CHAPTER

6

USE OF FISH GROWTH HORMONE: AUGMENTING PRODUCTIVITY IN AQUACULTURE

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INTRODUCTION

Natural growth rate of organisms has evolved to provide maximum fitness in wild environments and it matches with seasonal and spatial availability of food resources, variation in physical conditions, and associated with other correlated characteristics such as predation risk and disease resistance (Thorpe *et al*., 1998). Enhancement of natural growth rate of fish in aquaculture has been extensively explored, with gains arising from improvements in husbandry, nutrition, and genetical selection (Li *et al.,* 2003). Growth enhancement can be advantageous for aquaculture by shortening production time, and enhancing feed conversion efficiency. Therefore, controlling growth through endocrine approaches have been extensively explored. The development and differentiation of seawater-type chloride cells of branchial epithelia are regulated by GH, IGF-I and cortisol. Gene transfer technology has produced a great impact in modern biology and biotechnology (Powers et al., 1998), principally in applications of somatotropins such as insulin like growth factor-1 (IGF-1), growth hormone, thyroid hormones, prolactin, placental lactogen, and sex steroids (McLean and Delvin, 2000). Significant growth gain in fish could be achieved through application of these growth factors with some practical difficulties. However, use of growth hormone for growth improvement has been the central focus than the other hormones in different studies. Growth Hormone (GH) otherwise known as “Somatotropin” is a single chain polypeptide of approximately with

22 kDa molecular weight containing about 190 amino acid residues, produced by the pituitary gland and responsible for number of anabolic processes among vertebrates. Besides regulating body growth, GH is involved in reproduction, immunity and osmoregulation and metabolic regulation such as lipolytic activity and protein anabolism in fishes (Rousseau and Dufour, 2007). The different activities of growth hormone have been explored and used for both in reach and commercial purposes. In fish, the availability of naturally available fish growth hormone is meager, thus the recombinant growth hormone has been produced from different fishes for large scale production of hormones for commercial application. The prospect of using recombinant DNA technology for the production of vertebrate growth hormones has generated some interest for the potential use of recombinant growth hormone to stimulate growth and improve feed efficiency in aquaculture.

Similarly, growth enhancement in fish using molecular techniques is equally beneficial to aquaculture and is more effective than traditional breeding programmes to develop new fish strains. The technology which uses molecular technique is called as transgenic fish technology. In principle, the technology can be used to improve growth rate of the fish, control sexual maturation, sterility and sex differentiation, increasing disease resistance, cold resistance and modify the biochemical characteristics of the flesh to enhance the nutritional qualities. However, since fish can be readily improved by application of molecular techniques, it is timely to consider what genetically modified (GM) fish are likely to offer in the future, both in terms of benefits and disadvantages (Maclean, 2003). Growth hormone gene has been utilised extensively for construction of transgenic fishes to enhance growth and thus, species showing high growth rate is widely used to isolate growth hormone gene for the production of transgenic fish. The application of transgenic fish not only limited to enhance aquaculture production but also useful in biomedical sciences for various purposes. The transgenic fish produced through the incorporation of growth hormone transgene may have effects on feed conversion efficiency, color, body shape, behavior, carcass yield, body composition, muscle structure, hypoxia tolerance, disease resistance and reproductive traits. Though transgenic fish have lot of benefits to increase fish production of aquaculture industry, the regulatory agencies have yet to approve transgenic fish for commercial production. This

chapter explains the effects of administration of GH on fish physiology including behaviour, immunity and growth and use of growth hormone gene in transgenic fish production for growth improvement.

EXOGENOUS APPLICATION OF GH ON FISH GROWTH

A common therapy that is used to enhance growth in domestic animals is using GH and it has also become increasingly popular in aquaculture for fin fishes and shell fishes. Initially, mammalian GH was found to accelerate the growth of fish (Pickford and Thompson, 1948). Thereafter, there have been many studies demonstrating the efficacy of mammalian GH in the acceleration of growth of various species. In fish, approaches to accelerate growth by GH administration have been studied and increased growth has been achieved by various modes of delivery like injection, and oral administration of hormones (Schulte *et al*., 1989). The effect of administering exogenous natural mammalian and piscine growth hormone has been studied in many fishes including, salmonids, tilapias, basses, eels, carps, mullets, and channel catfish (Donaldson *et al*., 1979). These studies suggest that the exogenous growth hormone stimulates appetite and improves feed conversion and thus enhances fish growth (Matty, 1986). Exogenous growth hormone stimulate appetite in fish by a direct action on centers in the hypothalamus that influence feed intake or by inducing a number of metabolic changes that feed back on the hypothalamic centers to affect appetite (Markert *et al*., 1977). Earlier experiments suggested that exogenous growth hormone may improve feed conversion by one or more possible mechanisms 1) stimulation of lipid mobilization and oxidation 2) an action on the rate of protein synthesis or breakdown 3) stimulation of insulin synthesis and release. However, the mechanism (s) underlying improvements of feed conversion efficiency in GH treated fish are not entirely understood. Earlier experiments reported that elevated GH has no effect on gut enzyme activity (Lemieux *et al*., 1997), as an alternative it stimulates intestinal Na+ dependent and Na+ independent amino acid transport (Sun, 1992), which may partially explain improved feed conversion efficiency in treated fish. Depending upon the dose, treatment duration, and growth stage, a more than double of individual weight relative to controls may be achieved (McLean *et al*., 1996).

