, $p<0.05$) but not at time 2.

After the metabolic rate measurements were made at time 3 all fish were humanely killed. Maximal enzyme assays were performed on the heart, gill, liver, intestine, red muscle and white muscle for each remaining fish ($n = 30$). Levels of each of two enzymes (citrate synthase, CS, and lactate dehydrogenase, LDH, measured in the direction of lactate oxygenation) were found to be similar between treatment groups for the respective tissues. Total CS activity levels and LDH levels were highest in the heart and red muscle. There were limited differences in the relative organ masses of fish exposed to different treatments, for fish scaled to constant body mass. The organ masses were scaled using log organ weight vs. log body mass relationships, and expressed as the percentage of adjusted body mass (Figure 1).

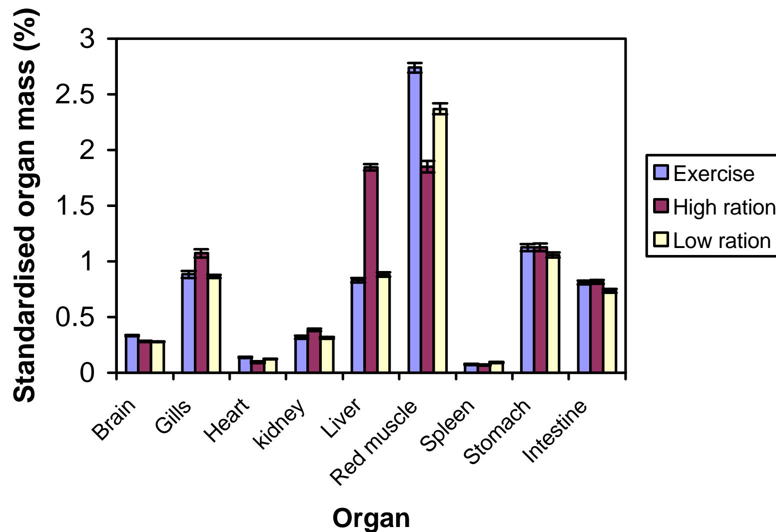

Figure 1 Standardised organ masses (Mean and SE) expressed as a percentage of adjusted body mass for all tissues except carcass and white muscle.

Multiple linear regression of scaled individual organ masses provided good relationships with MMR (r^2 = 0.71) and BMR (r^2 = 0.49), for which the most important organs were red muscle, brain, kidney and intestine. These tissues are the most metabolically active tissues in the carp *Cyprinus carpio* (Itazawa & Oikawa 1986). It is concluded that in pike differences in ration and exercise influenced individual physiology, and were reflected in between- and within-individual variation in BMR and MMR.

References

Calow P. 1985 Adaptive aspects of energy allocation. In: *Fish Energetics: New Perspectives.* (Eds Tytler P. & Calow P.), pp. 13-31. John Hopkins University Press, Baltimore.

Garland T., Jr. & Else P. 1987 Seasonal, sexual and individual variation in endurance and activity metabolism in lizards. *Am. Journ. Physiol*. **252:** 439-449.

Itazawa Y. & Oikawa S. 1986 A quantitative interpretation of the of the metabolism-size relationship in animals. *Experientia* **42:** 152-153.

Metcalfe N.B., Taylor A.C. & Thorpe J.E. 1995 Metabolic rate, social status and life history strategies in Atlantic salmon. *Anim. Behav*. **49:** 431-436.

Yamamoto T., Ueda H. & Higashi S. 1998 Correlation among dominance status, metabolic rate and otolith size in masu salmon. *J. Fish Biol.* **52:** 281-290.

GROWTH POTENTIAL AND PERFORMANCE OF

FARMED SOUTHERN BLUEFIN TUNA, *Thunnus maccoyii*

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Lack of information on the physiology of farmed southern bluefin tuna (SBT) requires the use of alternative methods to complement the direct assessment of growth. Relationships between growth rate and indirect measurements including biochemical growth correlates (RNA, DNA and protein concentration and ratios between them), organ weights and plasma IGF-I concentrations have been assessed over several years. Significant positive relationships between individual specific growth rates and indices of RNA content (RNA

concentration, RNA: protein, RNA: DNA) in the epaxial muscle have been observed (Carter et al. 1998). In the latter study SBT were fed at near maintenance but the muscle RNA: protein ratio (8.3±1.4g protein/mg RNA) was at least four times higher than would be predicted from other fishes based on their weight and temperature (Houlihan et al. 1995). RNA:protein was similar to that in unfed mammals (Millward et al. 1973) and suggested there may be differences in protein turnover of tuna compared with other teleosts. The current research aimed to gain a more detailed understanding of nutrient utilisation and growth of SBT through investigation of potential indicators of protein metabolism such as biochemical growth correlates over 24 hours following feeding.

Methods

SBT (13.9 ± 2.9 kg; mean ± sd) held in a 30m cage on the Tuna Research Farm, South Australia were used (Carter et al. 1998). Five fish were removed prior to feeding in the morning and further groups of five removed at 2, 4, 8, 12 and 24 hours after the morning feed. The fish were killed quickly by pithing, weight and fork length measured and tissue samples taken (Carter et al. 1998). Measurements were made of tissue protein, RNA and DNA (Carter et al. 1998) and plasma IGF-I concentrations. There were no significant ($P>0.2$) differences between plasma cortisol concentrations at 0h and the other times and suggested that repeated returns to the cage did not increase stress levels.

Results and Discussion

Muscle growth correlates were not different between times (Table 1). There was a suggestion that muscle temperature increased to peak at 8h following feeding.

The relationship between the RNA:protein ratio (capacity for protein synthesis) and protein synthesis (PS) has been described by PS (%/d) = 2.3RNA:Protein–6.4 (Carter et al. 1993). The mean RNA:protein ratio predicted a muscle protein synthesis rate of 3.7 %/d. This is similar to those found in other fish and around 1.3 times the rate predicted when weight and muscle temperature are taken into account (Houlihan et al. 1995; McCarthy et al. 1999). Consequently, these data don’t provide any strong evidence that RNA:protein ratio and protein turnover in tuna is significantly different from other teleosts. Furthermore, rates of protein synthesis in tuna muscle are likely to be explained by the relationship

between temperature and synthesis established for endotherms and ectotherms (McCarthy et al. 1999). However, it is important to note that rates of protein synthesis are also determined by RNA activity. The high RNA:protein ratio found in the earlier study (Carter et al. 1998) are not easily explained and may have related to a long period of poor nutrition.

Table 1. Change in muscle protein, RNA and DNA concentrations (mg/g muscle tissue) and ratios and temperature prior to (0h) and following feeding.

	0h	**2h**	**4h**	**8h**	**12h**	**24h**	**P**
Protein (mg/g)	145 ±14	141 ±11	137 ±19	153 ±12	154 ±11	150 ±21	ns
RNA (mg/g)	0.71 ±0.09	0.55 ±0.03	0.49 ±0.05	0.67 ±0.09	0.73 ±0.11	0.69 ±0.07	ns
DNA (mg/g)	0.46 ±0.11	0.36 ±0.10	0.55 ±0.13	0.48 ±0.10	0.40 ±0.12	0.42 ±0.13	ns
RNA: protein	4.9±0.6	3.9±0.2	3.6±0.3	4.4±0.7	4.7±0.6	4.7±0.6	ns
RNA:DNA	2.2±0.7	2.1±0.5	1.2±0.3	2.2±1.0	2.8±0.9	2.4±0.7	ns
Temp. (°C)	25.5 ±0.7	25.0 ±0.4	26.1 ±0.4	27.1 ±0.2	26.5 ±0.6	25.7 ±0.4	<0.07

The growth rates of the tuna in the current experiment were not known. However, IGF-I concentration (38.8 ± 16.1 ng / ml) varied between individual fish and was significantly correlated with muscle RNA ($r = 0.60$; $P < 0.01$) and protein ($r = 0.53$; $P < 0.01$) concentrations and more weakly with the RNA: protein ratio ($r = 0.35$; $P < 0.05$). These data are suggestive of the relationships between whole animal growth and mechanisms of protein accretion found previously (Millward et al. 1973; Carter et al. 1998; McCarthy et al. 1999) as well as with IGF-I concentrations. These measures have potential for understanding SBT growth performance particularly in relation to farming, feeding and feed development.

Acknowledgements

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References

Carter, C.G., Houlihan, D.F., Buchanan, B. and Mitchell, A.I. 1993. Protein-nitrogen flux and protein growth efficiency of individual Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 12: 305-315.

Carter, C.G., Seeto, C.G., Smart, A., Clarke, S. and van Barneveld, R.J. 1998. Correlates of growth in farmed juvenile southern bluefin tuna *Thunnus maccoyii* (Castelnau). Aquaculture. 161: 107-119.

Houlihan, D.F., McCarthy, I.D., Carter, C.G. and Marttin, F. 1995. Protein turnover and amino acid flux in fish larvae. ICES marine Science Symposium. 201: 87-99.

McCarthy, I.D., Moksness, E., Pavlov, D. and Houlihan, D.F. 1999. Effects of water temparature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*). Canadian Journal of Fisheries and Aquatic Science. 56: 231-241.

Millward, D.J., Garlick, P.J., James, W.P.T., Nnanyelugo, D.O. and Ryatt, J.S. 1973. Relationship between protein synthesis and RNA content in skeletal muscle. Nature. 241: 204-205.

