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Review

INTACT PROTEIN ABSORPTION BY THE FISH GUT. 2. APPLICATION POTENTIAL AND LIMITATIONS

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Summary

One of the major roles of the fish gut is to exclude antigenic materials. Nevertheless, considerable evidence has accumulated to suggest that the vertebrate gut, in general, is naturally permeable to ingested macromolecules. This has led to the proposal that it may be possible to employ the gut's inherent leakiness as a portal for delivering production–related peptides and proteins within the diet. This article presents an overview of research findings related to the methods employed to manipulate reproduction and growth in cultured fish using the oral route of administration. Consideration is given to the potential application of macromolecule uptake as this relates to disease and the maintenance of health of cultured inventory. In addition, the limitations which are imposed upon the commercial application of the process are briefly examined.

Key words: immunostimulants, peptide antibiotics, LHRH, GnRH, growth hormone

INTRODUCTION

It is well established that the vertebrate gut is permeable to a wide variety of ingested macromolecules, ranging in size from peptides through to particulate matter. In our previous review article (McLean et al., 1990a), we

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considered the mechanisms for macromolecule absorption in the fish gut and potential physiological role that this natural phenomenon might play; particularly as this related to the uptake of intact proteins. While the literature covering the mechanisms of absorption has expanded over the last few years, particularly for teleosts, this has generally only verified previous studies. With respect to physiological significance however, there has been significant progress within the field. Specifically many studies have examined the possibility for applying the natular leakiness of the fish gut to benefit production processes. This gathering interest has been spurred on by the increased availability of recombinant peptides and proteins, derived from both homeotherm and poikilotherm sources. The following endeavours to provide an overview of recent research findings as these relate to the manipulation of production-oriented processes using oral delivery of such peptides and proteins. Attention will be levelled at methods for controlling or governing reproduction, health and growth regulation in cultured teleosts. In addition, consideration is given to the limitations that must be overcome prior to anticipating practical application of the phenomenon.

INDUCED OVULATION

An ability to artificially induce maturation in fish provides a number of practical advantages. Firstly, initiation methods permit induction of spawning in species that would otherwise fail to breed in captivity. Secondly, they allow synchronisation of maturation in species which normally spawn in captivity. Thirdly, induced ovulation methods provide a means of obtaining gametes earlier than would otherwise be possible. Fourthly, in species or strains that mature asynchronously, induced ovulation technologies may be used to facilitate hybridisation (Donaldson and Devlin, 1996). Finally, the techniques may be employed to hasten maturation for roe production or as a means of reducing time to maturation in sex-reversed stocks (Henry et al., 1998). Presently, most commercial operations employ hypophysectomy techniques or inject or implant releasing hormones. While more costly than traditional methods of inducing ovulation, an ability to deliver maturation-inducing peptides and proteins orally would be invaluable because: 1) there would be a reduction in stress for species which may be vulnerable to handling, 2) treatments could be given to ornamental species too small for injection, and 3) the expense and risks associated with the use of needles would be eliminated.

The first suggestion that maturation could be controlled in teleosts following feeding of biologically active components, was derived from studies which used amphibian and mammalian pituitary preparations. For example, Owen (1937), fed bitterling with desiccated mammalian anterior pituitary, as 5 mg supplement daily, and observed that 68% of treated fish developed

nuptial coloration. The feeding of loach with 1 frog pituitary gland per day, for 30 days, resulted in partial maturation and an increase in mean egg diameter when compared to controls (Kubota, 1953). Total dietary replacement with dried beef pituitary gland powder induced ovulation and shortened brood interval by 10–15 days in adult swordtail, while accelerating sexual differentiation in offspring (Regnier, 1937, 1938). The feeding of lake trout with 1 cc of anterior lobe two times a week for 8 months, from age 1 to 9 months, increased egg size and induced precocious maturation in females by 12 months (Cantilo and Gonzales-Regalado, 1942). More recent studies have reported induced ovulation of goldfish orally administered with salmon pituitary extract (Suzuki et al., 1988a, b). Importantly, the latter authors also examined changes in the endocrine status of treated fish, and detected the presence of salmon gonadotropin within the circulation, which they considered to be responsible for the accompanying elevations in testosterone and 17α–20β–dihydroxy–4–pregnan–3196one. By extrapolation, it is likely that the earlier studies with crude pituitary preparations had like effect, thus confirming the passage of physiologically active gonadotropin from gut lumen to circulation. However, because crude pituitary preparations and partially purified gonadotropins express varying potency, induce immune response upon sustained use, have poor storage characteristics and are unstandardised (Harvey and Carolsfeld, 1993), they offer limited advantage as a material for use as dietary supplements or otherwise.