Administration of growth hormone has shown to improve the growth satisfactorily in many of the fish species. Interestingly, in some of the fishes it is possible to increase the growth to double the size than the actual individual size of fishes, and it depending upon the factors like dose, duration and time of the administration of the hormone (McLean et al., 1997). A study on Atlantic salmon resulted that the fish treated with GH exhibited significant increase in the metabolic rate during parr and pre-smolt stages that the control fish (Sedikki et al., 1996). The biological activity of purified protein from recombinant growth hormone of *Pangasianodon hypophthalmus* (rPhGH) was determined by Sekar et al. (2015) in rohu (*Labeo rohita*) fingerlings to promote growth. The fingerlings of *L. rohita* were injected with the hormone at three different concentrations, and a significant increase in growth was observed in fingerlings administered with rPhGH at a dosage of 1.0 mg g^{-1} body weight. Similarly, growth promoting effect of growth hormone has been studies in different fishes such as trout (Sato *et al*., 1989), eel (Sugimoto *et al*., 1990), common carp (Fine *et al*., 1993), flounder (Jeh *et al*., 1998) and giant catfish (Promdonkoy *et al*., 2004). Administration of the hormones in most of the teleost fishes has showed elevates the growth than the untreated fish in any mode of administration. Several methods such as injection (Guillén II et al., 1998; Li et al., 2003), immersion treatment (Schulte et al., 1989; Moriyama and Kawauch, 1990) and oral administration with (Tsai et al., 1997; Ben-Atia et al., 1999).

Other than the major impact on growth improvement, the administration of exogenous growth hormone lead to increase in endocrine growth regulatory component, and changes in the tissue proximate composition in most of the studied fishes (Fleming et al., 2002). Rainbow trout when injected with bovine GH led to elevation of endogenous GH (Kieffer et al., 1994). The effect of exogenous GH administration on the serum IGF-I and IGF-II concentrations were studied by Shamblott et al. (1995). Also, elevated levels of T3 and hepatic 50-deiodinase that converts thyroxine to T3 was observed in salmonids injected with GH (MacLatchy et al., 1992). Additionally, predictable changes in body composition and conformation can be achieved over elevated levels of GH through shifts in lipid, protein and mineral metabolism. It was observed that, the body of salmonids appear less deep-bodied and slender, thus reducing the condition factor (k) invariably when GH is administered (McLean and Donaldson, 1993;

Johnsson and Bjornsson, 1994). The changes in proximate composition of treated fish were extensively studied (Gill et al., 1985; McLean and Devlin, 2000). The ash content of the fish increased significantly owing to the augmented mineral deposition within bone leading to increased bone formation and desorption (Takagi et al., 1992). GH treatment is concomitant with rises in fatty acid and plasma glucose concentrations (Leatherland and Nuti, 1981) and also with elevated hepatic lipolysis (O'Connor et al., 1993). Protein synthesis in muscle and other tissues (Cheema and Matty, 1978; Foster et al., 1991; Fauconneau et al., 1996) and body moisture levels are elevated (McLean and Devlin, 2000) with exogenous GH treatment.

