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BIOENGINEERING AND BIOTECHNOLOGICAL STRATEGIES FOR REDUCED WASTE AQUACULTURE

Ian Mayer* and Ewen McLean**

* *Department of Zoology, Stockholm University, 106 91 Stockholm, Sweden*
** *University of Aalborg, Faculty of Technology and Science, Department of Civil Engineering, Aquaculture Biotechnology Section, Sohngaardsholmsvej 57, DK-9000, Aalborg, Denmark*

ABSTRACT

While recent years have witnessed dramatic advances in the reduction of aquaculture waste production, primarily due to advances in feed technology, the co-implementation of new bioengineering and biotechnological strategies are vital for alleviating the environmental impact of the rapidly expanding global aquaculture industry. The deployment of a new generation of automated feeding devices, and continued advances in recirculation technologies for land-based systems are amongst the more significant bioengineering advances that have resulted in reduced waste production. Advances in feed technologies will continue to play a pivotal role in the reduction of aquculture waste. Further, the advent of modern recombinant DNA technologies now allows for the economic production of a variety of feed supplements, most notably microbial phytases. Other, often overlooked, biotechnological strategies for achieving improved growth and conversion efficiencies include such physiological modifications as sustained exercise and compensatory growth. Somewhat more controversial biotechnological methods which may be beneficial in reduced waste management include endocrine manipulations and genetic engineering. Again, recent advances in recombinant DNA and transgenic technologies have also led to renewed interest in these strategies.

KEYWORDS

Aquaculture; bioengineering and biotechnological strategies; feed conversion efficiencies; genetic engineering; growth; hormonal manipulation; reduced waste.

INTRODUCTION

Global aquaculture has experienced rapid expansion over the last two decades and presently accounts for over 10% of total aquatic production. Furthermore, it is predicted that the next decade will witness even greater expansion. For example, while in 1990 world aquaculture production reached 15 x 10^6 tonnes, it is estimated that this figure will more than double by the turn of the century (Hempel, 1993). With this growth however, has come an increasing awareness of the detrimental environmental impact of the industry on a worldwide basis. While the contribution by aquaculture to total aquatic pollution is minimal compared to domestic, agricultural and industrial sources, there is nevertheless a growing concern over the regional impact of aquaculture on the environment. To date, the Nordic region has been one of the most widely studied areas with respect to aquaculture impact and figures for 1989 indicate that Nordic aquaculture accounted for a total annual loading to the environment of 3.5×10^3 t total P and 19.3×10^3 t total N (Braaten, 1991).

Since aquaculture competes for increasingly valuable water resources, and as the environmental impact of aquaculture tends to be more localised and visually apparent, an essential pre-requisite for the sustained global growth of the industry will be implementation of enabling technologies which allow for minimizing environmental degradation, while permitting expansion. The following review will briefly consider a number of bioengineering and biotechnological technologies which are either presently being employed to reduce the environmental impact of aquaculture or being considered as a potential means of advancing waste management problems.

BIOENGINEERING STRATEGIES

The application of engineering principles and equipment to aquaculture has been central to the rapid global development of the industry. In recent years, the impact of engineering upon production systems has increased dramatically, influencing not only the production environment through, for example, the development of sophisticated water quality monitoring devices, but also in the design of land based units. Significant reductions in aquacultural waste production and more effective methods of waste handling have been facilitated by advances in bioengineering. With respect to the reduction of aquaculture feed waste output, the following bioengineering advances have had, and will likely continue to have, significant impact.

Oxygen injection

The administration of oxygen has a twofold benefit on waste reduction in intensive aquaculture. Firstly, oxygen supplementation reduces water requirements by allowing increased stocking densities. Reducing the volume of effluent water leads to increased concentrations of waste products, which assists in maximising the efficiency of various waste removal methodologies. Secondly, oxygen injection facilitates the microbial breakdown (nitrification) of toxic nitrogenous wastes, and optimizes biofilter efficiency (Malone *et al.*, 1993). To date, oxygen injection systems have been developed to utilize high pressure oxygen gas, liquid oxygen and or oxygen derived from on-site generators. Presently, a wide variety of oxygen injection systems are employed by intensive land based aquaculture systems (Colt and Watten, 1988), mainly to optimise/minimise water use and as a means of maintaining fish growth at above optimal temperatures (Blom *et al.*, 1993).

Automated feeding devices

It is well established that teleosts, and other animals, express feeding periodicity. Therefore, optimal feed utilization is likely to occur only during times of maximal appetite. Accordingly, the industry has adopted automated feeding systems that are programmed to deliver feed during these periods. In addition, a new generation of "smart" automated feeding devices are now either presently available, or being adopted to reduce feed waste based on the principle of being able to determine the point of near satiation. This period is determined following the detection of uneaten pellets during feeding (Fig. 1a) either by an acoustic (Bergheim *et al.*, 1991) or video system (Foster and Ito, 1993). In response to pellet detection, an integrated control unit terminates feeding until the next predetermined feeding period. A further modification of this principle for land based systems has recently been developed by Summerfelt *et al.* (1995) in which the uneaten feed is detected by a hydroaucoustic probe located within the effluent pipe (Fig. 1b). The probe is coupled to a microprocessor controller assembly which switches the feeder off after a selected number of feed pellets are detected in the discharge. The use of time-restricted demand feeders, where food delivery is restricted to a number of predetermined periods during the day, represents an alternative method of feeding management. Using this strategy with caged rainbow trout *Oncorhynchus mykiss* , Alanärä (1992) reported both improved growth and reduced feed wastage, when compared to animals constantly provided feed on demand.