A recent innovation in the control of fish reproduction has been the use of gonadotropin–releasing hormones (GnRH; see Donaldson and Devlin, 1996). The advantages of employing releasing hormones relate to the fact that they act early in the hormonal chain, stimulating the natural release of gonadotropin, exhibit wide species potency, are easy to manufacture and, therefore, economical. Moreover, GnRHs are highly stable over a range of temperatures and may, in theory, be stored indefinitely. In addition, many analogue forms of this molecule are 50–100 times more potent than natural forms, such that only small quantities of the peptide are required to induce maturational processes. Some analogue forms incorporate terminal residues which have been swapped with ethylamide, while others include D amino acids, or both. These substitutions have the effect of making the molecule more resistant to proteolysis, such that they are excellent contestants for oral delivery use. Indeed, several studies have already demonstrated the potency of orally delivered LHRH analogues. For example, McLean et al. (1991), using intubation techniques, gave 0.2–20 $\mu g\ g^{-1}$ body weight des–Gly^{10} (D–Ala^{6}) LHRH to coho salmon, and observed a dramatic increase in circulating gonadotropin levels, thereby illustrating retained bioactivity of the absorbed molecule. Oral and dietary administration, using various LHRH analogues, either in combination with dopamine agonists, as single or multiple treatments, at various doses (1–5 $\mu g\ g^{-1}$ body weight), with different species, have also resulted in successful ovulation and or spermiation (e. g., Thomas and

Boyd, 1989; Solar et al., 1990a, b; Sukumasavin et al., 1992; Breton et al., 1995). Clearly, the manipulation of reproduction in fish via dietary incorporation of LHRH analogues is practical and workable even on an economic basis. Moreover, due to the interests and financial competence of the aquarist, it is likely that control of reproduction, using dietary–based treatments, will represent the first commercial application of the phenomenon.

CONTROL AND TREATMENT OF DISEASE

An important area of concern regarding disease control relates to the global (mis)use of antibiotics. An example of the problems associated with the application of antimicrobials in aquaculture is the emergence of strains of *Aeromonas salmonicida* that exhibit plasmids encoding antibiotic resistance (see GESAMP, 1997). This has resulted in increased efforts to develop effective vaccines and, in Europe and North America, a dramatic decline in the use of chemotherapeutants has been registered. In the context of the present review however, oral vaccination can only be considered based upon proteinaceous antigenic components. The advantages of oral vaccination are diverse and provide a means to reduce labour costs, save time, negate inventory handling, eliminate needle use, which may cross–contaminate, and abolish problems of treatment water discharge. Trials with synthetic peptide–based virus vaccines in terrestrial livestock have provided promising results, although contradictions have been reported from large fields–tests (Taboga et al., 1997). For fish, recombinant subunit vaccines, consisting of nucleoproteins, could be incorporated into feeds, although research in this area will be restrained due to developmental costs.

An alternative to traditional antibiotics which may be delivered in feeds, are third generation antimicrobials: the peptide antibiotics (for reviews see: Hancock, 1997). The advent of peptide antibiotic technology heralds the arrival of a novel group of drugs which avoid rapid resistance problems as seen with other antimicrobials. This is because peptide antibiotics act to kill bacteria by physical disruption of the cell membrane. While no study has examined the efficacy of peptide antibiotics in fish, due, in the main, to their present scarcity, these products will, no doubt, become more readily available over time. Since they are resistant to proteolysis, peptide antibiotics represent good candidates for dietary incorporation studies. In a like manner, a number of peptide and protein products have been shown to stimulate the natural immune system of domestic livestock, humans and teleosts. These co–called »immunostimulants« act by enhancing both humoral and non–specific defence mechanisms. Of note, in the context of the present review, are peptides, derived from fishery byproducts, or produced following enzymatic hydrolysis of cod protein, which enhance vaccine efficiency (Andersen, 1992), and macrophage activities (Bøgwald, et al., 1996) of salmonids. A comprehensive

review of the potential that immunostimulants offer the aquaculture industry has been provided by Raa (1996).