ADMINISTRATION OF GROWTH HORMONE AND FISH BEHAVIOUR

The effects of growth hormone (GH) on various types of behavior in teleost fish are well established but the underlying mechanism behind the behavioral change has not been fully understood. In salmonid fish, growth hormone increases locomotor activity, aggression, and feeding activity and at the same time reduces predator avoidance behavior (Johansson, et al., 2005). The impact of behaviour changes of growth hormone has been well demonstrated in mammals and fishes. However, it is not clear that the behavioural effect of growth hormone is mediated directly in the brain or by pheripheral changes (Johansson, et al., 2005). Growth hormone administration has shown to influence on the fish behaviour, it was understood that, GH administration increases energy demand and thereby elicit appetite which might reduce anti-predator response and promote competitive ability. This hypothesis was tested by Johnsson and Bjornsson (1994) and found that exogenous GH elevates feeding motivation, and thereby provide dominance status in juvenile rainbow trout, comparing the behaviour, growth rate, food conversion and hypo-osmoregulatory ability of GH-treated and control groups. GH-treated fish consumes twice as much as control fish over the 2 days of feeding and exhibited higher food conversion efficiency. Feeding competition trials involving size-matched and control fish showed GH-treated fish were significantly more dominant. Similarly, GH treatment promoted growth rate in brown trout and increased RNA levels, but had

not observed effect on dominance (Johnsson et al., 1996). Jonsson et al. (1998) observed agonistic interactions to clarify the role of GH in social interactions, in size-matched pairs of juvenile rainbow trout consisting of two control fish, two GH-treated fish. In this observation GH did not appear to affect fighting ability or social status directly, though it is likely to do so indirectly through affecting body size. In another study, Both Rainbow trout and Brown trout treated with GH appear more willing to risk exposure to predators, whether it be simulated attacks using a model thereon (Jonsson et al., 1996a; 1996b) or the presence of a predator Brown trout (Johnsson et al., 1996) was not sure. The growth-promoting effect of GH, however, appears to be much more pronounced under conditions of unrestricted food supply in the hatchery than in the wild, where food availability is limited; suggesting that the payoff associated with increased growth investment is higher in the former case (Johnsson et al., 2000). Moreover, work with domesticated Salmonids, which show similar increases in growth, aggression and willingness to risk exposure to predators as observed in GH-treated fish (Einum and Fleming, 2001; Johnsson *et al*., 1996), has shown that survival consequences for greater growth in the wild depend on the predation environment, being expressed only when risk is high (Biro et al., 2004).

ROLE OF GH ON OSMOREGULATION

Besides the growth promoting role, growth hormone promotes the acclimation to seawater in most of the teleost fish through the aid of insulin-like growth factor I. GH, IGF-I and cortisol regulate the development and differentiation of seawater-type chloride cells of branchial epithelia (Sakamoto and McCormick, 2006). The administration of GH may increase the number and size of gill chloride cells, Na^+, K^+, $2Cl^-$ co-transporter (NKCC), Na^+, K^+-ATPase and ion transporters associated with salt secretion (McCormick, 2001). The role of GH on salinity tolerance and salt secretory mechanisms is reported in salmonids, tilapia and killifish (Sakamoto and McCormick, 2006). Several authors reported that growth hormone receptors found in the liver, gill, gut and kidney of several marine fishes and the numbers of GH receptors are increased in the osmoregulatory organs of rainbow trout after exposure to sea water (Sakamoto and Hirano, 1991). According to McCormick et al. (2002), plasma levels of GH and IGF-I increase during smolting of salmonids

along with increase in gill chloride cell size and number, NKCC levels, Na^+,K^+-ATPase activity indicates salinity tolerance (Hoar, 1988). But, in marine eel and sea bream, (*Sparus sarba*) GH may not play an osmoregulatory role because of their marine origins or a limited capacity to hyper-osmoregulate (Deane and Woo, 2004).

ROLE OF GH ON FISH IMMUNITY

Activation of immune functions during the seawater acclimatization of some euryhaline fish species is appears to be linked with increased level of GH in the circulation which improves seawater adaptability (Dominguez et al., 2005). In fish, the non-specific defense elements such as cytotoxic, phagocytic, hemolytic and lysozyme activities and specific defense mechanism mainly immunoglobulin levels were elicited by administration of GH (Yada, 2007). In higher vertebrates, the GH secreted in the lymphoid tissues has an effect on the proliferation of leucocytes (Jeay et al., 2002). The structural resemblances of GH and its receptors to cytokines and its receptors indicate the functional association of GH on immune system and its role (Venters et al., 2001). The *in vivo* administration of GH in fishes showed an increase in superoxide anion activity, lysozyme activity and phagoctytic activity (Narnaware et al., 1997; Yada et al., 2002). Administration of bovine GH in *Oreochromis mossambicus* and *Oncorhynchus mykiss* stimulates somatic growth and also enhances antibody production against this growth hormone (Leedom et al., 2002; Biga et al., 2005). Hypophysectomized fish exhibit plummeted level of immunoglobulins (Ig) in the circulation and administration of GH in hypophysectomized fish elevated and restore the level of Ig in comparison with normal fish which indicates the importance of GH in the specific immunity of fish (Yada and Azuma, 2002). The administration of GH stimulates the proliferation of leucocytes in the peripheral blood of Chum salmon (Sakai et al., 1996). The extra-pituitary expression of GH mRNA in the lymphoid tissues and phagocytic leucocytes was noticed in several fishes and not in some fishes which indicates the degree of variation in the endocrine regulation of the immune function in different fishes (Yada, 2007). According to Yada (2007), the paracrine secretion and regulation of GH is also an important mechanism in the peripheral defense of host against the invaded pathogens.