ROLES OF GROWTH HORMONE AND PROLACTIN IN GOBIES DURING ADAPTATION TO VARIOUS ENVIRONMENTS

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Growth hormone (GH) and prolactin (PRL) belong to a family of hormones that share similarities in structure and function; it has been proposed that these hormones evolved from a common ancestral gene through duplication and subsequent divergence. GH regulates growth in all vertebrates, including fish (Chen et al., 1994). GH has also been implicated in seawater (SW) adaptation of salmonids importantly and also of some euryhaline teleost species (Sakamoto et al., 1993, 1997; Mancera and McCormick, 1998). PRL is the most versatile of the pituitary hormones. It shows lactogenic, luteotropic, mitogenic, somatotropic, metamorphic, antimetamorphic, and osmoregulatory activities. Among teleosts, the most prominent action of PRL is its osmoregulatory role in freshwater (FW) adaptation (Hirano, 1986). In teleosts, therefore, this family of hormones seem to play important roles in environmental adaptation. However, the studies are limited to the osmoregulation in the several euryhaline species, but not in gobiidae.

Gobiidae is the largest family in fishes. They are widely distributed ecologically: they live horizontally from open sea to inland streams, and vertically from mud surface on land to bottom layer in waters. Thus, even

among related species, they develop diverse physiological functions to adapt these various environments, and provide good models.

The present investigation was directed toward characterizing the expression patterns in pituitary GH and PRL of an ureotelic goby *(Mugilogobius abei)* after exposure to ammonia, as well as those of the estuarine, amphibious mudskipper *(Periophthalmus modestus)* during terrestrial adaptation and adaptation to different salinities.

Results and Discussion

Using PCR-amplified, mudskipper cDNA clones of GH and PRL, as probes in Northern analysis, we detected a 1.7 kb transcript for PRL and a 1.6 kb for GH in pituitaries of *M. abei* and *P. modestus*.

1. M. abei during adaptation to polluted environments

A few species of teleosts are known to produce urea in response to elevated external ammonia or alkalinity. *M. abei,* surviving in heavy polluted streams, has a very high ability for ammonia tolerance. When exposed to ammonia, they excrete a large amount of urea and also increase the glutamine synthesis (Iwata et al., 2000).

Furthermore, the growth rate became higher after exposure to 2 mM NH_4Cl for 4-8 weeks than that of control without ammonia. After a 1-week exposure to ammonia, these fish had 3-times higher levels of GH mRNA but similar levels of PRL mRNA compared with controls. This increase in GH mRNA coincided with the elevated syntheses of urea and glutamine, and preceded the growth. Therefore, GH may transduce the environmental cues to enhance nitrogen metabolism, including syntheses of urea and glutamine, and then promote growth consequently. In fact, GH is known to promote growth partly through regulation of protein metabolism in fishes as in mammals. This can be comparable to the case of salmonid smoltification where GH plays a role in growth as well as in SW adaptation (Bjˆrnsson, 1997).

2. Mudskipper during adaptation to different salinities and terrestrial environment

The mudskipper spend the greater parts of their lives out of water and can also adapt either to FW or SW. The mudskipper were transferred from 1/3 SW either to FW, SW, or to aquaria without water. Fishes transferred to different salinities were sampled after 10 hours and 7 days. Water deprived fishes were sampled only after 10 hours.

When they are transferred to FW, the plasma sodium levels showed a transient decrease. There was no significant difference in plasma sodium during adaptation to other environments, although the 20% increase was observed in fish kept out of water. Plasma cortisol levels increased in fish kept out of water as well as in FW. Prolactin mRNA levels increased after FW transfer, supporting its important role in the FW adaptation of fishes. PRL mRNA levels in the mudskipper kept out of water were also 4-times higher than those in 1/3 SW fish. These PRL expression should be intriguing to explore the regulators such as tyrotropin-releasing hormone and novel prolactin-releasing pepetide, since increased plasma levels of sodium and cortisol are both known to suppress the PRL expression in several species (see Shepherd et al., 1999). In the mudskipper skin, the tissue resistance increased during FW adaptation and terrestrial adaptation, although Cl^- secretion diminished only during FW adaptation. Therefore, PRL may decrease the integmental permeability to electrolytes and water in order to maintain the electrolytes in FW and water in terrestrial environment. GH mRNA levels were elevated significantly 7 days after SW transfer, similarly as in several teleosts such as salmonids, chichlid and killifish (Sakamoto et al., 1993, 1997; Mancera and McCormick, 1998).

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References

Bjornsson, B. Th. 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiol. Biochem. 17:9-24

Chen, T. T., A. Marsh, M. Shamblott, K.-M. Chan, Y.-L. Tang, C. M. Cheng, and B.-Y Yang. 1994. Structure and evolution of fish growth hormone and insulinlike growth factor genes. In Fish Physiology: Molecular Endocrinology of Fish, N. M. Sherwood and C. L. Hew editors. Academic Press, New York. Vol. 13, 179-209

Hirano, T. 1986. The spectrum of prolactin action in teleosts. Prog. Clin. Biol. Res. 205:53-74

Iwata, K., M. Kajimura, and T. Sakamoto. (submitted). Functional ureogenesis in the gobiid fish, *Mugilogobius abei*. J. Expt. Biol.

Mancera, J. M. and S. D. McCormick. 1998. Evidence for growth hormone/insulin-like growth factor I axis regulation of seawater acclimation in the euryhaline teleost *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 111:103-112

Sakamoto, T., B. S. Shepherd, R. S. Nishioka, S. S. Madsen, K. Siharath, N. H. Richman III, H. A. Bern, and E. G. Grau. 1997. Osmoregulatory actions of growth hormone and prolactin in an advanced teleost. Gen. Comp. Endocrinol. 106:95-101

Sakamoto, T., S. D. McCormick, and T. Hirano. 1993. Osmoregulatory actions of growth hormone and its mode of action in salmonids: A review. Fish Physiol. Biochem. 11:155-164

Shepherd, B. S., T. Sakamoto, S. Hyodo, R. S. Nishioka, C. Ball, H. A. Bern, and E. G. Grau. 1999. Is the primitive regulation of pituitary prolactin ($tPRL_{177}$ and $tPRL_{188}$) secretion and gene expression in the euryhaline tilapia *(Oreochromis mossambicus)* hypothalamic or environmental? J. Endocrinol. 161:121-129

AN INDIVIDUAL APPROACH TO MODELLING EFFECTS OF ENVIRONMENTAL FACTORS ON FEED INTAKE AND GROWTH OF ATLANTIC SALMON

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

This paper explains how results from investigations based on using individually identifiable fish can be used to model the effects of environmental factors on growth performance and feeding of Atlantic salmon, *Salmo salar* L. at different stages of its life cycle. Special reference will be made to potential applications of these findings in production planning of farmed salmon and in different approaches to experimental design.

The main aim of these studies was to produce a more detailed understanding of the inter-relationships between feed intake and growth for individual fish held in groups so that this knowledge could then be used by those working on growth related studies of salmonids and be helpful to the salmon farming industry in the development of efficient rearing protocols.

Materials and methods

A series of experiments was designed to determine the effects of photoperiod (using artificial lighting), ration level, sea water and sexual maturation on feed intake and growth rates of Atlantic salmon, *Salmo salar* L. at various stages of the life cycle.

Fork lengths (mm) and wet weights (g) were measured at regular intervals throughout each of the experiments. Specific growth rates (SGR, percent body weight per day) of individual fish between sampling dates were calculated using the following equation (Ricker, 1979): $SGR = ((\ln(W_t) - \ln(W_0)) \times 100)/t$, where W_0 and W_t are the initial and final weights of each fish (g) and t is the number of days between weighings.

X-radiography (Talbot and Higgins, 1983; Stead et al., 1996) was employed to measure feed intake of individual fish and radioimmunoassays (Stead et al. 1999) were used to determine levels of the male and female steroid hormones, 11-Ketotestosterone and estradiol-17β, respectively.

Food intake of individual fish was estimated as the mean of three measurements made at approximately biweekly intervals over a defined period of growth (Stead et al., 1996).

At each sampling date throughout each experiment, the weight, specific growth rates, food intake, weight-specific food conversion ratios (data not shown) and condition factors (data not shown) were compared. Analysis of variance (ANOVA) was used to examine differences within and between treatments where appropriate otherwise the antedependence method of Kenward (1987) was employed.

Results

Freshwater growth of Atlantic salmon parr may be increased or decreased during autumn and winter by manipulation of different photoperiod regimes, that is, under extended or shortened day lengths of 16 and 8 hours of light, respectively, when applied over consecutive time periods. Fish reared under extended day lengths had significantly greater weights, lengths, specific growth rates and food intake rates than those fish on shorter day lengths ($P<0.05$).

In Atlantic salmon smolts, fed at ration levels of 0.5, 1.0 and 3.0 percent body weight per day, growth performance in fresh water was not a significant determinant of subsequent seawater growth ($P>0.05$). Specific growth rates increased with increasing ration level in both freshwater and sea water ($P>0.05$). Salinity appeared to effect the feed intake-growth relationship more at higher rations than at lower ration levels.

During the early stages of reproductive development, immature fish had similar feeding rates and growth rates to those fish that were maturing. Thereafter, immature fish had higher growth rates. Two phases of sexual maturation were identified for maturing fish: an early phase (October – April) was characterised by slowly rising hormone levels concomitant with relatively high rates of feed intake and growth, and a late phase (May-October), steroid levels increased more rapidly and growth rates decreased in association with inappetence.

Discussion

The results illustrate the complexity of the interrelationships of feed intake and growth rate with photoperiod, ration, seawater and sexual maturation.

Although many underlying mechanisms which are involved in the regulation of a response to an environmental change are still to be answered, this paper seeks to explain how using individual based models can provide useful information that may be considered as part of a production plan for farmed salmon.

The findings presented in this paper also raise some interesting questions regarding best approaches to experimental design and statistical analyses when using groups of individual fish reared under culture.

References

Kenward, M.G. 1987. A method for comparing profiles of repeated measurements. Applied Statistics 36, 296-308.

Ricker, W.E. 1979. Growth rates and models, *In* Fish Physiology, vol. VIII. *Edited by* W.S. Hoar, D.J. Randall and J.R. Brett. Academic Press Inc., New York. pp. 677-743.