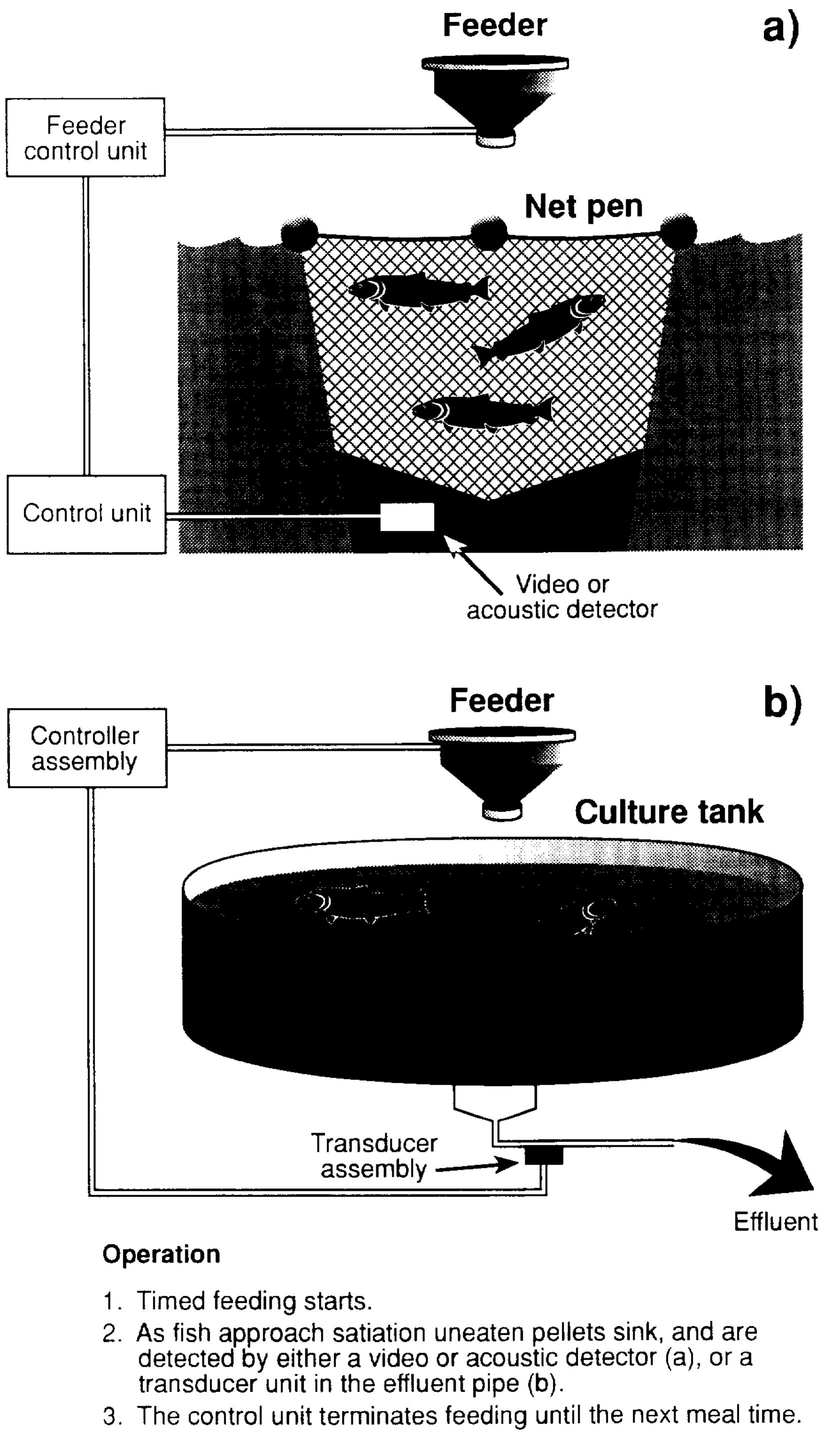

Figure 1. Automated feeding systems based on the detection of uneaten feed pellets by either (a) a video or ancoustic detector, or (b) by a transducer unit in the effluent pipe (after Summerfelt *et al.*, 1995).

It is well known that the establishment of dominance hierarchies can occur in fish populations (e.g., Olla *et al.*,1992). For example, it has been demonstrated that Atlantic salmon *Salmo salar* fed automatically, where feed dispersion is localised, may result in a 40% wastage due to a few dominant fish overfeeding close to the dispenser (Thorpe *et al.*, 1990). Appropriately, the automated feeder industry has responded to these observations by developing more efficient feeders which dispense feed more evenly over the entire water surface.

Onsite re-pelleting technologies

During transport and handling, feeds often release dust and small particles which may account for a significant percentage of total weight. Indeed, dust content may amount to 2% in extruded feeds to as much as 9% for pelleted feeds (Seymour and Johnsen, 1990). Where such feeds are utilised in automated feeders, both dust and small particles remain uneaten and thus contribute a direct loading to the water column. A bioengineering strategy now being introduced to eliminate this source of pollution is the adoption of sieving the pellets through a vibrating screen and then re-pelleting the collected dust and particles (Bergheim *et al.*, 1991). An alternative and, or supplementary strategy could be implementation of technologies for retrieval, and reutilization of uneaten pellets.

Recirculation technologies

The ever increasing demands on water resources, coupled with the expanding awareness of the environmental impact of intensive aquaculture has led to the generation of interest in the application of landbased recirculation systems as a means of further controlling the culture environment. The advantages of employing such techniques are that they require less water, and by their nature allow for complete control over waste production. To date, the major limitation of such high density systems has been their prohibitively high capital cost. However, where cultured species are of high value, water resources limiting, or environmental conditions restrictive, these technologies are becoming more economically feasible. For example, in the United States, recirculation system technologies are increasingly being adopted to enhance production of high value aquaculture products including ornamental species, alligators, soft crabs, crayfish, stiped bass and sturgeon.