GROWTH HORMONE –MYOSTATIN INTERACTION ON MUSCLE GROWTH AND QUALITY

During earlier times, the quality of any livestock meat is mainly considered on the sensory factors such as texture, flavor, color and visible fat. However, the quality is described on the basis of the composition of poly-unsaturated versus saturated fat and microbiological contamination mainly because of increasing health concern and food safety awareness from the consumers. Feed management strategies can preferably affect the biochemical composition, flavor and texture quality of carcass of livestock. Similarly, genetic variation also has a great influence on feed intake, metabolism and there by conversion of food into desirable ratio of fat and muscle, location of fat distribution, muscle fibre type and quantity as well as taste (Williams, 2008). Improved genetic tools helps to increase the resistance of animals against the potential pathogens through improved genetic makeup which in turn reduce the microbial contamination on carcasses and processed meat. Myostatin (MSTN) is a transforming growth factor β (TGF-β) family member and negative regulator of muscle growth (McPherron et al., 1997). Myostatin plays an essential role in the regulation of developmental pathway for the differentiation of progenitor cells into muscle and fat tissues. During the nonexistence of myostatin, the cells preferentially transform into muscle tissue instead of fat tissue. Myostatin and GH have negative relationship and expression patterns in several vertebrates (Marcell et al., 2001; Liu et al., 2003).

In MSTN gene knockout mice, the skeletal muscle mass is 2–3 folds larger than the wild-type mice, which accomplished through hyperplasia and hypertrophy of muscle fibers (McPherron et al., 1997). According to Jiang et al. (2002) variations in the myostatin gene results in reduction of myostatin expression and hence the muscular hypertrophy which yields higher average daily gain as well as improved muscling (Clop et al., 2006). Xu et al. (2003) found that over expression of MSTN prodomain in zebrafish, binds with MSTN domain and inhibits its regular function and allow the increased number of muscle fibers development. The transgenic coho salmon produced through all-salmon GH/metallothionen (MT) gene construct, showed overexpression of GH and lesser expression of

MSTN which is evident from 11-folds larger growth rate than the control coho salmon in 15 months (Devlin et al. 1994). According to Hill et al. (2000), the transgenic coho salmon have appreciably more numbers of small-diameter muscle fibers that indicates their larger body size which is achieved as a result of increased muscle hyperplasia. Similarly, Roberts et al. (2004) proved that the transgenic coho salmon, fast growing fish than its wild counterpart exhibit under expression of both MSTN transcripts and protein suggests that MSTN does act as a negative regulator of growth. Thus, this study provides evidence that the anabolic effects of GH could be attributed through MSTN. Hyperplasia and hypertrophy in transgenic salmon indicates its better muscle quality due to improved muscle texture than their wild counterparts (Roberts et al., 2004).

MOLECULAR TECHNIQUES FOR GROWTH ENHANCEMENT

There are many ways to enhance growth including inbreeding, gynogenesis, androgenesis, selection, intraspecific crossbreeding, interspecific hybridization, polyploidy, sex reversal and breeding, nuclear transplantation and transgenesis. Cloned populations have been produced via gynogenesis and androgenesis (Dunham, 2004), but direct cloning of an individual fish of interest has not yet been accomplished. A significant impact of gene transfer technology has been observed in modern biotechnology (Powers et al., 1998). The fish species used for gene transfer studies can be divided into two main groups: those which are used in aquaculture (Fletcher and Davies, 1991; Hew et al. 1995; Chen and Lu, 1998) and those which can be used as a model fish for basic research (Chen and Lu, 1998). Some of the major food fish species are tilapia (*Oreochromis* sp.), carp (*Cyprinus* sp.), channel catfish (*Ictalurus punctatus*), and salmon (*Salmo* sp., *Oncorhynchus* sp.), while gold fish (*Carassius auratus*), zebrafish (*Danio rerio*), and medaka (*Oryzias latipes*) are used in basic research. Genetic engineering of farm animals offers great potential for improvement of selected genetic traits of agricultural significance. Several species of fish have also been used to exploit this technology for commercial purposes and examples include induction of freeze resistance in transgenic salmon using an anti-freeze protein gene (Fletcher et al., 1988) and production of growth enhanced