Stead, S.M., Houlihan, D.F., McLay, H.A. and Johnstone, R. 1996. Effect of ration and seawater transfer on food consumption and growth of Atlantic salmon, *Salmo salar L.,* smolts. Can. J. Fish. Aquat. Sci. 53: 1030-1037.

Stead, S.M., Houlihan, D.F., McLay, H.A. and Johnstone, R. 1999. Food consumption and growth in maturing Atlantic salmon, *Salmo salar* L. Can. J. Fish. Aquat. Sci. 56:1-10.

Talbot, C., and P.J. Higgins. 1983. A radiographic method for feeding studies on fish using metallic iron powder as a marker. J. Fish Biol. 23: 211-220.

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GENE EXPRESSION AND PROTEIN DEGRADATION PATHWAYS IN RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

The efficiency with which fish utilise their food is related not only to food consumption, but is also influenced by rate of protein turnover. There is evidence that fish that have lower protein turnover for a given amount of protein consumed are able to convert more of the ingested food into growth. Protein synthesis rates appear relatively similar between animals when variation in food consumption is allowed for, but protein degradation seems to hold the key in explaining the efficiency of conversion of food to growth (Houlihan et al., 1995). Protein degradation rates are generally derived from protein growth and synthesis measurements. This indirect approach is necessary partly due to the difficulties in measuring a process that is carried out by a variety of enzyme pathways. In order to make progress in this difficult but key area we are using several molecular approaches.

Both non-lysosomal and lysosomal pathways of protein degradation are involved in the control of the amino acid regulation, both these pathways are highly controlled and regulated (Attaix *et al*., 1999). The non lysosmal ATP dependant ubiquitin proteasome pathway, is the major route of degradation of muscle protein in mammals that releases amino acids and is dramatically upregulated during acute wasting diseases (Wing *et al*., 1995). During this route

of proteolysis targeted proteins are ubiquitinated prior to proteolytic cleavage by the proteasome. This is a tightly regulated process and mRNA levels for the ubiquitin proteasome pathway reflect the changes in proteolytic activity. In liver tissue the major route of protein breakdown is via the lysosome where cathepsins are especially important. The pathways that regulate protein turnover have not been studied in detail in fish. We have investigated pathways of protein breakdown in fed and starved rainbow trout using a variety of molecular approaches.

Results and Discussion

Northern blot analysis of RNA extracted from rainbow trout muscle tissue demonstrates an increase in abundance of polyubiquitin mRNA in fish that have had food withdrawn when compared to fed controls (figure 1). There is a corresponding increase in mRNA abundance of the proteasome subunit N3. These results confirm a general increase in gene expression for components of the ATP dependant protein degradation pathway (Wing et al., 1995). No up regulation for these genes was observed in RNA extracted from liver.

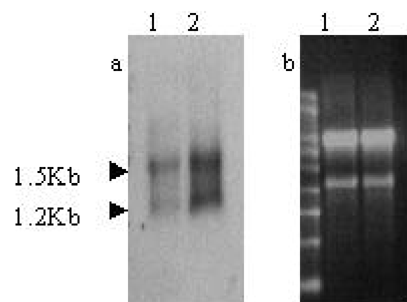

Figure 1. (a) Northern blot analysis of rainbow trout polyubiquitin mRNA in muscle tissue. 10µg of total RNA was separated on a 1.2% agarose gel, lane 1, fed fish, lane 2, food withdrawn. The RNA was transferred to nylon membrane and fixed by UV light. The membrane was probed with a trout polyubiquitin cDNA probe labelled with ^{32}P-dCTP, and washed to high stringency (0.2 x SSC, 65°C). (b) Ethidium bromide stained gel to demonstrate equal loading of RNA.

To identify the proteins / genes differentially expressed in the liver during starvation we are using proteomics to examine changes in protein expression patterns. Protein profiles generated by high resolution 2 D PAGE, coupled with digitised gel image analysis software allows the position and intensity of hundreds to thousands of proteins to be monitored, giving a global picture of these in proteins in a tissue under different metabolic states. In-gel trypsin digests of individual protein spots, combined with mass spectrometry to accurately size the tryptic peptides produces characteristic peptide fingerprints. The peptide masses were used to search the NCBI data base using the MASCOT search program (Perkins, *et al*., 1999).

One protein spot that was found to consistently more abundant in the starved fish was cut from the gel and the trypsin digest finger print produced allowed the protein to be identified as cathepsin D (Brooks *et al*., 1997). Figure 2 shows the abundance of the cathepsin D in six individual fish, three fed and three starved. This enzyme, a member of the pepsin family is a lysosomal endopeptidase involved in proteolysis.

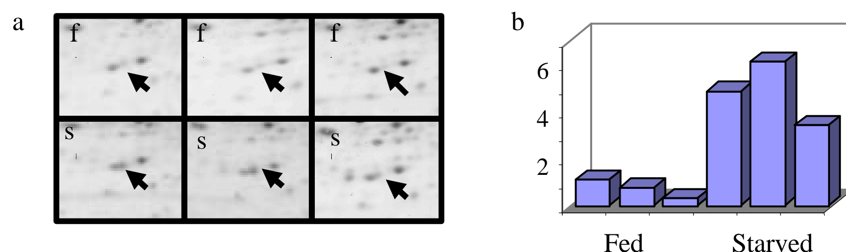

Figure 2.D protein patterns (a) were digitised using Phoretix 2-D software. Relative intensity of the cathepsin D protein in livers of fed and starved trout the protein spots were normalised for each gel (b).

In this study we have demonstrated that components of both the lysosomal and non lysosomal pathways of protein degradation are enhanced after a period of food withdrawal. Questions can now be addressed as to how these pathways are regulated in normal growth and if they can be correlated to feeding efficiency.

References

Attaix, D., Combaret, L. & Taillandier, D. 1999. Mechanisms and regulation in protein degradation. Protein Metabolism and Nutrition, Proceedings VIII, 51-67.

Brooks, S., Tyler, C.R., Carnevali, O., Coward, K. & Sumpter, J.P. 1997. Molecular characterisation of ovarian cathepsin D in the rainbow trout, *Oncorhynchus mykiss.* Gene 201: 45-54

Houlihan, D.F., Carter, C.G. & McCarthy, I.D. 1995. Protein synthesis in fish. In: *Biochemistry and Molecular Biology of Fishes*, vol. 4, chapter 8, Hochachka and Mommsen (eds.), Elsevier Science B.V., pp 191-220

Perkins, D.N., Pappin, D.J.C., Creasy, D.M. & Cottrell, J.S. 1999. Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis 20: 3551-3567

Wing, S.S., Haas, A.L. & Goldberg, A.L. 1995. Increase in ubiquitin-protein conjugates concomitant with the increase in proteolyis in rat skeletal muscle during starvation and atrophy denervation. Biochem. J. 307: 639-645.

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PHYSIOLOGICAL CONDITION OF

ORNAMENTAL FISH *Xiphophorus helleri*

FED AND STARVED

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Abstract

Ornamental aquarium fish, swordtail (*Xiphophorus helleri*), were fed twice a day with commercial tropical fish food, "Kantal", *ad libitum* and starved at 23°C for 15 days. To determine physiological condition, nucleic acids concentrations of the white and red muscles of the fish were analyzed by fluorometry. The DNA concentrations (µg mg^{-1} wet weight) of white and red muscles of starved fish were higher than those of the fed fish. The values of RNA concentrations of fed fish were always higher than those of starved fish in both muscles tested. However, the difference between the RNA values for starved and fed were lower in the red muscle. Red muscle values of RNA showed greater variations among individuals fasted. The RNA concentrations in red muscles of fed fish are superior to those in white muscle. This suggests that red muscle has a storage function similar to liver. The RNA/DNA ratio of the fed fish was always higher than the starved fish for both muscles. This indicates that RNA/DNA ratio of the white muscle can be used as an index of physiological condition of this species.

Key words: Physiological condition, Nucleic acids, Ornamental fish

Introduction

Fish of the family Poeciliidae, an ornamental fish, were used because of their ease of handling and their resistance in conditions of culture and high economic

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return of aquaculture (Fernando *et al*., 1991), compared to other species of ornamentals fishes (Olivier & Kaiser, 1997).

The swordtail fish, *Xiphophorus helleri*, a red variety, is one of the commercial ornamental fishes and is more frequently seen in aquarium store; the fish were obtained from the original green form, among other varieties. They are a prolific species among 270 breeding fish (Mills, 1984; Mills & Vevers, 1986), are resistant and energetic, and are aggressive with smaller animals, particularly males. They are distributed geographically in the South of Mexico (meridian zone) and Guatemala, where they live in rivers that outlet at the Atlantic Ocean. Their presence has also been noticed in the rivulet at the southwest of Queensland, Australia (Arthinton, 1989).

Studies of physiological aspects are limited specifically to what is referred to as growth, whether by weight or instantaneous growth (RNA/DNA). The juveniles of the red fish, *Sciarnops ocellatus*, had demonstrated that the levels of RNA/DNA were sensitive to environmental factors such as temperature, salinity and feeding regimes (Chung *et al.*, 1988, 1991, 1993).

The objective of this work is to determine nucleic acid levels in white and red musculatures and to use RNA/DNA ratio as physiological condition of sword-tail fish *Xiphophorus helleri* in laboratory conditions, with the same feeding and suppression.

Materials and Methods

Fish (2.82 ± 0.28 g wet weight) were obtained from a commercial store, in Maracay city (Aragua State, Venezuela), acclimated to a laboratory, then placed in an experimental aquarium of 60 liters. They were fed *ad libitum*, or starved during a period of 15 days. After the experimental time had passed, 6 samples of each treatment were sacrificed to extract samples from the epaxial white muscle, dorsal musculature and the red muscle under the lateral line.