In the recent past, advanced water treatment technologies, as exemplified by multi-stage biofilters (e.g. bead filters, biodrums, trickling filters), together with combined biochemical treatment units including, oxygen injection (*vide supra*) ozonation, UV-treatment, anaerobic denitrification, and the use of zeolites for ion exchange (Rosenthal and Black, 1993), have all become more prevalent in enclosed, partial water exchange-based systems. UV water treatment decreases microbial loading of the culture water, as well as minimizing the build-up of pathogens. Ozonation enhances biodegradation of those waste substances not easily attacked by the bacterial complement of biofilters. Intermittent ozonation is now successfully employed in a number of culture systems to specifically break down long chain organic substances that can then be more easily managed by biofilters. Further advances in technology and design of recirculation systems will undoubtebly reduce the capital costs associated with these plants, such that they will become economically viable for the cultivation of a greater variety of species in the future.

There are numerous other bioengineering strategies available that may, either individually, or when orchestrated with other systems, assist in decreasing the environmental impact of aquaculture. In addition to, and in co-ordination with such strategies, a number of biotechnological advances offer considerable promise as methods to control and reduce aquaculture waste. While, for reasons of clarity, the following considers individual concepts, it is emphasized that greatest benefit will likely accrue with the application of one or several such technologies during the production process.

BIOTECHNOLOGICAL STRATEGIES

Feed technology

Feed is the main source of pollution in intensive aquaculture (see De Pauw and Joyce, 1991; Pillay, 1992; Handy and Poxton, 1993). Therefore, development of methods which reduce feed waste and increase feed conversion efficiencies (FCE) are of high priority. Indeed, advances in feed technology, especially those directed towards improving FCEs, have led to dramatic reductions in aquaculture waste production. An illustration of the importance of minimal improvements in FCE as a means of reducing waste has been provided by Maroni (1985). This author estimated that a change in FCE from 1.0 to 1.5, results in a corresponding elevation in pollution loading, including a 186% increase for organic matter (as chemical oxygen demand, COD), 70% for total N, and 86% for total P.

A number of "cleaner" feed technologies are now being successfully employed, particularly in the salmon industry. One of the major advances has been the adoption of extruded feeds over the more traditional pelleted feeds. Extruded feed conveys a number of advantages. Most notably, the extrusion process (heat, pressure and moisture) gelatinizes the largely undigestible complex polysaccharides (e.g. starch) producing more digestible carbohydrates. The increased feed digestibility leads directly to a reduction in faecal waste. Further, it has been demonstrated that salmon fed extruded diets show improved FCEs compared to fish fed pelleted feeds (Seymour and Johnsen, 1990). In addition, extruded feeds have lower dust levels and a higher stability in water compared to pelleted feeds. Seymour and Johnsen (1990) showed that a commercial extruded pellet remained 84% intact (by weight) after 24 h in water, whereas two types of commercial pressed pellets of the same size showed a 50% breakdown after just 17 and 53 min.

Extrusion technology has also facilitated the production of feeds containing a higher fat content (so-called "high energy diets"; e.g., Alsted, 1988). Such diets, which contain 30% fat as compared to previous levels of about 20% are now commonly available. Johnsen and Wandsvik (1991) have reported that salmon fed such high energy diets show improved FCEs compared to fish fed, on an equal energy basis, lower fat diets. Further, it has been observed that use of high energy diets results in a 35% reduction in N loading (as ammonia) at the point of effluent discharge (Johnsen *et al.*, 1993). In a feeding study on Atlantic salmon the use of a high energy diet has facilitated a reduction in the total protein content of the feed (Johnsen and Wandsvik 1991). This protein sparing technique, besides allowing for a reduction in feed costs, has the added benefit of potentially reducing both N and P loading to the environment.

The dietary P requirement for optimum growth in fish is 0.5-0.8% of the feed (Enell, 1994). Feed components of animal origin, such as fishmeals, contain a high concentration of P (1.5-3.2%) with the consequence that the majority of P is excreted. The most desirable method of reducing the P content in fish feeds would be to substitute the fishmeal with plant-derived components since these contain lower total P. At the same time, such a strategy would shift the industry away from fishmeal dependency. A major constraint to fish meal replacement however, relates to the form in which the majority of P is stored in plants: as the hexaphosphate ester of myoinositol (MIH), or phytic acid (Graf, 1986, Fig. 2). However, the availability of this form of P is limited when animals lack appropriate endogenous phosphatase activity (Walsh *et al.*, 1993); which includes many species of cultured fish. MIH has also been shown to retain antinutritional activity. For example, in mammals, MIH chelates a number of minerals (eg. Ca, Zn, Fe) in the gut (Cantor and Perney, 1992), and may reduce protein bioavailability (Conneely, 1992). Nevertheless, it has been known for some time that these problems can be overcome by the addition of phosphohydrolase enzymes, or phytases, to the diet (e.g. Nelson *et al.*, 1968), which releases P from the MIH core (Fig. 2).

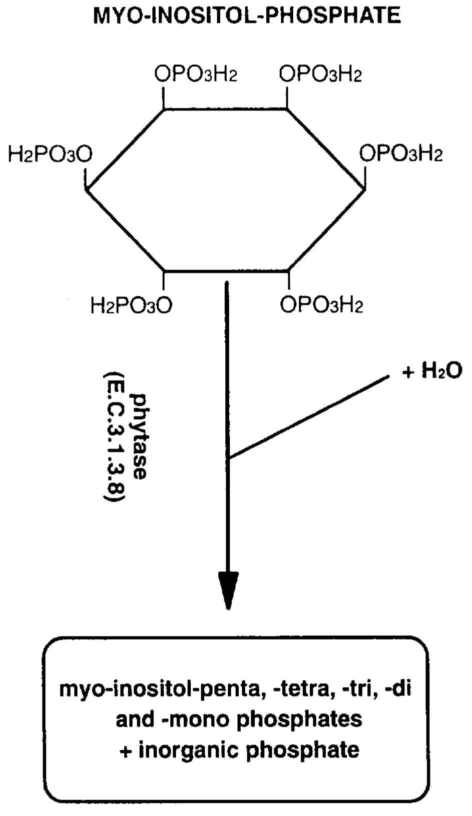

Figure 2. Enzymic breakdown of myo-inositol phosphate (phytic acid) by phytase.