GROWTH MANIPULATION

The advantages of treating cultured fish with growth enhancing proteins and peptides are extensive and do not only relate to their growth promoting ability. Acceleration of growth by endocrine manipulation reduces the time required to produce market–sized animals (Down and Donaldson, 1993); provides the option of producing larger animals of greater value over a normal production cycle (McLean et al., 1993); furnishes a method for adjusting marketing strategies, wherein produce is available »out of season« (McLean and Donaldson, 1993); reduces environmental impact due to enhanced feed conversion efficiency (Mayer and McLean, 1995); elevates immune responsiveness in treated individuals (Kitlen et al., 1997); enhances osmoregulatory capacity (Shrimpton et al., 1995); permits manipulation of end–product quality characteristics (McLean et al., 1997); and enhances development of roe and induces precocious maturation in sex–reversed broodstock (McLean et al., 1994). To date, several studies have shown that natural and recombinant GHs are absorbed by the fish gut, subsequently transported into the circulation in a physiologically active form, and exert growth promoting activity (e. g., Dagani and Gallagher, 1985; McLean and Donaldson, 1988, 1990; Le Bail et al., 1989; McLean et al., 1990b, 1993; Hertz et al., 1991; Zhang, 1993; Tsai et al., 1994). The optimal time for applying GH, in the commercial setting, has been considered by Mayer and McLean (1995), whereas the social and ethical issues arising from the use of GH in food animals has been critiqued by McLean et al. (1997).

LIMITATIONS TO COMMERCIAL APPLICATION

There is little doubt that the mucosal barrier represents the major obstruction to orally active proteins and peptides. The efficiency of this barrier has been illustrated by comparing marker protein uptake in agastric and gastric species and matching rectal versus oral administration studies (e. g. McLean and Ash, 1986, 1987). In combination, such observations indicate that it may be possible to increase absorption of orally delivered peptide and protein drugs to levels close to that seen during rectal administration. Several studies have examined this prospect (Table 1), using various strategies, including: protection of the bioactive from the stomach and gastric secretions; defence against lumenal enzymes; modification of the epithelial layer — in essence, punching holes into the gut wall, and increasing protein and peptide presence by delivery within a protective »vehicle«. While some of these methods have

*Table 1. A wide variety of methods have been investigated for their utility to enhance the gastrointestinal uptake of, or to protect marker proteins and peptides from the degradative action of the fish gut. The following examples illustrate the diversity of tactics examined and the species investigated. HRP = horseradish peroxidase; HGG = human gamma globulin; SBTI = soyabean trypsin inhibitor; LPC = lysophosphatidylcholine; Quil A expresses detergent properties; *the antacid employed was sodium bicarbonate.*

Tablica 1. Ispitivan je velik broj metoda glede njihove uporabljivosti za postizanje veće gastrointestinalne apsorpcije proteina, ili zaštite proteinskih markera od degradacijske aktivnosti probavnoga trakta. Sljedeći primjeri pokazuju raznolikost ispitanih taktika i istraživanih vrsta.

Formulation	Marker protein/peptide	Impact	Species examined	Reference
delivered in feed with yeast	GH	Increased growth	Striped mullet	Tsai et al., 1994
alginate capsules	GH	Increased growth	Rainbow trout	Moriyama et al., 1990
administered with deoxycholate	GH	Increased growth	Common carp	Hertz et al., 1991
administered with LPC	GH	Increased growth	Coho salmon	McLean et al., 1990b
admixed with feed	GH	Increased growth	Coho salmon	McLean et al., 1993
administered with LPC	LHRHa	Induced spermiation	Sablefish	Solar et al., 1990a
administered with antacid*	LHRHa	Induced ovulation	Sablefish	Solar et al., 1990b
administered with SBTI	HRP	Increased absorption	Rainbow trout	McLean and Ash, 1990
administered with Quil A	HGG	Increased absorption	Tilapia	Jenkins et al., 1994
alginate capsules	HGG	Increased absorption	Atlantic salmon	O'Donnell et al., 1996

proven effective, sometimes resulting in a 1000–fold increase in absorption, others have proven less satisfactory (Table 1).