fish using novel growth hormone (GH) genes (Dunham et al., 1987; Brem et al., 1988; Penman et al., 1990) or an Insulin-like Growth Factor (IGF) gene (Chen et al., 1995). Although several species of fish have been used to produce lines of transgenic fish, in only a few cases has germline transmission and stable long-term transgene expression been satisfactorily demonstrated. The main problem with obtaining germline transmission has been the widespread mosaicism of the transgene due to delayed integration into the host genome during early embryonic development (Maclean and Rahman, 1994; Gong and Hew, 1995). Transgenic gene knockout technology (Wong and Van Eenennaam, 2008; Thresher et al., 2009) and gene editing technology that mutates the fish without inserting exogenous DNA has also been accomplished (Tan et al., 2013). Further, another biotechnological tool based on a bacterial CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-associated protein-9 nuclease (Cas9) from *Streptococcus pyogenes* (Cong et al., 2013) has gained much importance in knocking down the myostatin gene, thereby augmenting muscular growth in mammals. CRISPR/Cas9 system has been widely used in plants and animals to trigger mutagenesis. Jao et al. (2013) opined that CRISPR/Cas9 have the potential to develop high rates of homozygous/biallelic mutagenesis and allow phenotypic evaluation in the transformed fish that obtain the mutation in every cell.

TRANSGENIC FISH FOR GROWTH ENHANCEMENT

Transgenic fish have been produced for numerous species of fish including non-commercial model species such as the loach, *Misgurnus anguilIicaudtis* (Maclean et al., 1987), Medaka, *Oryzias latipes* (Ozato et al., 1986), topminnows and zebra fish (Rainbow zebra fish) (Gong et al. 2002). Several experimental trials have also produced and evaluated transgenic farmed fish species including gold fish (Zhu et al., 1985), silver carp, common carp, mud loach, Atlantic salmon, rainbow trout (Chourrout, 1986), chinook salmon, coho salmon, Nile tilapia (Brem et al., 1988) and channel catfish (Dunham et al., 1987). Additionally, gene transfer has also been accomplished in a game fish, Northern pike (Gross et al., 1992). Growth performance of the transgenic salmon showed an average of 11-fold increase of growth compared to nontransgenic controls

(Devlin et al., 1995). Nam et al. (2001) found that 35-fold increases in growth of transgenic mud loach, *Misgurnus mizolepis* developed by transgene produced from the same species through microinjecting the mud loach growth hormone (mlGH) gene fused with the mud loach β-actin promoter than their non-transgenic siblings. Most of the farmed transgenic fish aimed for the growth improvement, and many of these fishes have used for studying and standardizing growth pattern. Result of growth evaluation of different studies has showed positive result in many of the fishes. But, some of the studied has proved that the technology is unsuccessful due to many unknown reasons. Some of the studies have been quoted in the following Table.

Table 6.1: Studies showing enhancement of growth achieved in different target organisms worldwide with citation

FAMILY AND SPECIES	CONSTRUCT	GROWTH	COUNTRY	SUPPORTING CITATION
Salmonidae				
Atlantic salmon, *Salmo salar*	opAFP-csGH	2–6-Fold	Canada	Du et al. (1992) and Fletcher et al. (2004)
Coho salmon, *Oncorhynchus kisutch*	ssMT-ssGH	Up to 11-fold	Canada	Devlin et al. (1994a,b)
Coho salmon *Oncorhynchus kisutch*	opAFP-csGH	3–10-Fold	Canada	Devlin et al. (1995)
Chinook salmon, *O. tshawhytscha*	opAFP-csGH	6-Fold	Canada	Devlin et al. (1995)
Rainbow trout, *O. mykiss*	opAFP-csGH	3.2-Fold	Canada	Devlin et al. (1995)
Cutthroat trout, *O. clarki*	opAFP-csGH	6-Fold	Canada	Devlin et al. (1995)
Arctic charr, *Salvelinus alpines*	Various constructs	Up to 14-fold	Finland	Pitkanen et al. (1999)
Rainbow trout *O. mykiss*	ssGH-ssGH	None	Finland	Pitkanen *e*t al. (1999)
Cichlidae				