Several microbes synthesize phytases, but that produced by *Aspergillus ficuum* is particulaly valuable due to its thermostability and high specific activity; both useful traits when considering the wide variety of pelleting conditions used by the aquaculture feed industry. Moreover, the advent of modern recombinant DNA technologies now allows for the economic production of phytases. However, careful consideration must be given to pH optima in the selection of phytase preparations, since gastrointestinal pH activities in teleosts are wide ranging. Thus, although phytases could be active in the pellet prior to ingestion, the effectiveness of phytases may be compromised at different levels of the fish gut.

Remarkable results have been reported for enhanced P utilization following seeding of plant-based feeds with phytases in the livestock and poultry industries (e.g. Cantor and Perney, 1992; Cole, 1992). The application of phytases to fish feeds as a means of reducing P excretion has however, only recently been examined (Ketola, 1994; Rodehutscord and Pfeffer, 1995; Schäfer *et al.* 1995). In these studies, plant based (soybean meal) diets were supplemented with phytases, the incorporation of which resulted in significant increases in P digestibility and reductions in P excretion. For example, the supplementation of soybean-based diets with microbial phytase to increased P digestibility from 25 to 57% in rainbow trout (Rodehutscord and Pfeffer, 1994), and reduced P excretion by 30% in common carp, *Cyprinus carpio* (Schäfer *et al.*, 1994).

While preliminary studies with dietary phytase supplementation of plant-based fish feeds are encouraging, this application of biotechnology, as a means of reducing P excretion, requires further study. In particular, future studies should be directed towards determining to what extent animal-based meals can be replaced by plant sources, and the optimal incorporation levels for phytases with respect to the latter. This strategy would combine the advantages of reducing the industry's dependency on costly fishmeals while decreasing the potential for P pollution. Information with regard to the potential longterm deleterious effects of dietary phytase supplementation upon normal metabolism in teleosts remains to be further explored.

Reduced N waste has also been achieved following adjustment of dietary protein as fish grow larger. It has been shown that N retention varies considerably with the size of fish, or more accurately with growth rates (Houlihan *et al.*, 1988). Adult fish with lower growth retain 15-20% of absorbed N as opposed to 40% in rapidly growing, young animals (Handy and Poxton, 1993). Therefore, N waste can potentially be minimized by progressively reducing dietary N content, as the growth rate in fish decelerates.

FEEDING, BEHAVIOUR AND MANAGEMENT STRATEGIES.

It has been demonstrated that fish show feeding periodicity due to changes in appetite associated with cycles of emptying and filling of the stomach (Jobling, 1993). For example, both Arctic charr, *Salvelinus fontinalis,* and rainbow trout feed most actively at dawn and dusk (Jørgensen and Jobling, 1989; Landless, 1976), although at certain times of the year (winter months) Arctic charr may consume 70% of their daily feed at night. Juvenile Atlantic salmon appear to feed during daylight hours irrespective of season (Higgins and Talbot, 1985), while larger salmon in sea cages feed most actively during the early morning and late afternoon (Kadri *et al.*, 1991). Further, the correct timing of meals can also incite behavioural responses, such as reduced aggression and activity levels, which lead to improved feed conversion efficiencies (Seymour, 1989). Thus significant improvements in FCEs can be obtained by administering feed at times appropriate to the behaviour of the species in question, and by adapting feeding strategies to the time at which fish exhibit maximal appetite.

Intensive cultivation exposes all animals to abnormal conditions, and this is nowhere more evident than during the feeding of fish. In devising optimal feeding strategies which also reduce feed waste, it is crucial that the cultured animal's "normal" morphological, physiological and behavioural requirements are addressed (Huntingford and Thorpe, 1992). For example, teleost feeding behaviour and efficiency of feed utilization, can be enhanced when pellets are modified to optimize characreristics such as taste, colour, contrast, shape, texture, etc. (e.g. Sutterlin and Sutterlin, 1970; Wankowski and Thorpe, 1979; Clarke and Sutterlin, 1985; Mackie and Mitchell, 1985; Strandmeyer, 1992). Another factor which may assist in reducing feed-based pollution of the aquatic environment relates to selection of appropriate pellet size ranges, since optimum feed particle size will not only change with growth, but will also vary between species. For example, Tabachek (1988) demonstrated that Arctic charr showed optimum growth when fed on pellets with particle sizes smaller than those recommended for Atlantic salmon.

Behavioural interactions within the holding unit also affect feeding opportunity and growth. Stocking density employed, current velocity used, feeding regime (including distribution and timing) applied, environmental manipulations exploited, the efficiency of grading during production, use of duo- or polyculture techniques, species selection, and type of rearing system operated, may all influence competition, aggression and growth during culture (e.g. Noeske and Spieler, 1984; Jobling, 1987; Merola and De Souza, 1988; Baker and Ayles, 1990; Kadri *et al.*, 1991; Holm, 1992). Such factors therefore, should be considered as a means of reducing aquaculture waste and improving FCE.

ECO-PHYSIOLOGICAL MODIFICATIONS

Eco-physiological modifications as a means of improving FCEs and reducing aquacultural waste has until recently been largely overlooked. Two physiological modifications in particular have shown promising potential for their application to aquaculture, namely sustained exercise and compensatory growth.