Tissues were frozen at –20ºC until the moment of analysis. Determinations of RNA and DNA were made taking 15 mg of both tissues and were processed by the fluorometric method (Canino & Calderone, 1995). Two-way analysis of variance was used to determine the effects of feeding and the kind of tissue on the RNA and DNA levels.

Results and Discussion

The set of organisms submitted to fasting survived 15 days of the treatment. Analysis of variance revealed that there were significant differences between RNA and DNA levels and RNA/DNA ratios in both muscles types and feeding conditions, as well as an interactive effect between the variables (Table 1).

DNA levels were different with respect to the tissue, being major on red and white muscle of the no feeding fish (138.66 ± 6.66 µg g^{-1} wet mass and 136.01 ± 8.66, respectively), compared to red and white muscles of the feeding fish (130.66 ± 21.33 and 110.66 ± 0.66 µg g^{-1} wet mass, respectively) (Table 2). Thus, the feeding condition appeared to have a major effect on the DNA values.

Table 1. The results of two ways analysis of variance for comparision of RNA, DNA and RNA/DNA ratio in muscles of *Xiphophorus helleri* fed and fasted during 15 days (*p < 0.05; ** p < 0.01; *** p < 0.001, Ns: p > 0.05).

Variations	Value		
	DNA	RNA	RNA/ DNA
Among tissues	11.88 **	8.41 **	6.34 **
Feeding conditions	4.41 **	18.21 ***	18.57 ***
Interaction	1.30 Ns	19.34 ***	19.40 ***

The basic levels of RNA/ DNA in the white and red tissues of *Xiphophorus helleri* under conditions of feeding show that the white muscle tissue has a higher rate of growth than the red tissue. It is probably related to the white tissue's capacity to grow and to provide metabolic substrates to other tissues, while red musculature seems to be more related to the energetic contributions to

fast movement of the fish. It is marked by a great variation over the energetic substrates of both tissues, and their mobilization is related to physiological processes such as instantaneous growth (Lemus *et al*., 1993).

Table 2. Average values (± SD) of RNA and DNA in white and red muscles of *Xiphophorus helleri* fed and fasted for 15 days.

Experiment conditions		DNA $\mu g\ g^{-1}$ wet tissue	RNA $\mu g\ g^{-1}$ wet tissue
Fasting	White muscle	136.01 ± 8.66	126.6 ± 4.01
	Red muscle	138.66 ± 6.66	120.02±10.93
Feed-ing	White muscle	110.66 ± 0.66	157.32±10.66
	Red muscle	130.66 ± 21.33	125.33 ± 6.66

The levels of RNA/DNA ratio on the white musculature of the feeding organisms was 1.41 ± 0.10, higher than that observed in the same tissue in conditions of initiation (0.48 ± 0.17), demonstrating that there exists a significant increase on the rate of instantaneous growth. However, the red musculature showed less difference with high variation on the levels of RNA/DNA under feeding conditions (Fig. 1).

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References

Arthinton, A.H. 1989. Diel of *Gambusia affinis holbrookki*, *Xiphophorus helleri*, *X. maculatus* and *Poecilia reticulata* (Pisces: Poecilidae) in streams of southeastern Queensland, Australia. Journal of Asian Fisheries Society 2:193-212.

Chung, K.S., M. Nirchio, G.J. Holt & C. Arnold. 1988. Acidos nucleicos en la musculatura blanca y roja de juveniles del pez rojo *Sciaenops ocellatus*. Boletín del Instituto Oceanográfico de Venezuela 27:123-127.

Chung, K.S., J.G. Holt & C. Arnold. 1991. Influencia de la privación de la dieta y realimentación sobre el crecimiento y la relación ARN/ADN en los juveniles del pez rojo *Sciaenops ocellatus*. Acta Científica Venezolana 42:355-344.

Chung, K.S., G.J. Holt & C. Arnold. 1993. Influence of salinity and food deprivation on growth and RNA-DNA ratio in red drum *Sciaenops ocellatus* (Pisces: Scianidae). Revista de Biología Tropical 41:187-195.

Fernando, A, V.P.E. Phang & S.Y. Chan. 1991. Diets and feeding regimes of poeciliid fishes. Journal of Asian Fisheries Society 4:99-107.

Lumus, M., K S. Chung & G..J. Holt. 1993. Efecto de la temperatura sobre el crecimiento de juveniles de *Petenia kraussii* (Pisces: Cichlidae): Relación ARN/ADN. Revista de Biología Tropical 41:45-48.

Mills, D. 1984. Community fishes. A fish keeper's guide. Salamander Book Limited, London. 117 pp.

Mills, D. & G. Vevers. 1986. Guía práctica ilustrada de los peces de acuario. Editorial Blume S.A. Barcelona, España. 208 pp.

Olivier, A. & H. Kaiser. 1997. A comparison of growth, survival rate, and number of marketable fish produced of swordtails, *Xiphophorus helleri* Heckel (Family Poeciliidea), between two types of culture system. Aquaculture Research 28:215-221.

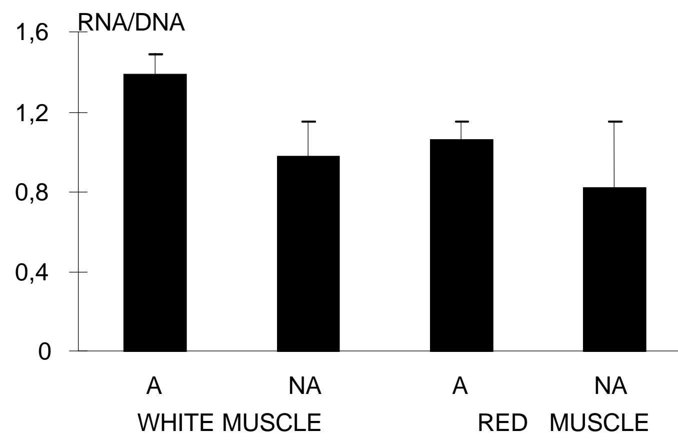

Fig 1. Average value of RNA/DNA ratios in white and red muscles of X*iphophorus helleri* in fed (A) and fasted (NA) conditions.

GROWTH EFFICIENCY

IN TRANSGENIC TILAPIA (*Oreochromis sp*.)

CARRYING A SINGLE COPY

OF AN HOMOLOGOUS cDNA GROWTH HORMONE

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Abstract

Growth hormone (GH) has been shown to have a profound impact on fish physiology and metabolism. However, detailed studies in transgenic fish have not been conducted. We have characterized the food conversion efficiency, protein profile and biochemical correlates of growth rate in transgenic tilapia expressing the tilapia GH cDNA under the control of human cytomegalovirus regulatory sequences. Transgenic tilapia exhibited about 3.6 fold less food consumption than non-transgenic controls ($P<0.001$). The food conversion efficiency was significantly ($P<0.05$) higher (290%) in transgenic tilapia (2.3±0.4) when compared to the control group (0.8±0.2). Efficiency of growth, synthesis retention and anabolic stimulation and average protein synthesis were higher in transgenic than in non-transgenic tilapia. Distinctive metabolic differences were found in transgenic juvenile tilapia. We had found differences in hepatic glucose and in agreement with previous results we observed differences in the level of the enzymatic activity in target organs. We conclude

that GH-transgenic juvenile tilapia show altered physiological and metabolic conditions and are biologically more efficient.

Introduction

Previously we have reported the generation of transgenic tilapia with improved growth performance (13,14). These tilapia express low ectopic levels of tilapia GH (tiGH) (13,14,15,16,17). Here we present the results of a series of correlated simultaneous experiments in which growth, food consumption, conversion efficiency and the activity of key metabolic enzymes in muscle, liver and plasma were monitored for transgenic tilapia. The aim of the study was to examine the relation between consumption and growth and between biochemical correlates of growth rate in transgenic and non-transgenic tilapia to help to understand the effects produced by the ectopically expressed GH.

Results

We have previously obtained and characterized a fast-growing GH-transgenic tilapia line (13, 14, 15,16,17). The enhanced growth produced by the ectopic expression of tiGH in this transgenic tilapia could be due to increased food consumption and/or improved food conversion. To differentiate these two possible mechanisms, the total food consumption was carefully recorded weekly. When relative food consumption rate was calculated, it was found that transgenic tilapia, when compared to non-transgenics, had a lower food consumption rate (Table 1). Furthermore, the food conversion efficiency was increased by 290% (Table 1).

Table 1. Experiment 1: Growth efficiency in transgenic and non-transgenic juvenile tilapia.

Parameters	Transgenic	Non-Transgenic	T/NTx100
Initial weight (W1,week 1) (g)	104.8 ± 5.9	109.7 ± 5.9	---
Final weight (W2,week 5) (g)	126.2 ± 8.6	137.5 ± 10.7	---
Total food intake (TFI) (g)	9.1 ± 1.8*	33.0 ± 5.2*	28 %
Relative food consumption rate [TFI/(W1 x 35 days)]	0.002 ± 0.003*	0.009 ± 0.01*	22 %
Food conversion efficiency (total fish weight gain/TFI)	2.3 ± 0.4**	0.8 ± 0.2**	290 %

Tilapias were weekly weighted and he food intake determined to calculate the parameters of growth efficiency. Values are the average ± SE (N=10). *P<0.001, **P<0.05 (Student t-Test).

Transgenic tilapia grow 60%-80% faster than non-transgenic siblings (14). However, under the experimental conditions employed here, specific growth rates were similar in both experimental groups (0.6 ± 0.2 %day^{-1}). This fact could be explained by rearing under laboratory conditions that are not optimal for growth. Nevertheless, the data obtained and the analyses conducted by us are valid as we compared experimental groups with similar specific growth rates but different biochemical and metabolic requirements due to the transgene expression.