Nile tilapia, *Oreochromis niloticus*	opAFP-csGH	2–4-Fold	UK	Rahman et al. (1998; 2001) and Rahman and Maclean (1999)
Nile tilapia *Oreochromis niloticus*	ssMT-ssGH	None	UK	Rahman et al. (1998; 2001) and Rahman and Maclean (1999)
Tilapia, *O. hornorum* Hybrid	hCMV-tiGH	82%	Cuba	Martinez et al. (1996)
Ictaluridae				
Channel catfish, *Ictalurus punctatus*	RSVLTR-rtGH,	Up to 26%	USA	Dunham et al. (1992)
Channel catfish *Ictalurus punctatus*	mMT-hGH	None	USA	Dunham et al. (1987)
Heteropneustidae				
Heteropneustes fossilis	Zpb-ypGH	30–60%	India	Sheela et al. (1999)
Cyprinidae				
Goldfish, *Carassius auratus*	mMT-hGH	None	PR China	Zhu et al. (1985)
Common carp, *Cyprinus carpio*	mMT-hGH	None	PR China	Zhu et al. (1989)
Common carp *Cyprinus carpio*	cbA-gcGH	42–80%	PR China	Zhu (1992) and Wang et al. (2001)
Catla, *Catla catla*	RSVLTR-rtGH	None	India	Sarangi et al. (1999)

Common carp *Cyprinus carpio*	ccbA-ccGH	4-Fold	Israel	Hinits and Moav (1999)
Rohu *Labeo rohita*	CMV-roGH	4-Fold	India	Venugopal et al. (2004)
Rohu *Labeo rohita*	gcbA-roGH	4.5–5.8-Fold	India	Venugopal et al. (2004)
Esocidae				
Northern pike	RSVLTR-bGH	30%	USA	Gross et al. (1992)
Cobitidae				
Mud loach, *Misgurnus misolepis*	mlb-actin-mlGH	Up to 35-fold	Republic of Korea	Nam et al. (2001; 2002)

Techniques for Gene Transfer

Gene transfer research in fish began in mid 1980's using microinjection (Zhu et al., 1985; Dunham et al., 1987). Zhu et al. (1985) published the first report of transgene microinjection into the goldfish fertilized eggs. As pronuclei are extremely difficult to visualize in live one-cell fish embryos, in almost all fish gene transfer experiments, the foreign gene was microinjected into one-to- four cell embryo cytoplasm (Hayat, 1989). Ozato et al. (1986) used a slightly different approach and injected the transgenes in the oocytes of the Medaka, which had been removed from the ovaries nine hours before ovulation. The chicken delta-crystallin gene was injected and found in four of the eight Medaka embryos examined (Ozato et al. 1986). An average of about 5% of the surviving microinjected embryos possesses the foreign DNA. However, microinjection is a slow and tedious procedure (Powers et al. 1992), and can also result in high mortality of the eggs (Dunham et al. 1987). Following microinjection, new techniques such as retroviral integration, electroporation, sperm mediated transfer, liposomal-reverse-phase-evaporation, and high velocity microprojectile bombardment were developed (Chen and Powers, 1990) for fast and efficient production of

large numbers of transgenic individuals. The first successful gene transfer utilizing electroporation produced integration rates and survival similar to that for microinjection (Inoue et al., 1990). Further, Powers et al. (1992) successfully demonstrated that electroporation can be more efficient than microinjection with integration rates sometimes as high as 30-100%. Walker (1993) found that hatching rates were higher for electroporated embryos than for microinjected channel catfish embryos and post-fertilization electroporation treatments had higher hatching rates than electroporation of sperm and eggs prior to fertilization. Electroporation technique is quite easier and less skill required than microinjection and reasonably more numbers of first generation individuals can be produced rapidly. Further, electroporated embryos are more likely to keep alive than microinjected embryos during the initial phase of incubation (Dunham and Winn, 2014).

In most of the published data on transgenic fish, integration rate is rather low (<5%) although this figure varies widely (Hackett, 1993; Fletcher and Davies, 1991). Nuclei are not usually visible and DNA is injected into the cytoplasm; this could have an effect on the extent and timing of integration of the microinjected transgene. Integration of DNA constructs into the fish genome at stages beyond the one cell stage, results in mosaicism. Mosaicism may occur either in somatic or germ cells of the developing fish. Transfer of the transgene from the founder generation to their progeny in less than the expected Mendelian ratio may indicate that the transgenic fish have germline mosaicism (Guyomard et al., 1989; Penman et al., 1990; Culp et al., 1991). Other unexpected levels of transgene genetic transmittance may be due to transfer of unintegrated transgenes to the offspring (Stuart et al., 1988; Guyomard et al., 1989) or multiple transgene integration sites (Penman et al., 1990; Stuart et al., 1990; Tewari et al., 1992).