Several studies indicate that sustained exercise of salmonids leads to the hypertrophy of muscle cells, increased protein deposition, and improved growth rates and feed conversion efficiencies (reviewed by Davison, 1989; Christiansen and Jobling, 1990). For example, Arctic charr fry exercised at 1.1-2.3 body length/s grew faster than fry reared in standing water (Christiansen *et al.*, 1989). Twenty seven weeks following enforced exercise, fry in the above study were 21% heavier than controls and, furthermore, showed significantly lower body lipid and higher body protein levels compared to the unexercised fish. Moreover it was noticeable that during periods of sustained exercise fish schooled naturally, which resulted in reduced levels of agnostic behaviour (Christiansen and Jobling, 1990). The improved growth rates and FCEs observed in exercised charr would thus appear to have resulted from the combined effect of both physiological and behavioural changes (Jobling *et al.*, 1993).

When food supply is increased following a period of starvation or restricted feeding, fish in common with other animals may display a growth spurt often referred to as compensatory growth (Weatherly and Gill, 1981; Dobson and Holmes, 1984). The physiological basis and control mechanisms of compensatory growth are still poorly understood, although starved-refed animals characteristically show increased food intake and improved food conversion efficiencies compared to animals raised on the normal feeding regime. For these reasons compensatory growth has recently attracted interest for its practical application to intensive fish cultivation. For example, Miglavs and Jobling (1989) showed that juvenile Arctic charr raised on a restricted, followed by satiated, ration showed improved FCEs compared to fish continually fed a satiated ration, although this effect was transitory. Similarly, in a feed cycling experiment Quinton and Blake (1990) showed that rainbow trout maintained on a feeding cycle of 3 wk starvation and 3 wk feeding exhibited better growth rates and FCEs compared to the constantly fed controls, even though the controls were given twice the amount of feed. The preceding studies together with a variety of others, illustrate that optimal feed utilization efficiencies may be enhanced through the development of elite feeding strategies.

ENDOCRINE MANIPULATIONS

The last two decades have witnessed a tremendous increase in our understanding of the endocrine regulation of growth in animals. Coupled with advances in recombinant (r) DNA technologies enabling the production of almost limitless supplies of a wide variety of growth regulators, it is now feasible to contemplate the manipulation of animal production characteristics, including improved FCEs, using such biosynthetics.

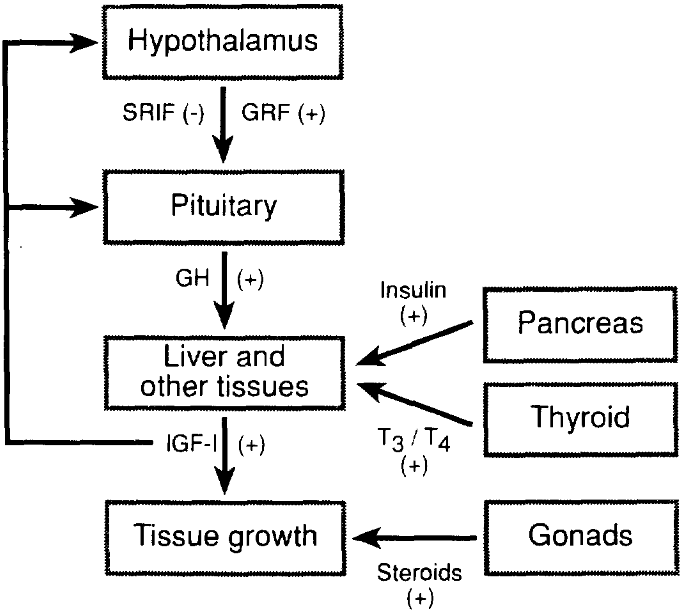

Figure 3. Endocrine regulation of growth in teleosts.

The endocrine regulation of growth is complex, and is summarized in Fig. 3. While many hormones have been implicated in affecting somatic growth and metabolism in poikilotherms, the most significant is growth hormone (GH). It is well established that treatment of teleosts with exogenous GH increases appetite, accelerates growth, improves feed conversion efficiencies (reviewed by McLean and Donaldson, 1993), and in salmonids enhances smolt fitness (Hoar, 1988); the practical benefits of the latter being that salmon return higher FCEs in saltwater. Moreover, administration of GH to salmonids decreases carcass fat content (McLean *et al.*, 1992, 1994). Not only will these benefits be financially advantageous to the fish farmer, but if GH is administered optimally, a significant reduction in waste output, during cultivation, may be achieved. Interestingly, GH has also been shown to retain efficacy in crustaceans (Charmantier *et al.*, 1989; Toullec *et al.*, 1991), and molluscs (Paynter and Chen, 1991; Kawauchi and Moiyama, 1991), but whether the observed growth acceleration in such species is related to increased FCE or not, remains to be fully elucidated. However, until recently the application of this biotechnology to the commercial culture of teleosts remained economically impractical due to the high costs associated with GH purification. The rapid development of recombinant (r) DNA technologies has now made it feasible to produce large quantities of highly purified rGHs. This technological advance has stimulated renewed interest in the commercial application of GH in aquaculture (McLean and Donaldson, 1993).

The major technical constraint hindering the commercialization of GH in aquaculture is the lack of effective and economic methods for protein administration. To date, growth acceleration in teleosts has been achieved using a variety of methods of GH delivery, including injection (Gill *et al.*, 1985; McLean *et al.*, 1991), constant infusion (Down *et al.*, 1988), immersion (Agellon *et al.*, 1988; Schulte *et al.*, 1989; Cribbs *et al.*, 1991) and sustained-release therapies (McLean *et al.*, 1992,1994). However, all these methods of administration, even allowing for the potential availability of inexpensive rGHs, would technically be prohibitively costly to deliver on a commercial basis. The above methods of GH administration by their nature, are labour intensive and time consuming. As well, such treatment regimes would also involve excess handling or manipulation of stocked fish. Therefore, from a practical point of view the most desirable methods of GH delivery would be those which require limited handling of individuals. Recently, McLean *et al.* (1994) evaluated the long term growth performance of coho salmon following single treatment with a sustained release pellet containing rpGH, and found that considerable economic benefit could be gained following such treatment, without apparent detrimental effect. Fish which received the highest GH dose using this system, maintained a weight and length advantage over the controls for 77 and 95 wks respectively. Moreover, at the end of the 95 wk trial the GH-treated fish exhibited significantly lower body lipid levels compared to controls. This study, which represents the longest evaluation of the effect of GH therapy on teleosts, clearly illustrated the benefits that can be gained using GH treatment, with minimal manipulation of treated animals. However, even more desirable than single implantations of sustained and controlled release GH delivery systems, would be administration via the feed.