The evolution of oral delivery systems include not only basic physiology–oriented research, but also studies upon drug stability and properties, optimal dosage(s) required, storage life of presentation formulae, the need for excipients within the final product, actual production costs, including development and manufacture of specialised equipment for preparation, legal servicing (patenting etc.), field trial expenses etc. Clearly, while the oral route is highly attractive, it is likely that such formulations will be more expensive than traditional drug delivery technologies due to the aforementioned development and licensing costs, and this may slow commercial take–up.

Sažetak

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APSORPCIJA INTAKTNOGA PROTEINA U PROBAVNOME TRAKTU RIBA. 2. MOGUĆNOSTI APLIKACIJE I OGRANIČENJA

Jedna od glavnih uloga probavnoga trakta u riba jest izlučivanje antigenskih tvari. No, brojni dokazi upućuju na to da je, općenito uzevši, probavni trakt kralježnjaka prirodno propustan za makromolekule unesene u organizam hranom. Na osnovi toga predloženo je da bi se prirođena propusnost probavnoga trakta mogla iskoristiti za unošenje proizvedenih peptida i proteina u organizam hranom. Ovaj rad daje pregled rezultata istraživanja dobivenih metodama koje su korištene radi utjecaja na reprodukciju i rast uzgajanih riba unošenjem tvari oralno. Razmatrana je moguća primjena apsorpcije makromolekula u organizam što se tiče bolesti i održavanja zdravlja uzgajanih riba. Osim toga, ukratko su ispitana ograničenja koja se postavljaju glede komercijalne promjene ovoga procesa.

Ključne riječi: immunostimulansi, peptidni antibiotici, LHRH, GnRH, hormon rasta

REFERENCES

Andersen, D. P. (1992): Immunostimulants, adjuvants and vaccine carriers in fish: applications to aquaculture. Annul Rev. Fish Dis. 2, 281–302.

Bøgwald, J., Dalmo, R. A., Leifson, R. M., Stenberg, E., Gildberg, A. (1996): The stimulatory effect of a muscle protein hydrolysate from Atlantic cod,

Gadus Morhua L., on Atlantic salmon, *Salmo salar* L., head kidney leucocytes. Fish Shellf. Immunol. 6, 3–16.

Breton, B., Roelants, I., Mikolajczyk, T., Epler, P., Ollevier, F. (1995): Induced spawning in teleost fish after oral administration of GnRH–A. pp. 102–104 in: Reproductive physiology of fish. (F. W. Goetz and P. Thomas, editors). FishSymp '95, Austin, Texas.

Cantilo, E., Gonzales–Regalado, T. G. (1942): Investigaciones realizadas con el extracto anterohysofisario en el desarrollo del *Salvelinus fontinalis*. Rev. Med. Vet. (Buenos Aires) 24, 323–338.

Dagani, G., Gallagher, M. L., (1985): Effects of dietary 17a–methyltestosterone and bovine growth hormone on growth and food conversion of slow– and normally–growing American elvers (*Anguilla rostrata*). Can. J. Fish. Aquat. Sci. 42, 185–189.

Donaldson, E. M., Devlin, R. H. (1996): Uses of biotechnology to enhance production. pp. 969–1020 in: Principles of salmonid culture (W. Pennell and B. A. Barton, editors). Elsevier, Amsterdam.

Down, N. E., Donaldson, E. M. (1993): Hormonal enhancement of growth. pp. 109–120, In: Recent advances in aquaculture, vol. 4., Blackwell Scientific Publications, Oxford.

GESAMP (1997): Towards safe and effective use of chemicals in coastal aquaculture. GESAMP Reports and Studies, 65, 45pp.

Hancock, R. E. (1997): Peptide antibiotics. Lancet 349, 418–422.

Harvey, B., Carolsfeld, J. (1993): Induced breeding in tropical fish culture. Ottawa: IDRC.

Henry, J. C., McLean, E., Mayer, I., Donaldson, E. M. (1998): Approaches to advancing maturation in sex–reversed Atlantic salmon (*Salmo salar* L.). Aquaculture International, 6, in press.