Table 2. Experiment 2: Biochemical correlates of growth rate in juvenile tilapia.

Coefficient	***Transgenic***	Non-Transgenic	(T/NT)
Protein synthesis, Ks (% day^{-1})	0.35	0.17	2
Protein synthesis per day (% mg day^{-1})	0.07 8± 0.02*	0.028 ± 0.004*	2.7
Protein growth, Kg (%day^{-1})	0.26 ± 0.06**	0.15 ± 0.03**	1.7
Ration consumption, Kr (% day^{-1})	0.7	2.4	3.6
Growth efficiency, Kg/Kr (%)× 100	37.4	6.45	5.7
Protein synthesis retention efficiency, Ks/Kg (%)× 100	133.5	64.5	2
Anabolic stimulation efficiency, Ks/Kr (%)× 100	50	4	12.5

Average ± SD (N=10), *P<0.01, **P<0.05 (Student t-Test).

Average protein synthesis and protein growth (p<0.05) was higher in transgenic than in non-transgenic tilapia (Table 2) Transgenic tilapia also showed a lower ration consumption (Tables 1 and 2). Therefore, the efficiency of growth, synthesis retention and anabolic stimulation were higher in transgenic tilapia.

Table 3. Biochemical and morphometric analyses conducted in juvenile tilapia.

Parameters	Plasma		Liver		Muscle	
	Transgenic	Non Transgenic	Transgenic	Non Transgenic	Transgenic	Non Transgenic
GOT	284±54 µU/mg	246±66 µU/mg	21.4±2.3 mU/mg*	49.3±6.5 mU/mg*	99.2±13.6 mU/mg*	57.5±13.3 mU/mg*
GPT	26.3±6.7 µU/mg	18.2±10.4 µU/mg	6.0±1.1 mU/mg*	28.4±3.4 mU/mg*	6.8±0.9 mU/mg*	3.3±0.6 mU/mg*
LDH	ND	ND	2.1±0.2 mU/mg	2.1±0.4 mU/mg	7.5±1.2 mU/mg	6.4±0.6 mU/mg
Lactate	ND	ND	550±83 µM	400±55 µM	4.9±0.2 mM	5.6±0.3 mM
Pyruvate kinase	ND	ND	0.20±0.02*	0.10±0.01*	-	-
Glucose	1.2±0.2 mM	1.5±0.2 mM	40.8±2.8 mM*	54.2±3.9 mM*	0.34±0.06 mM	0.38±0.06 mM
Glycogen	ND	ND	0.28±0.06 mmol/g	0.27±0.03 mmol/g	ND	ND
Hepat. Index	ND	ND	2.4±0.1 %	2.4±0.2 %	ND	ND

Values are the average ± SD (N=10). *P<0.05 (Student t-Test). ND not determined.

The GH exerts its growth-promoting action through different metabolic pathways. Previous results had shown differences in free alanine and aspartic acid levels in the muscle of juvenile transgenic tilapia (14). An increase in the GOT and GPT transaminases was found at this stage of life in transgenic fish, but not in lactate dehydrogenase enzyme activity, neither in the lactate nor glucose levels in muscle tissue (Table 3). Transgenic juvenile tilapia had lower hepatic glucose and a higher pyruvate kinase activity, showing an enhanced glycolysis when compared to non-transgenics (Table 3). There were no differences regarding the levels of lactate and glycogen, neither in the hepatosomatic index between transgenic and non-transgenic tilapia (Table 3). In adult animals, no differences were found in the parameters measured (Table 4).

Table 4. Biochemical analyses conducted in adult tilapia.

Parameters	Plasma		Liver		Muscle	
	Transgenic	Non Transgenic	Transgenic	Non Transgenic	Transgenic	Non Transgenic
GOT	582±71 µU/mg	596±60 µU/mg	115±11 mU/mg	161±33 mU/mg	19.2±2.1 mU/mg	23.1±2.9 mU/mg
GPT	325±73 µU/mg	188±32 µU/mg	67±12 mU/mg	80±10 mU/mg	6.2±0.4 mU/mg	7.7±1.2 mU/mg
LDH	ND	ND	ND	ND	10.1±1.8 mU/mg	14.0±3.7 mU/mg
Glucose	7.5±0.7 mM	7.0±0.4 mM	21.2±2.5 mM	18.6±1.3 mM	3.5±0.1 mM	3.4±0.1 mM
Glycogen	ND	ND	0.35±0.03 mmol/g	0.34±0.08 mmol/g	ND	ND

Values are the average ± SD (N=8). P>0.05 (Student t-Test). ND not determined.

The total contents of RNA, DNA and protein were measured in juvenile and adult muscle of transgenic and non-transgenic tilapia. No differences were found except in the d RNA/protein index in transgenic and adult muscle, respectively

Discussion

The transfer of GH transgenes has resulted in growth acceleration of economically important fish species (11). However, how much of this growth improvement is due to higher ration consumption or to better growth efficiency has not been determined. This is a fundamental question for biological studies and for cost-effective analysis.

Higher growth rates have been shown to be the result of reduced maintenance costs and increased metabolic efficiency (18). In bovine GH (bGH)-injected striped bass hybrids an increase in the specific growth rate and food conversion efficiency without significant alteration of food consumption rate has been reported (8,9). Due to the treatment with bGH, the relative nitrogen retention increases by 20% together with the intestinal nutrient absorption (27). Four

weeks of GH treatment do not significantly alter water, non-protein nitrogen, protein ash and fiber content when expressed as percent of fresh tissue weight. The mean DNA concentrations (mg g^{-1} tissue) do not show any appreciable change but the mean RNA/DNA and protein/DNA ratios are significantly higher for the treated fish (8,9). Furthermore, GH treatment results in a significant variation in the level of some amino acids (27). Also, trout with higher protein growth efficiency are more efficient in their retention of synthesized protein (28). It has been reported food conversion efficiency in rainbow trout is stimulated after the application of ovine growth hormone (29) .

Transgenic F70 tilapia showed a higher protein synthesis rate and protein growth and lower ration consumption, resulting in higher efficiency of growth, synthesis retention and anabolic stimulation It has been proved no differences in the digestibility test among transgenic, non-transgenic and wild type tilapia (17). Therefore, these transgenic tilapia are metabolically more efficient, capable of supporting growth with better food conversion efficiency. Similar results have been reported by Krasnov et al. (30) in rapidly growing transgenic Arctic char. They found specific growth rate and muscle protein content equal with respect to non-transgenic siblings. However, the rate of NH_4 excretion appeared equal in control and transgenic fish, therefore indicating that the rapid growth correlates with higher efficiency of protein retention. In transgenic carps expressing the trout GH transgene, an increase in muscle protein content of about 7.5% and variation in some aminoacid levels were also reported (31). Studies carry out by Zongbin et al, (32) in the MThGH-transgenic F_2 red carp (Cyprinus carpio L.red var.) showed feeding rates of transgenic significantly lower than of the non transgenic control, however the specific growth rates of the transgenic in wet weight, dry weight, energy and protein and the conversion efficiencies of the transgenic F_2 in all these parameters were also higher than non-transgenic fish.
The results obtained in wild type or GH-injected fish are essentially in accordance with the results obtained in transgenic tilapia expressing ectopic tiGH. Differences in the magnitude of the effect may respond to the levels of GH present in each case.

Transgenic tilapia will need to partition a lower proportion of ingested energy into basal metabolism and the replacement of existing body tissue, making more available for growth. How are transgenic tilapia obtaining the energy required to support a better and more efficient growth rate? It looks like GH-transgenic fish utilize the energy released by oxidation of aminoacids more efficiently.
Transgenic tilapia F70 express ectopic tiGH in various tissues including the liver, muscle, gonads and brain (13, 15). For biochemical analyses we selected

the muscle, liver and plasma. Studies in the muscle correlate well with estimates for the whole body (33) and are the portion of the animal used for commercialization and human consumption. The liver is an important organ for biochemical studies and is the target of GH action to induce the expression of insulin-like growth factors (IGF) which, together with GH, provoke the growth-promoting action (10). The plasma connects all organs of the body and reflects the nutritional status of the organism, affecting among other factors, the synthesis of GH and IGF (34,35).

Biochemical studies were conducted in juvenile and adult tilapia. We have shown that the effect of ectopic tiGH on growth performance is more pronounced in juvenile transgenic tilapia (13,36), therefore reflecting better the biochemical processes induced by ectopic GH. Adult tilapia, on the other hand, will be used for human consumption and it is of special interest to compare in these animals the biochemical profile of transgenic and non-transgenic tilapia. Juvenile transgenic tilapia have reduced free levels of alanine and aspartic acid in the muscle when compared to non-transgenic controls (14). It is probably these gluconeogenic amino acids are used to produce energy (37). The increase in the GOT and GPT transaminases in the muscle correlated well with the decrease in alanine and aspartic acid levels as these enzymes are involved in the production of energy from these amino acids (38). Although it is not common the oxidation of amino acids by muscle cells, this reaction could be favored in GH-transgenic tilapia. Gluconeogenesis from alanine has been reported in rainbow trout (39) and coho salmon (*Oncorhynchus kisutch*) (40) hepatocytes and in the eel *Anguilla japonica* (41).

In the liver, the opposite effect was recorded. In this tissue the activity of GOT and GPT was lower in juvenile transgenic tilapia, thus suggesting that in the liver gluconeogenic aminoacids are not used for energy production. Increase activity in hepatic GOT and GPT has been described for Red Sea bream (*Chrysophrys major*) that conserve the glycogen and metabolize proteins during starvation at low temperature (42).