The gastrointestinal absorption of large peptides such as GH however, can be severely restricted due to the wide variety of physical, chemical and immunological factors operative within the fish gut, which act to prevent the uptake of macromolecules (reviewed by McLean and Donaldson, 1990). Even given such limitations though, it is now evident that bioactive peptides such as GH can gain access to the fish blood stream in a physiologically active state. Indeed, recently McLean *et al.* (1993) have shown that growth rates and FCEs can be enhanced in both diploid and triploid coho salmon following dietary delivery of rpGH (20 g/g body wt/d). Further, the same authors reported enhanced FCEs where the GH was co-delivered with a putative penetration enhancer and an antacid, the latter added to the diet to neutralise the hydrolyzing action of the gut's proteolytic enzymes.

Most recently, Tsai *et al.* (1994) elegantly demonstrated that growth can be enhanced in teleosts by feeding diets incorporating yeasts transgenic for GH. In these studies, an episomal plasmid was constructed for the intracellular expression of rainbow trout GH cDNA in *Saccharomyces cerevisiae*. Tsai and colleagues subsequently showed that juvenile striped mullet, *Mugil cephalus*, fed diets containing 2% rGH yeast exhibited significantly improved growth rates and FCEs compared to control fish after just 4 wk treatment. Although the precise mechanism(s) of GH action on lipid metabolism in fish is still unclear, it has been suggested (Higgs *et al.*, 1976) that GH stimulates lipid mobilization, sparing dietary protein which can then be utilized for body growth. The mobilization of lipids at the expense of proteins presumably has the direct effect of reducing excretory N. but the potential for this has yet to be illustrated in fish.

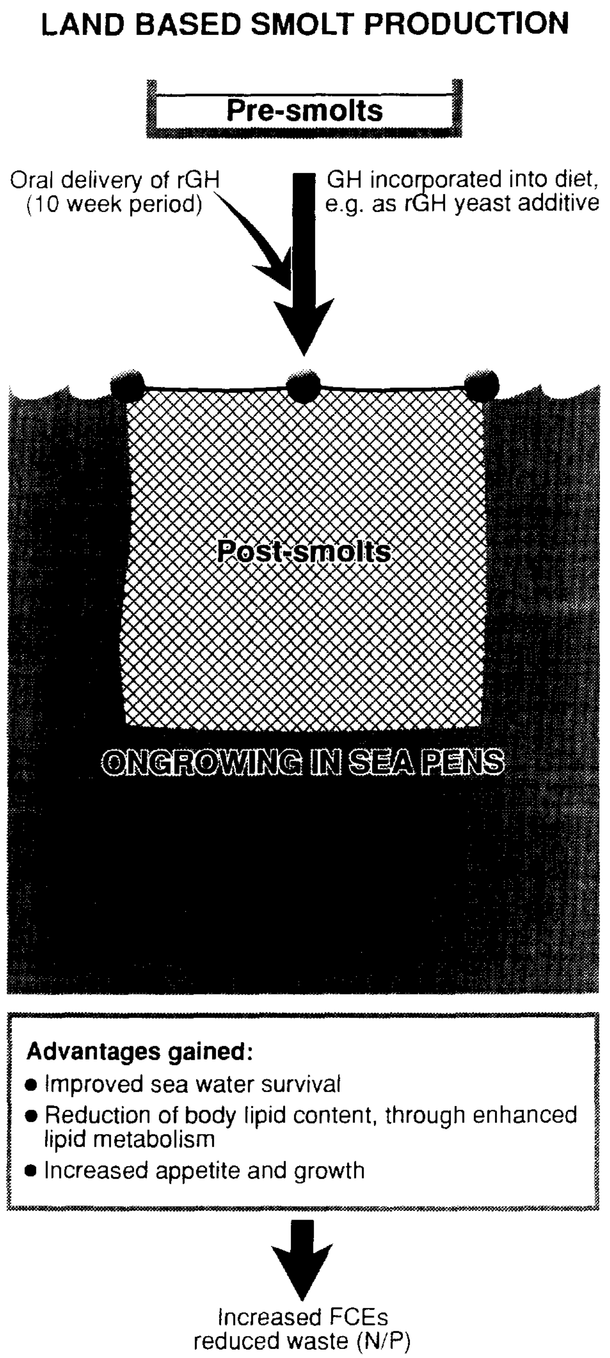

Figure 4. Possible scenario for the future use of GH biotechnology in intensive salmon cultivation.

A possible scenario for the future use of GH in intensive salmon cultivation is illustrated in Fig. 4. Administration of rGH via the diet (incorporation of yeasts) for a predetermined period (ca. 10 wk) prior to smoltification, and subsequent transfer to seawater. This strategy would convey the following advantages:

i) improved seawater survival;
ii) reduced body lipid content through enhanced lipid metabolism;
iii) increased appetite and growth;
iv) reduced waste loading due to improved FCE.

In mammals, the effectiveness of exogenous GH is reportedly enhanced by monoclonal antibodies (MAb) raised against specific epitopic sites of the molecule. Although the binding of hormones by antibodies usually decreases the activity of the former, certain MAbs potentiate the action of GH. Again, MAb-GH complexes have been shown to significantly enhance FCEs (Holder and Aston, 1989), although their application to aquaculture organisms remains untested. Other uses of MAbs might be in the reduction of fat deposition by immunization against adipocytes. However, the practical usefulness of MAbs in aquaculture are limited by the lack of an economic method of delivery.