β-actin Gene Promoter and its Role in Growth Enhancement

The choice of promoter/enhancer could determine the success in obtaining expression of transgenes in transgenic fish. Tissue specificity of promoters and enhancers depends on tissue-specific transcription factors and their availability which may affect the expression of the

transgene. A transgene has three components, a promoter sequence, a structural gene and a regulatory 3' sequence. The promoter is responsible for the transcription of the structural gene, which is then responsible for the desired phenotypic character. These two gene components can be isolated from different species or from the same species. Some researchers have suggested that constructs made of fish promoters and fish genes are expressed more efficiently than mammalian constructs and/or promoters in fish cells (Winkler et al., 1992; Betancourt et al., 1993). The aberrant or nil expression was sometimes attributed to the use of promoter sequences from more distantly related species (Chourrout, 1986; Brem et al., 1988; Penman *et al*. 1990).

Regulatory sequences of viral origin such as Rous Sarcoma Virus have been effectively used to drive GH transgene expression in Carp by Zhang et al. (1990) and 20% enhanced growth was reported in transgenics as compared to non-transgenic controls. However, the use of transgene constructs which are as near as possible homologous both with respect to regulatory and structural gene sequence is to be recommended, largely because they are more effective (Friedenreich and Schartl, 1990; Alam et al., 1996), but also because of the probability that use of the subsequent fish in aquaculture would have increased customer acceptance. This is especially true with respect to sequences of viral origin, which should surely be avoided because it may cause adverse effect if the progeny of the transgenic organism is ever likely to enter the human food chain. Moreover, the possible use of transgenic fish in the aquaculture industry has raised social and moral considerations which have encouraged the development of 'all-fish' gene constructs (Du et al., 1992; Hackett, 1993).

After achieving the isolation and characterization of the common carp (*Cyprinus carpio*) β-actin gene (Liu et al., 1990b) at first, a variety of fish gene constructs containing carp β-actin regulatory sequences (Liu et al., 1990a; Liu et al., 1990b) have been developed. These constructs were tested for their potency in fish tissue culture cells (Moav et al., 1992), and by transient expression in early fish development was accomplished (Moav et al., 1992; Moav *et al*. 1993). The β-actin gene promoter from medaka fish has been isolated and used to drive a lacZ reporter gene in embryos from the same species (Takagi et al., 1994). Strong Green Fluorescence Protein (GFP) expression has also been shown in medaka fish using a medaka β-actin gene promoter (Hamada et al., 1998). The

β-actin gene promoter from zebrafish has been isolated by Higashijima et al. (1997) and these authors also showed that this sequence could drive the GFP gene efficiently in Zebrafish. The use of transgenic constructs comprising `all-fish' sequences has already been reported in several fishes. Du et al. (1992) have used a growth hormone gene from Chinook salmon (*Oncorhynchus tschawytscha*) driven by a promoter sequence from an antifreeze gene of the ocean pout (*Macrozoarrces americanus*) for the production of transgenic Atlantic salmon (*Salmo salar*) and found growth enhancement of 2 to 6-fold higher than controls. Similarly, Devlin et al. (1994b) reported extraordinary growth (up to 11-fold over controls) in Coho salmon (*Oncorhynchus kisutch*) following introduction and expression of an all-salmonid construct consisting of a sockeye salmon (*Oncorhynchus nerka*) GH gene spliced to a metallothionein promoter of the same origin.

Status of Transgenic Fish

Genetically modified fish species are not popularized or accepted for wide distribution because of the issues on food safety and environmental risk. The general public has little understanding of biology and the vagaries of how their food is grown and where it comes from, so public education of the positive and negative aspects of transgenic food and its effects is paucity and is most warranted (FAO, 2001). In order to study the impact and make public more aware, different researchers have studied the impact of transgenic fish on health. Berkowitz and Kryspin-Sorensen (1994) have elaborately discussed a food safety issue posed by genetically modified fish. Additionally, Dunham (2004) analyzed the theoretical food safety of GH transgenic salmon. The scientific organizations like FAO, WHO, US National Academy of Sciences etc., have stated that transgenic fish will not cause any human health risk in most of the consumers but rarely which may develop allergic reaction in sensible people. Added to health hazard, the other impact of transgenic fish is that it can cause risk to native gene pools and natural ecosystems. According to the detailed review by Dunham (2011) transgenic fish have relatively lower fitness than their wild counterparts. Hence, if the transgenic fish escape to natural environment, transgene from the fish would be likely eliminated due to its inferiority, which prevent the adverse environmental effects with due course of time (Dunham and Winn, 2014). In an observation, "GloFish",

the ornamental transgenic fish developed were unable to establish in wild in the US due to their inferior fitness than normal zebrafish and hence, Glofish in not an environmental threat.