Hertz, Y., Tchelet, A., Madar, Z., Gertler, A. (1991): Absorption of bioactive human growth hormone after oral administration in the common carp (*Cyprinus carpio*) and its enhancement by deoxycholate. J. Comp. Physiol. 161B, 159–163.

Jenkins, P. G., Wrathmell, A. B., Harris, J. E., Pulsford, A. L. (1994): Systemic and mucosal immune responses to enterically delivered antigen in *Oreochromis mossambicus*. Fish Shellf. Immunol. 4, 255–271.

Kitlen, J. W., Hejbøl, E. K., Zinck, T., Byatt, J. C., Varming, K., McLean, E. (1997): Growth and respiratory burst activity in rainbow trout treated with growth hormone and bivalent vaccine. Fish Shellf. Immunol. 7, 297–304.

Kubota, Z. (1953): On the maturing process of loach *Misgurnus anguillicaudatus* (Cantor). III. promotive action of mature of the ovary of loach bred with the bait mixed hormone. J. Shimonoseki Coll. Fish. 3, 111–116.

Le Bail, P. Y., Sire, M. F., Vernier, J. M. (1989): Intestinal transfer of growth hormone into the circulatory system of rainbow trout, *Salmo gairdneri*. Interference by granule cells. J. exp. Zool. 251, 101–107.

Mayer, L., McLean, E. (1995): Biotechnological and bioengineering strategies for reduced waste aquaculture. Water Sci. Technol. 31, 85–102.

McLean, E., Ash, R. (1986): The time–course of appearance and net accumulation of horseradish peroxidase (HRP) presented orally to juvenile common carp *Cyprinus carpio (L.)*. Comp. Biochem. Physiol. 84A, 687–690.

McLean, E., Ash, R. (1987): The time–course of appearance and net accumulation of horseradish peroxidase (HRP) presented orally to rainbow trout *Salmo gairdneri* (Richardson). Comp. Biochem. Physiol., 88A: 507–510.

McLean, E., Ash, R. (1990): Modified uptake of the soluble protein antigen, horseradish peroxidase (HRP), following oral delivery to rainbow trout *Oncorhynchus mykiss*. Aquaculture 87, 373–379.

McLean, E., Donaldson, E. M. (1988): The oral administration of production–orientated proteins and peptides to salmonids. pp. 113–123 in: Proceedings of the 39th Annual North–West Fish Culture Conference, Richmond, B. C., Canada.

McLean, E., Donaldson, E. M. (1990): The absorption of bioactive proteins by the fish gastrointestinal tract: A review. J. Aquatic Anim. Health 2, 1–11.

McLean, E., Donaldson, E. M. (1993): The role of growth hormone in the growth of poikilotherms. pp. 43–71, In: The Endocrinology of Growth, Development and Metabolism in Vertebrates. M. P. Schriebman, C. G. Scanes and P. K. T. Pang (editors), Academic Press, New York.

McLean, E., Ash, R., Teskeredžić, E., Teskeredžić, Z. (1990a): Intact protein absorption by the fish gut. Pathways of absorption and potential physiological significance. Ribarstvo Jug. 45, 108–113.

McLean, E., Donaldson, E. M., Dye, H. M., Souza, L. M., (1990b): Growth acceleration of coho salmon (*Oncorhynchus kisutch*) following oral administration of recombinant bovine somatotropin. Aquaculture 91, 197–203.

McLean, E., Parker, D., Warby, C. M., Sherwood, N. M., Donaldson, E. M. (1991): Gonadotropin release following oral administration of LHRH and its superactive analogue (des Gly^{10}(D–Ala^{6})LHRH ethylyamide) to 17β–estradiol primed coho salmon. J. Fish Biol. 38, 851–858.

McLean, E., Teskeredžić, E., Donaldson E. M., Sonza, 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., Donaldson, E. M., Mayer, I., Teskeredžić, E., Teskeredžić, Z., Pitt, C., Souza, L. M. (1994): Evaluation of a sustained–release polymer–encapsulated form of recombinant porcine somatotropin upon long–term performance characteristics of coho salmon, *Oncorhynchus kisutch*. Aquaculture 122, 359–368.