One of the major biological restrictions to the application of GH by the fish culture industry relates to its relatively rapid metabolism (plasma half-lives for GHs range between 15-44 min, McLean and Donaldson, 1993), and chemical instability. The development of more stable GH analogues for use in sustained release and oral delivery systems is highly desirable. Further, analogue forms of GH are apparently more potent in stimulating salmonid growth. For example, Down *et al.* (1989) found that a rbGH analogue in which amino acid residues 33-39 were deleted was more effective in enhancing growth and FCEs in coho salmon compared to unaltered rbGH.

An alternative strategy to exogenous GH administration would be to manipulate those regulatory mechanisms that control endogenous GH secretion. The neural regulation of GH is primarily under the control of two hypothalamic peptides (Fig. 3): growth hormone-releasing factor (GRF) which stimulates GH secretion, and somatostatin (SRIF) which inhibits GH release (reviewed by Harvey, 1993).

It has been reported, as in homeotherms, that exogenous GRF induces GH release in teleosts (Peter *et al.*, 1984; Lou *et al.*, 1990). Indeed, preliminary studies indicate that weekly injections of 5 µg (1-24)h GRF stimulate growth in chinook salmon *O. tshawytscha* (E. McLean and E.M. Donaldson, unpublished observations). Although further studies on teleosts are needed, it has been demonstrated in homeotherms that GRF administration increases both growth and FCEs (reviewed by Dawson and Buttery, 1991). In addition, Moseley *et al.* (1987) reported that GRF treatment increased N retention in mammals, resulting in decreased urinary N excretion. Therefore, GRF administration may prove an alternative method to GH treatment as a means of improving growth and FCEs in cultured teleosts.

Since somatostatin (SRIF) is the primary hypothalamic factor inhibiting GH release, its immunoneutralization may provide an alternative to GH therapy as a means of enhancing somatic growth in fish. Indeed, immunization against SRIF in some homeotherms has resulted in enhanced growth rates and improved FCE (McLean and Donaldson, 1993), and preliminary trials indicate that anti-SRIF treatment accelerated growth in carp, (E. McLean, E. Teskeredzic, and E.M. Donaldson, unpublished observations). Injection of coho salmon *O. kisutch*, with anti-SRIF resulted in increased endogenous GH levels, suggesting that the enhanced growth rates observed for carp resulted due to elevated GH levels (Diez *et al* ., 1992). Recently, Mayer *et al.* (1994) showed that intraperitoneal injections of the identical anti-SRIF preparation (SOMA-10, 1Eµg/g body wt/ 5 d) resulted in a significant increase in growth in chinook salmon compared to control fish over a 40 d trial period (Fig. 5). In combination, the preceding studies suggest that passive and active immunizations of fish against SRIF may provide a more (consumer) acceptable means of manipulating growth and FCE in cultured fish than for example with exogenous GH. However, further studies with such concepts must be undertaken in order to ascertain the long term performance characteristics and FCE returns for treated animals.

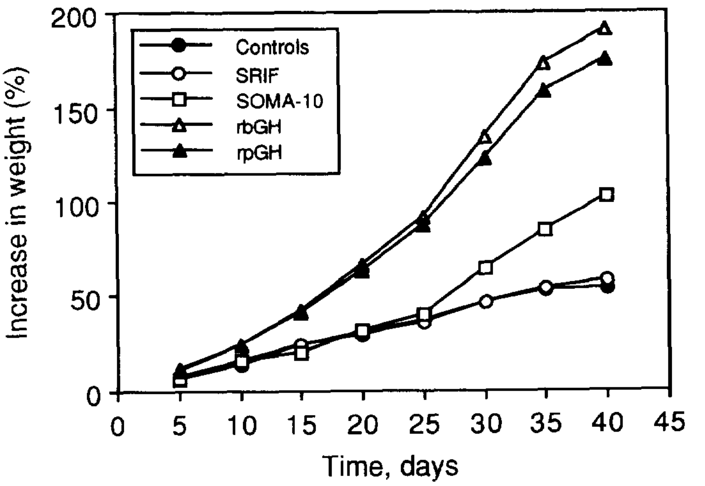

Figure 5. Antisomatostatin-induced growth acceleration in juvenile chinook salmon.

Increasing evidence indicates that insulin-like growth factor I (IGF-I) mediates many of the growth-promoting effects of GH (Bern *et al.*, 1991). It would seem logical therefore, that exogenous IGF-I might also accelerate growth, and perhaps enhance FCEs in poikilotherms in a manner similar to that recorded for GH. However, preliminary research in mammals with recombinant IGFs has indicated that this factor is clearly less effective than GH (Scanes and Baile, 1993). It would appear that IGF-I mimics some but not all the growth promoting effects of GH, which may indicate that the actions of IGF-I are local rather than general. Indeed, early data would appear to support this concept since Skyrud *et al.* (1989) reported a null effect for IGF-I upon growth in charr.

Recently interest has focused on the apparent growth-promoting properties of placental lactogen (PL, chorionic somatomammotropin), a member of the prolactin-GH gene family (Rand-Weaver *et al.*, 1993). Evidence from mammalian research suggests that PL is influential in the regulation of maternal metabolism and foetal growth, and further, in common with GH, stimulates both body growth and IGF-I production (Nicoll, 1993). In coho salmon, Devlin *et al.* (1994) have demonstrated that both growth rates and FCEs were dramatically increased following injections of rbPL. Furthermore, relative to a control group of fish injected with an identical concentration of rbGH, rbPL-treated fish returned superior FCEs and appeared to lose their parr markings more rapidly. These observed effects were somewhat unexpected since rbPL expresses only 23% amino acid sequence homology to salmon GH (Rand-Weaver *et al.*, 1993), and further, earlier studies indicated that hPL therapy had no somatotropic effects in fish (Clarke *et al.*, 1973). However, in view of the findings of Devlin *et al.* (1994) further studies with respect to the application of PL to fish culture as a means of enhancing growth, FCEs and smoltification would appear highly warranted.