The potential for gluconeogenesis could be assessed indirectly by measuring kinetic parameters of liver pyruvate kinase (43). The lower hepatic glucose and higher pyruvate kinase activity could reflected that, in juvenile transgenic tilapia, glucose was used in the liver to produce energy. However, since the levels of glycogen remained unchanged, the glucose used for oxidation and energy production was not obtained from hepatic glycogen. Although these results reflect a metabolic disbalance in the liver of juvenile transgenic tilapia,

the maintenance of the hepatosomatic index denotes that this disbalance is probably within physiological levels. The injection of high supraphysiological concentrations of recombinant tiGH in juvenile *O.aureus* tilapia results in the increase of the hepatosomatic index (10).

An increase in the RNA/DNA ratio was found in the muscle of juvenile transgenic tilapia. This result reflected an increase in the protein synthesis in these tilapia. Similar results have been reported for GH-treated fish (8,9). In adult transgenic tilapia, an increase in the RNA/protein ratio reflected an effect of ectopic tiGH on ribosomal capacity.

Biochemical analyses in adult tilapia showed no differences between transgenic and non-transgenic animals. This result is important for the evaluation of the possible effects of consuming transgenic tilapia as it further documents that transgenic tilapia F70 are safe as food (44).

In conclusion, the results reported by us support that (a) transgenic tilapia have a better food conversion efficiency, protein synthesis and growth efficiency adding more value to this transgenic line and supporting that differences in protein turnover are important determinants of growth efficiency in fish (18) and (b) we have found differences in the hepatic glucose values and the muscle GOT and GPT activity to compare transgenic and non transgenic fish. The energy required for the accelerated growth in juvenile transgenic tilapia could be produced from hepatic glucose and the gluconeogenic amino acids alanine and aspartic acid oxidation in muscle.

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

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Abstract

Trout were injected (1.5 µg g BW^{-1} wk^{-1}), or fed on diets supplemented with (10 and 100µg g BW^{-1} 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 µg g BW^{-1} 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 ± 0.15 g and length: 138.2 ± 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 ± 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 µg g BW^{-1} wk^{-1}); GHby injected (1.5 µg g BW^{-1} wk^{-1}) and three diets, two of which

contained GHby calculated to deliver doses of 10 and 100 μg g BW^{-1} wk^{-1}. Injected volumes were 200 μl. 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 μg g BW^{-1} wk^{-1}) and 0.1 g GHby mL^{-1} (100 μg g BW^{-1} 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 μg g BW^{-1} wk^{-1} GHby fed and control groups (n = 8/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

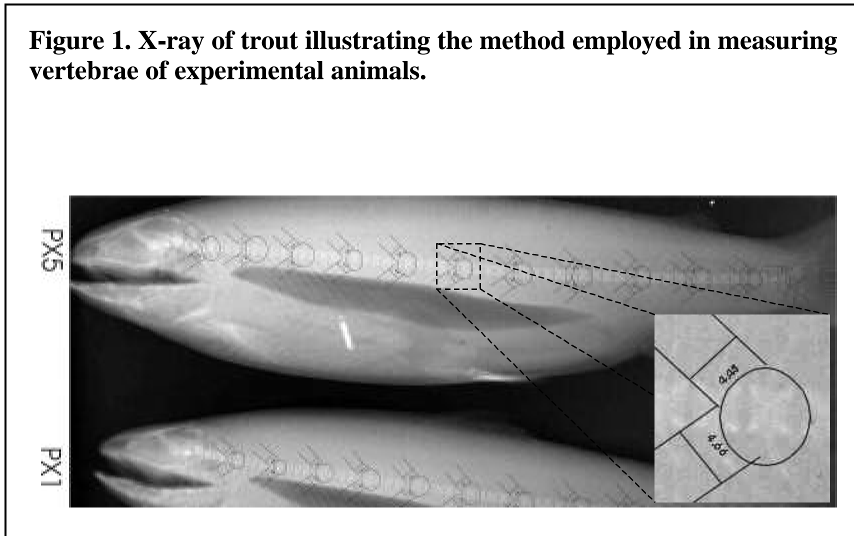
was built, where y_{ijk} represented observed data (Montgomery, 1997). Significant differences between treatments identified by ANOVA were further subjected to Duncan's multiple range test to determine which means differed (Montgomery, 1997).

The model applied was:

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

where μ 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).

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



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±0.1 to 1.46±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[a]	131.1±5.78[ac]	137.7±0.72[a]	209.1±2.83[ac]
Control inj.	32.6±0.40[a]	127.2±5.27[c]	138.5±0.68[a]	208.6±2.53[c]
GHby10	32.9±0.47[a]	137.0±6.19[ab]	138.4±0.78[a]	212.8±2.91[ab]
GHby100	32.6±0.68[a]	140.3±8.60[b]	139.7±1.50[a]	215.1±4.26[b]
GHby inj.	32.9±0.49[a]	151.5±5.95[d]	137.8±0.75[a]	220.5±2.35[d]
rbGH inj.	33.1±0.43[a]	157.8±7.35[d]	138.1±0.67[a]	222.1±3.12[d]

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_{wt}	SGR_{l}	FCE
Control	1.64±0.05[bc]	0.50±0.01[a]	0.822
Control inj.	1.60±0.04[c]	0.49±0.01[a]	0.833
GHby10	1.68±0.47[a]	0.50±0.02[a]	0.792
GHby100	1.71±0.06[a]	0.51±0.02[a]	0.801
GHby inj.	1.78±0.04[d]	0.55±0.01[b]	0.752
rbGH inj.	1.82±0.04[d]	0.56±0.01[b]	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^{a}	51.45 ± 0.80^{ab}	26.60 ± 0.59^{a}
Control inj.	83.86 ± 0.43^{a}	52.44 ± 0.85^{a}	25.95 ± 0.89^{a}
GHby10	83.51 ± 1.46^{a}	52.75 ± 0.69^{a}	26.40 ± 0.77^{a}
GHby100	84.60 ± 4.32^{a}	51.98 ± 1.64^{ab}	28.89 ± 0.68^{b}
GHby inj.	83.16 ± 0.42^{a}	50.95 ± 1.13^{b}	28.41 ± 0.92^{b}
rbGH inj.	82.81 ± 0.43^{a}	51.51 ± 0.88^{ab}	27.83 ± 0.60^{b}

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

Irrespective of treatment or time of examination, no effect was observed when comparing every 5th 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 $80\%^{+}$ level. Such a level of bioactivity would correspond to by-product injected fish receiving a dose of 1.2 µg g BW^{-1} $week^{-1}$ 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 $99\%^{+}$ 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.

References

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

Danzmann, R.G., van der Kraak, G., Chen, T.T. and Powers, D.A. 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.

Garber, M.J., DeYong, K.G., Byatt, J.C., Lellis, W.A., Honeyfield, D.C., Bull, R.C., Schelling, G.T. and Roeder, R.A. 1995. Dose-response effects of recombinant bovine somatotropin (Prosilac^{TM}) on growth performance and body composition of two-year-old rainbow trout (*Oncorhynchus mykiss*). J Anim Sci, 73: 3216-3222.

Gerrits, P.O. 1990. Staining procedures for tissues embedded in 2-Hydroxyethyl Methacrylate. Heraeus Kulzer, 2-4.

McLean, E. and Devlin, R.H. 2000. Application of biotechnology to enhance growth of salmonids and other fish. In: *Recent Advances in Marine Biotechnology*. M. Fingerman and R. Nagabhushnam (eds), pp. 17-55. Science Publishers Incorporated, Enfield, New Hampshire, USA.

McLean, E., Teskeredzic, E., Donaldson, E.M. and Souza, L.M. 1993. Accelerated growth of diploid and triploid coho salmon *Oncorhynchus kisutch* following dietary administration of recombinant porcine somatotropin. Fish Physiol. Biochem., 11: 363-369.

McLean, E., Ronsholdt, B., Sten, C. and Najamuddin 1999. Gastrointestinal delivery of peptide and protein drugs to aquacultured teleosts. Aquaculture, 177: 231-247.

Montgomery, D.C. (1997). Design and Analysis of Experiments. New York: John Wiley & Sons.

Rasmussen, R.S., Ostenfeld, T.H., Ronsholdt, B. and McLean, E. 2000. Manipulation of end-product quality in rainbow trout with finishing diets. Aquaculture Nutrition, 6: 17-23.

Ronsholdt, B., Nielsen, H., Faergemand, J. and McLean, E. 2000. Evaluation of image analysis as a method for examining carcass composition of rainbow trout. Ribarstvo, 58: 3-11.

Schep, L.J., Tucker, I.G., Young, G., Ledger, R. and Butt, A.G. (1999). Controlled release opportunities for oral peptide delivery in aquaculture. J. Contr. Rel., 59: 1-14.

Tsai, H.J., Kuo, J.C., Lou, S.W. and Kuo, T.T. 1994. Growth enhancement of juvenile striped mullet by feeding recombinant yeasts containing fish growth hormone. Prog. Fish.–Cult., 56: 7-12.

Weatherley, A.H. and Gill, H.S. 1987. The Biology of Fish Growth. London: Academic Press.

GROWTH HORMONE ENDOCRINOLOGY OF ATLANTIC SALMON DURING PARR-SMOLT TRANSFORMATION

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

The parr-smolt transformation (smoltification) of anadromous salmonids involves both physiological and biochemical changes with enhanced seawater (SW) adaptability as one of the characteristics features. Growth hormone (GH) is considered one of the main regulators of this developmental process, improving hypoosmoregulatory ability, as well as regulating growth and behaviour (Björnsson, 1997). Plasma GH levels increase significantly during smoltification, concomitant with improved SW tolerance and increased gill Na^+, K^+-ATPase activity (McCormick *et al*., 1995). Insulin-like growth factor I (IGF-I) can improve SW tolerance and may thus mediate long-term actions of GH in SW acclimatisation (McCormick, 1996).