McLean, E., Devlin, R. H., Byatt, J. C., Clarke, C., Donaldson, E. M. (1997): Evaluation of a controlled release formulation of recombinant bovine growth hormone on growth and seawater adaption in coho (*Oncorhynchus kisutch*) and chinook (*O. tshawytscha*) salmon. Aquaculture 156, 113–128.

Moriyama, S., Takahashi, A., Hirano, T., Kawauchi, H. (1990): Salmon growth hormone is transported into the circulation of rainbow trout, *Oncorhynchus mykiss*, after intestinal administration. J. Comp. Physiol. 160B, 251–257.

O'Donnell, G. B., Reilly, P., Davidson, G. A., Ellis, A. E. (1996): The uptake of human gamma globulin incorporated into poly (D, L–lactide–co–glycolide) microparticles following oral intubation in Atlantic salmon, *Salmo salar* L. Fish Shellf. Immunol. 6, 507–520.

Owen, S. E. (1937): The bitterling fish response to male sex hormones. Endocrinology 21, 689–690.

Raa, J. (1996): The use of immunostimulatory substances in fish and shellfish farming. Rev. Fish. Sci. 4, 229–228.

Regnier, M. T. (1937): Action des hormones sexuelles sur l'inversion du sexs chez *Xiphophorus helleri* Heckel. C. R. Acad. Sci., Paris 205, 1451–1453.

Regnier, M. T. (1938): Contribution a l'etude de la sexualite des Cyprinodonte vivipares (*Xiphophorus helleri, Lebistes reticulatus*). Biol. Bull. 72, 385–493.

Shrimpton, J. M., Devlin, R. H., McLean, E., Byatt, J. C., Donaldson, E. M., Randall, D. J. (1995): Increased gill corticosteroid receptor concentration and saltwater tolerance in juvenile coho salmon (*Oncorhynchus kisutch*) treated with growth hormone and placental lactogen. Gen. Comp. Endocrinol. 98, 1–15.

Solar, I. I., Baker, I. J., Donaldson, E. M., McLean, E. (1990a): A method of inducing ovulation in teleosts, by oral delivery of a protected, superactive LH–RH analogue. pp. 157–160 In: Proceedings of the 21st Annual Meeting of the International Association of Aquatic Animal Medicine. R. Francis Floyd (editor) Vancouver, B. C., Canada, May 13–17 1990, 187pp.

Solar, I. I., McLean, E., Baker, I. J., Sherwood, N. M., Donaldson, E. M. (1990b): Induced ovulation in the sablefish (*Anopoploma fimbria* Pallas, 1811) following oral delivery of Des–Gly^{10}(D–Ala^{6}) LHRH ethylamide. Fish Physiol. Biochem. 8, 497–499.

Sukumasavin, N., Leelapatra, W., McLean, E., Donaldson, E. M. (1992): Orally induced spawning of Thai carp (*Puntius gonionotus*, Bleeker) following co–administration of des Gly^{10} (D–Arg^{6}) sGnRH ethylamide and domperidone. J. Fish Biol. 40, 477–479.

Suzuki, Y., Kobayashi, M., Aida, K., Hanu, I. (1988a): Transport of physiologically active salmon gonadotropin into the circulation of goldfish, following oral administration of salmon pituitary extract. J. Comp. Physiol. 157B, 753–758.

Suzuki, Y., Kobayashi, M., Nakamura, O., Aida, K., Hanyu, I. (1988b): Induced ovulation of the goldfish by oral administration of salmon pituitary extract. Aquaculture 74, 379–384.

Taboga, O., Tami, C., Carrillo, E., Nunez, J. I., Rodriguez, A., Saiz, J. C., Blanco, E., Valero, M. L., Roig, X., Camarero, J. A., Andreu, D., Mateu, M. G., Giralt, E., Domingo, E., Sobrino, F., Palma, E. L. (1997): A large–scale evaluation of peptide vaccines against foot–and–mouth disease: lack of solid protection in cattle and isolation of escape mutants. J. Virol. 71, 2606–2614.

Thomas, P., Boyd, N. (1989): Dietary administration of LHRH analogue induces spawning in spotted seatrout (*Cynoscion nebulosus*). Aquaculture 80, 363–370.

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

Zhang, Q. (1993): Effects of recombinant tuna growth hormone (R–tGH) on growth of grass carp fingerling. J. Fish. China 17, 230– 234.

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