Finally, one novel area of endocrine manipulation is the application of gut peptides and β-adrenergic agonists. While these compounds have been shown to act as potent repartitioning agents in various livestock animals (Watkins *et al.*, 1990) knowledge of their occurrence and function in poikilotherms is very limited. While the application of these repartitioning agents in fish culture should be regarded with caution, it has recently been shown that the addition of ractopamine to the feed of the channel catfish, *Ictalurus punctatus* resulted in both increased weight gain and reduced fat deposition (Mustin and Lovell 1993).

GENETIC MANIPULATIONS

A wide range of genetic techniques have been examined as a means of improving the production performance of cultured teleosts, crustaceans and molluscs. Essentially this has entailed three areas of genetic manipulation, namely selective breeding, hybridization, and most recently transgenesis.

Changes to the native genetic information in fish stocks can be achieved through selective breeding programmes which are designed to promote favourable traits over a period of generations. However, for a trait to be chosen in a selective breeding programme, three pre-requisites must be met. Firstly, the trait must be of economic importance, secondly it must be possible to quantify, and finally, heritability must be greater than zero. Desirable traits chosen and successfully enhanced, in selective breeding programmes have included growth rate (e.g. Withler *et al.*, 1987), body quality (body protein or lipid content), and maturation. The latter trait is of importance since, when selecting for improved growth, there is a tendency for the rate of precocious maturation to be enhanced (Rowe and Thorpe, 1990). However, so far selective breeding programmes have not seriously improved FCE as a parameter for selection in fish.

Several studies have examined the potential for enhancing the production performance of aquacultured species using hybridization. This technique has been applied to the production of monosex and sterile animals (e.g., cichlids); in altering body conformation (e.g. ictalurids), and for increasing growth characteristics - heterosis. With respect to the latter, however, considerable variation in response has been observed (e.g. Harada *et al.*, 1986; Heap and Thorpe, 1987, Dunham, 1987; Pan *et al.*, 1989).

With the rapid advancement of molecular techniques it is now possible to isolate a gene of particular interest and subsequently transfer it to fertilized eggs of animals. The incorporation of such foreign gene constructs results in the production of transgenic animals with correspondingly modified genetic properties. In effect, transgenic technologies, where such are employed as a means of enhancing endogenous production of bioactive proteins and peptides, represent an alternative, although perhaps more sophisticated, means of drug delivery. Thus, fish transgenic for GH would effectively eliminate the need for costly GH administration. The first transgenic fish incorporating a GH gene was produced by Zhu *et al.* (1985), and since then transgenic technology in fish culture has developed rapidly (reviewed by Fletcher and Davis, 1991). Recently, Devlin and colleagues have succeeded in producing transgenic coho salmon which exhibit remarkable growth properties and improved FCEs (Devlin, R.H., personal communication), although future studies with these animals await further address, and it is of note that technical problems still exist with respect to the production of such animals, including:

i) low efficiency of producing transgenics (in salmonids the frequency of genomic integration of the GH gene is usually $< 4\%$);
ii) variability of expression the transgenes due to integration at different positions in the genome;
iii) health problems due to chronic expression of the gene product.

The expression of the foreign DNA should satisfy a number of criteria, including tissue specificity, correct timing and adequate amounts. These criteria are controlled by selecting an appropriate promotor/enhancer, ideally of endogenous origin. Other target genes could be those which control or promote digestibility (carbohydrates as opposed to proteins), and intestinal resorption to improve food uptake and conversion efficiencies, and utilization (e.g. carbohydrases). Enhancement of these characteristics through transgenic technology would not only reduce feed costs and provide a wider range of components for least cost diet formulation , but also significantly reduce waste (N and P) output.

The production of transgenic animals provides several advantages over the traditional selective breeding techniques. Genetic engineering allows for the manipulation of individual genes rather than the entire genome. This results in reduced time needed to express the desired character. Further, transgenesis permits the transfer of genetic information across species barriers. Transgenesis should be viewed as an addition to classical selection, in that there are good possibilities of combining transgenic techniques with selective breeding programmes, e.g. the input of transgenics in a breeding scheme would result in extra-genetic variance, resulting in an increased response to genetic selection.

Concern is growing on the possible ecological impact escaped transgenic fish may have on natural fish populations. Wild stocks provide a valuable source of genetic material. Thus, the primary concern of genetically altered fish escaping is the potential to introduce new genetic material or alter the existing genetic structure of native populations through inter-breeding. Further, genetically altered fish may threaten native populations through competition or through the introduction of disease. The worst scenario would be that morphologically distinct native populations would ultimately be replaced by "mongrel" populations with the corresponding irreversible loss of biological diversity. Therefore, the development of genetically altered strains of fish, either by selective breeding or transgenesis, requires the co-implementation of containing methodologies that can effectively block reproductive interactions with wild populations (Devlin and Donaldson, 1992). Biological containment can be most effectively achieved by chromosome set manipulation. For example, in salmonids, triploidy can be readily induced in newly fertilized eggs by means of heat, pressure or electric shock treatment. However, because male triploids show gonadal maturation, all-female monosex populations need to be produced prior to triploidization. In this regard, it is of note that for certain cultured species, there exists sexual dimorphism in growth (e.g. *Penaeus monodon*).

The last two decades have witnessed dramatic improvements in the reduction of aquacultural waste, due primarily to advances in feed technology. However, with the projected rapid expansion of global aquaculture it is imperative that new technologies are implemented to further reduce the environmental impact of aquaculture industry. If implemented correctly, new bioengineering and biotechnological strategies will not only benefit the aquaculture industry financially by optimising growth potentials in aquatic species, but will also lead to further reductions in waste production, an essential pre-requisite for the sustained growth of the industry.

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