During the parr-smolt transformation of the Atlantic salmon, a frequently observed feature is that the plasma GH profile has two distinct peaks, and it has been speculated that this is due to interplay between GH secretion rate and the metabolic clearance rate of the hormone (Björnsson *et al*.2000), which have been shown to increase after SW transfer in coho salmon and rainbow trout (Sakamoto *et al*. 1991). Further, Yada *et al*. (1992) demonstrated that in amago salmon, that response of GH mRNA to seawater exposure is related to the

development of preparatory mechanisms for SW entry, thereby speculating that GH mRNA increases before SW entry.

In order to elucidate the endocrine mechanisms that are underlying these changes in plasma growth hormone (GH) levels during the parr-smolt transformation, Atlantic salmons were kept in outside tanks, under natural condition from early February until early July. Approximately three times a month, pituitaries and blood were sampled for *in vitro* GH secretion studies, GH mRNA expression, total pituitary GH content, plasma GH and IGF-I levels.

Results and Discussion

During the parr-smolt transformation of the Atlantic salmon, a concurrent assessment of pituitary GH gene expression, storage and secretion was made to establish the chain of events leading to the observed changes in plasma GH. In mid- to late April, an increased secretion rate caused the plasma GH levels to rise, without triggering new synthesis of GH or the amount of GH stored in the somatotrophs to drop. This indicates that the pituitaries are secreting stored GH. From mid-April to mid-May, the GH secretion increased, causing parallel rise in GH gene expression. As a consequence, there is a drop in the total pituitary GH content before increasing synthesis of GH replenishes this.

A likely explanation for the “split-peak” in plasma GH profile, often seen during salmon smoltification (Björnsson *et al*., 2000; McCormick *et al*., 1995), is an increased metabolic clearance rate of GH (Sakamoto *et al*., 1991). Towards the end of this study, there was a gradual rise in plasma GH levels, in spite of a concurrent decrease in both GH secretion and GH gene expression occurred. This indicates a decreased demand for GH, perhaps due to down-regulation of GH receptors and thereby lower metabolic clearance rate. In conclusion, the study demonstrates that there is a complex interplay between GH production, storage, secretion and plasma levels during the parr-smolt transformation of the Atlantic salmon.

References

Björnsson, B. Th. 1997. The biology of salmon growth hormone: from daylight to dominance. Fish Physiol. Biochem. 17: 9-24.

Björnsson, B. Th., Hemre, G. I., Björnevik, M. and Hansen, T. 2000. Photoperiod regulation of plasma growth hormone levels during induced smoltification of underyearling Atlantic salmon. Gen. Comp. Endocrinol. In press.

McCormick, S. D., Björnsson, B. Th., Sheridan, M., Eilertson, C., Carey, J. B. and O´dea, M. 1995. Increased daylength stimulates plasma growth hormone and gill Na^+,K^+-ATPase in Atlantic salmon (*Salmo salar*). J. Comp. Physiol. B 165:245-254.

McCromick, S. D. 1996. Effects of growth hormone and insulin-like growth factor I on salinity tolerance and gill Na^+,K^+-ATPase in atlantic salmon (*Salmo salar*): interaction with cortisol. Gen. Comp. Endocrinol. 101:3-11.

Sakamoto, T. and Hirano, T. 1991. Growth hormone receptors in the liver and osmoregulatory organs of rainbow trout: characterization and dynamics during adaptation to seawater. J. Endocrinol. 130: 425-433.

Yada, T., Kobayashi, T., Urano, A. and Hirano, T. 1992. Changes in growth hormone and prolactin messenger ribonucleic acid levels during seawater adaptation of amago salmon (*Oncorhynchus rhodurus*). J. Exp. Zool. 262

GROWTH HORMONE PROFILES AND DEVELOPMENT OF SOMATOTROPHS IN ATLANTIC HALIBUT LARVAE

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Introduction

The Atlantic halibut is the largest flatfish species, and as other flatfish, has a complicated larval development. The pelagic larvae hatch after about two weeks and feeding starts six weeks later. After three to four months, they start to undergo metamorphosis. Following major changes in body shape, including the

migration of the left to the right side, the larvae settle as bottom dwelling. In Atlantic halibut aquaculture, the larval rearing is a critical rearing stage, with high incidence of mortality and abnormal development.

Growth hormone in teleost fishes is known to participate in the regulation of several important physiological processes including metabolism, growth, appetite, and osmoregulation. It is therefore likely that the hormone may be important for larval growth and development.

Therefore, this study was carried out in order to elucidate the growth hormone (GH) endocrinology of halibut larvae, by measuring GH tissue content as well as the histology of the pituitary somatotrophs

Materials and Methods

The study was carried out with unfertilized eggs and larvae from hatching through metamorphosis (from 22 to 859 day-degrees (D°)), collected at the halibut hatchery of Fiskey Ltd, Northern Iceland, over two consecutive years.

In order to study GH profiles during early development, a homologous radioimmunoassay was established. GH was isolated from adult halibut pituitaries collected at Fiskey Ltd, using methods modified from Johnson *et al* (1997). In the radioimmunoassay, this GH was used for standards and iodination, together with specific antibodies raised in rabbits, courtesy of Dr. P. Swanson.

For the immunohistochemistry of GH-producing somatotrophs in the pituitaries, anti-halibut antibodies were raised in rabbits and validated.

Results and Conclusions

Tissue GH analysis revealed that GH is detectable in unfertilized eggs. In developing larvae, tissue GH content per body weight increased during development from hatching to metamorphosis. The earliest stage at which GH was localized in the somatotrophs by immunohistochemistry, was at the age of 187 D°.

The present study demonstrates that there appears to be a maternal source of growth hormone in halibut eggs, similar to what has been demonstrated for thyroid hormones in eggs of different teleost species (Kobuke *et al* 1987). The study further demonstrates that endogenous production of growth hormone is initiated early in larval development, during the yolk-sack stage, prior to first feeding. The study establishes that GH can play a regulatory role during early development of the Atlantic halibut

References

Johnson LL, Norberg B, Willis ML, Zebroski H and Swanson P. 1997 Isolation, characterization, and radioimmunoassay of Atlantic halibut somatolactin and plasma levels during stress and reproduction in flatfish. *Gen. Comp. Endocrinol.* **105**: 194-209.

Kobuke L, Specker JL and Bern HA 1987 Thyroxine content of eggs and larvae of coho salmon, *Oncorhynchus kisutch. J. Exp. Zool.* **242**: 89-94.

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THE EFFECT OF SALINITY ON FOOD CONSUMPTION AND GROWTH OF NILE TILAPIA (*OREOCHROMIS NILOTICUS* L.)

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Introduction

To date there is little information available explaining the effects of salinity on food consumption and individual growth performance of brackishwater fish including Nile tilapia (*O. niloticus*). When individual food consumption rates are known it is possible to examine the feeding behaviour of fish and to compare the nutritional status of an individual fish with its physiological performance. In recent years, X- radiography has been used to measure food consumption rates of individual fish under a variety of experimental conditions (Talbot and Higgins, 1983; Stead, *et.al.*, 1999). Hence, the aims of this study were: (i) to identify the optimum salinity for growth performance of O. *niloticus* and (ii) to compare the interrelationships between individual estimates for food consumption, growth rate and protein content of O. *niloticus* under different salinities.

Materials and Methods

The experiment commenced on 19 June 1998, for a duration of 75 days at the Dept. of Zoology, University of Aberdeen. Three treatments (salinity) with two replicates for each salinity T_1 (freshwater 0 ‰), T_2 (10 ‰) and T_3 (20 ‰) were

maintained by adding crude salt with recirculated, filtered aerated freshwater. All the treatments were stocked with the same size fish (average length and weight, 8.5 ± 0.24 cm and 9.94 ± 0.15 g, respectively) at the rate of 20 fingerlings per tank. Before stocking in the experimental tanks, fish were anaesthetised and individually freeze branded by using a number of combinations of brand marks on one lateral side. The fish were hand-fed (45% protein) at the rate of 2% bw. day $^{-1}$. A starvation experiment was conducted on three groups of fish (n=20) maintained at different salinities.

Measurements of individual food consumption

Individual food consumption was measured using a modified version of the X-radiography technique as used by Stead *et.al.*, (1999).

Measurement of protein content

At the end of the experiment, protein content of white muscle in each fish were measured, following the method described by Lowry *et al*., (1951).

Results

- Nile tilapia *O. niloticus* reared in different salinities (freshwater 0, 10 & 20 ‰) and fed the same ration level (2% bw. day^{-1}), showed no significant differences in specific growth rates, food consumption rates and food conversion ratios between and within the treatments (Fig. 1).

- Protein content in white muscle of individual fish were not significantly different between salinity groups (Fig.1b).

- No significant relationships were observed between individual specific growth rates and food consumption rates in the treatment (salinities) groups (Fig. 2a & 2b). A significant ($P<0.05$) negative correlation were observed between individual SGRs and FCRs for all fish (Fig. 2a & 2c).

- During the starvation experiment for control fish, weight loss (SGR % day^{-1}) was similar in all treatments and the effects of starvation on weight loss did not differ significantly between salinities (0, 10 and 20

‰) (Fig. 1c). After 7 days of starvation, more than 50% of the fish at the highest salinity (20 ‰) developed body lesions which covered 5-25% of their body surface possibly due to a higher osmoregulatry cost at the higher salinity (20 ‰).

- In all salinities (0, 10 and 20 ‰), fish in the present study were observed to exhibit aggressive behaviour when feeding and it is possible that the 2% bw. day^{-1} ration level was a restricted level for the *O. niloticus* fingerlings.