Commercialization of genetically growth enhanced transgenic fish has apparently taken place in some countries such as Chile, China, Cuba and New Zealand. However, in locations such as Europe and Japan, conservative approaches to the development of transgenic fish prevailing politically for many more years. Several attempts have been made to produce transgenic fish for improving growth in aquaculture, but most of the study remained at experimental level. However, some of the companies have succeeded and produce transgenic fish in commercial level. AquaBounty Technologies (Niiler, 2000) have developed a genetically modified (GM), triploid Atlantic salmon, the AquAdvantage salmon. This transgenic fish was transformed with a construct with a promoter from an ocean pout and growth hormone-regulating gene from a Pacific Chinook salmon. This fish has been developed with aim of having year-round growth in the fish against the fish growth observed during summer and spring and also to achieve the fast growth than the native original salmon i.e attained marketable size in 16-18 months instead of three years observed in other fishes. This fish has shown positive result and the transgene allowed the fish to achieve the faster growth. In different experiment, the fish has been studied for different adverse effect caused by the fish and found that the most of the impact of the developed transgenic fish was not significantly different from the control (diploid salmon) salmon fish (Bodnar, 2010). Finally, in November, 2015, the Food and Drug Administration (FDA) approved AquaBounty Technologies' application after several trials to sell the AquAdvantage salmon to U.S. consumers. Thereafter, AquaBounty has potential licensees for their salmon in Scotland, New Zealand and the United, Canada, Chile and Europe. Added to this earlier, transgenic carps have been commercialized in China, and transgenic Nile tilapia in Cuba, although this is disputed. Moreover, the first commercial transgenic fish in the U.S. may be Zebrafish. These fish expressing various fluorescent pigments of jelly fish are marketed in the ornamental fish industry in early 2004 in the trade name of "Glofish". The fishes like medaka, zebrafish, mummichog and swordtails were widely used for transgenic fish production especially for the application as animal models for studies

of human diseases, vertebrate development, toxicology, and genetics which attributed because of its small size, rapid generation time and cost-effective laboratory culture techniques (Dunham and Winn, 2014).

CONSTRAINTS

Although it is quite feasible to accomplish gene transfer in commercial fish species, research facilities adequate to produce and confine transgenic fish are relatively few. The basic disadvantages of transgenic fish production of commercially important aquaculture fish species include the large size, seasonality of their egg production, more expensive holding facilities and large generation intervals, at least 6 months for tilapia and 1-2 years for carps where as 2-6 years in case of salmonids (Dunham and Winn, 2014). Mosacism in the parent generation of transgenic fish lengthens and slows research projects. Many commercial species have relatively long generation intervals compared to species that serve as laboratory fish models and other experimental models such as fruit fly, medaka, zebrafish and mice. Not surprisingly, lack of control on where in the genome the transgenes are inserted and other possible genetic factors such genetic background and epistasis have lead to variable responses in individual transgenic families or lines. These dictates combining transgenesis with traditional techniques such as selection to identify the high performance transgenic lines and optimize their gene expression and phenotype. This makes gene transfer a medium to long term breeding program rather than a short term one. However, transplantation of primordial germ cells is now possible opening the avenue for new gene knockout technology. Early transgenic fish was also hindered by a lack of fish promoters and much of the early research was conducted with viral promoters. If commercialization is the objective, much of that early research needs to be reiterated using fish promoters which will likely receive much better public perception and acceptance. Despite these obstacles, in actuality, molecular technique in fish research has made very good progress and results have, in some cases, been dramatically successful.

SUMMARY

Absolutely no doubt that Growth Hormone incorporation is beneficial for the growth, meat quality and other physiological functions of the fish. The

transgenic fish with Recombinant Growth Hormone transgene will the futuristic approach for increase the aquaculture production with limited and shrinking natural resources to supply feed the ever-increasing human population. Hence, it is need of the day to take a wise decision after considering all positive and negative impacts of transgenic fish by all the relevant scientific agencies and political bodies. Further, the transgenic fish production technology should be fine tuned able to produce the sterile transgenic fish population through more advanced technologies such as gene knockout and gene editing technologies. For getting approval of transgenic commercial fish production from all scientific agencies, there should be an absolute commitment from all the concerned people those who involved in the aquaculture farming to ensure infallible confinement in each stage of production cycle of fish to prevent the escapes of the fish.

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