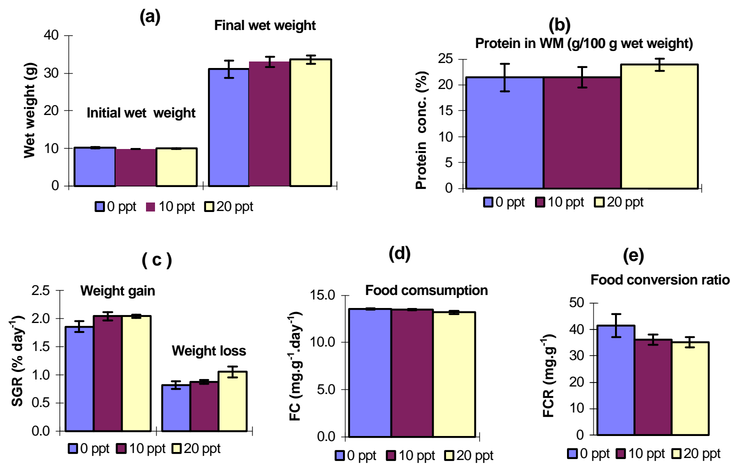

Figure 1: Comparisons of Tilapia growth under different salinity during the experimental period.

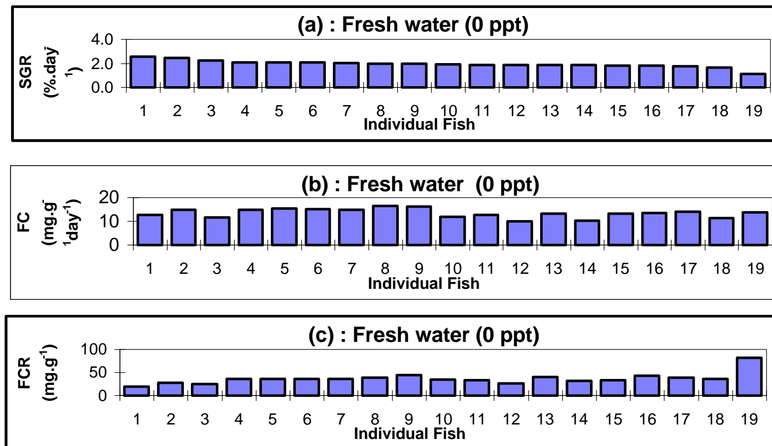

Figure 2: Relationships between SGR, FC and FCR for fish in freshwater (0‰). Individual SGR are ranked in descending order (a) and the respective FC are plotted directly below for each corresponding fish (b) and similarly for FCR (c).

Conclusion

Although changes in salinity (0, 10 and 20‰) did not appear to have a significant effect on growth performance of *O. niloticus,* the results reported the complexity of the interrelationships of food consumption and growth rate with salinity. Further experiments are required to establish unequivocally the mechanisms, which are involved in the regulation of a response in O.*niloticus* to salinity.

References

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Stead, S.M., D.F. Houlihan, H.A. Mclay and R. Johnstone. 1999. Food consumption and growth in maturing Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 56: 1-10.

Talbot, C., and P.J. Higgins. 1983. A radiographic method for feeding studies on fish using metallic iron as a marker. J. Fish. Biol. 23: 211-220.

EFFECTS OF DIETARY SOYBEAN MEAL ON THE APPARENT DIGESTIBILITY AND GALLBLADDER WEIGHT OF RAINBOW TROUT

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Attempts to replace the fish meal component of practical fish feed with soybean meals (SBM) have met with valuable success. However, applications of high level SBM in diets have resulted in reducing growth and poor feed conversion efficiency by anti-nutritional factors, amino acid imbalance, and lacking of phosphorus utilization of fish. No studies have been carried out on gallbladder relating to dietary SBM, although there were a few works with bile acid after feeding (Talbot and Higgins 1982; Avery et al. 1992). To evaluate the nutritional utilization of the SBM diet, in this study long-term feeding trial (120 day) was conducted to estimate the relationship between apparent digestibility of protein and lipid and gallbladder weight in rainbow trout fed SBM diets.

Materials and Methods

Eight experimental diets were used; 0%, 10%, 22%, 34%, 46%, 58%, 70% of SBM with approximately 44% protein content in seven formulated diets and commercial diet (CD) was used for control. Rainbow trout (mean weight 29g) was divided into 7 groups (73 fish each tank) with duplicate trials, and fed twice a day with *ad libitum*. Water temperature was maintained constantly at 17 . Digestibility of SBM diets was measured at 60 and 120 days of experimental period following Kim et al. (1996). The gallbladder of rainbow trout (n=8-9) starved for 40 hrs was weighted at 30, 60, 90, 120 day of experimental period for effect of long-term fed SBM diet. To find out changes of gallbladder weight ratio (GBWR) with time, the gallbladder of rainbow trout starved for 48 hrs was weighted after supplying 2% feed per fish weight at 120 days of experimental period. Then, the weight of gallbladder and the volume of serum bile acid of fish (n=8-10) from each treatment were measured at before feeding, after feeding 1 hr, 3 hrs, 6 hrs, 12 hrs, 18 hrs and 24 hrs, respectively.

Results and Conclusions

The digestibility of protein was 91.5~94.2% and 88.6~92.2% at 60 days and 120 days, respectively ($p>0.05$). The digestibility of lipid at 60 days of experimental period was 91.2~92.7% fed experimental diets contained less than 34% of SBM, while that of lipid at same time was 84.0~88.9% % fed experimental diets contained more than 34% of SBM ($p<0.05$).

Table 1. Gallbladder weight ratio (GBWR[1]) of rainbow trout fed with soybean meal diets.

SBM Content (%)	Experimental period (day)			
	30	60	90	120
0	0.31 ± 0.10^{abc}	0.30 ± 0.08^{bc}	0.26 ± 0.10^{ab}	0.30 ± 0.08^{a}
10	0.29 ± 0.13^{bc}	0.32 ± 0.08^{ab}	0.27 ± 0.10^{a}	0.32 ± 0.10^{a}
22	0.33 ± 0.08^{a}	0.34 ± 0.07^{a}	0.25 ± 0.09^{ab}	0.32 ± 0.08^{a}
34	0.31 ± 0.05^{abc}	0.26 ± 0.06^{cde}	0.25 ± 0.09^{ab}	0.28 ± 0.09^{a}
46	0.26 ± 0.05^{cd}	0.22 ± 0.06^{def}	0.23 ± 0.03^{bc}	0.20 ± 0.02^{b}
58	0.21 ± 0.02^{de}	0.21 ± 0.04^{ef}	0.14 ± 0.05^{cd}	0.14 ± 0.02^{bc}

70	0.19±0.04[e]	0.17±0.05[f]	0.12±0.03[d]	0.13±0.03[c]
CD	0.33±0.08[ab]	0.28±0.05[cd]	0.25±0.05[ab]	0.30±0.09[a]

[1] Weight of gallbladder/wet body weight × 100, values within column with same superscript are not significantly different (p>0.05).

The weight change of gallbladder that related digest of lipid was shown at Table 1. GBWR was significantly decreased in experimental groups, which fed diets contained more than 46% of SBM (p<0.05). The changes of GBWR were shown at Fig. 1.

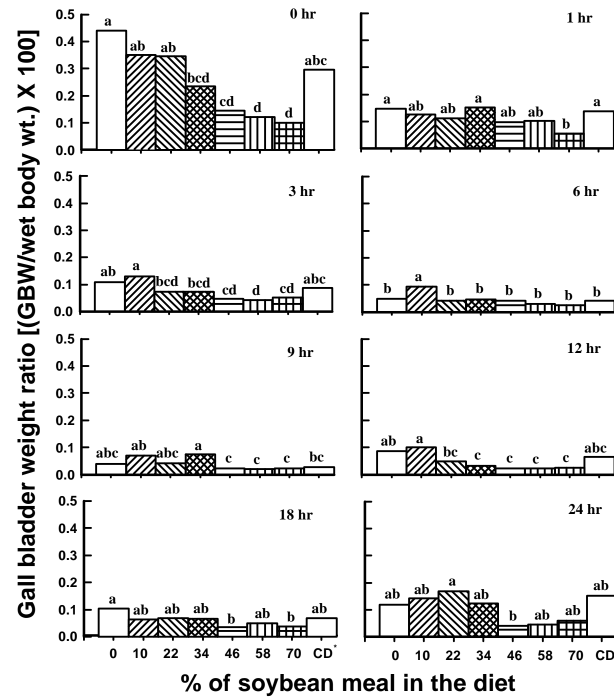

Fig. 1. Changes of gallbladder weight ratio (GBWR) in the rainbow trout tested with soybean meal diets. Hours indicate before and after feeding.

There was not significantly different GBWR of experimental fish fed diet contained SBM less than 22%, but the level of GBWR was significantly decreased at experimental fish fed diet contained SBM more than 34% ($p<0.05$). The level of GBWR was suddenly decreased after feeding, reached minimum level at after 9 hrs then slightly increased from 12 hrs and finally recovered by 27.3~60% of beginning of experimental time. The level of serum bile acid of control group was 20.2±3.8 µmol/l but that of 58% SBM group was 10.4 ±2.1.

Above results showed that rainbow trout could digest dietary protein well but digestibility of lipid was decreased due to reducing circulation of bile acid when the fish fed diet contained more than 34% of SBM.

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References

Avery, E. H., B. L. Lee, R. A. Freedland & C. E. Corneliust. 1992. Bile pigments in gallbladder and freshly-secreted hepatic duct bile from fed and fasted rainbow trout, *Oncorhynchus mykiss*. Comp. Biochem. Physiol. 101A :857-861

Kim, P. K., J. K. Jeon & H. T. Huh & J. Y. Jo. 1999. Effects of dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*). J. of Aquaculture 9 :265-278

Talbot, C. & P. J. Higgins. 1982. Observations on the gallbladder of juvenile Atlantic salmon *Salmo salar* L., in relation to feeding. J. Fish. Biol. 21 :663-669.