Synthetic Growth Hormone Secretagogue GHRP-6 Exhibits Enhanced Growth Activity and Immune System Stimulation in Teleost Fish and Shrimp

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Abstract

Aquaculture is part of the solution for meeting the growing world demand for food, mainly as an animal protein source. However, current yields are insufficient for aquaculture to play this crucial role. Growth-rate enhancement is one of the approaches that have been exploited in this regard. Also, losses caused by bacteria, viruses and parasites remain a significant problem. It has been demonstrated that growth stimulants contribute not only to growth enhancement but also to fish health improvement. Growth Hormone Releasing Peptide-6 (GHRP-6) is one of the earliest-developed, synthetic, peptidyl growth hormone secretagogue receptor agonists. These compounds mimic the effect of the endogenous ligand, ghrelin. This peptide has shown its benefits for both growth enhancement and immune system stimulation in fish and crustaceans. In the present study, we demonstrate that intraperitoneal administration of GHRP-6 induces liver insulin growth factor-I messenger RNA, and increases growth hormone levels in juvenile tilapia (Oreochromis sp.), in a time-course experiment. In addition, administration of GHRP-6 in formulated feed to tilapia larvae was assessed. Growth and immune parameters such as lectin titers and intestinal intraepithelial lymphocyte numbers were increased in treated larvae. We also evaluated the effect of GHRP-6 injection over feed intake in shrimp and its effects on shrimp growth when the peptide was administrated by successive immersion baths. GHRP-6 increased feed intake, body weight and size, the number of rostral spines and gill branches, protein concentration and haemocyte number in treated shrimps.

Keywords: Growth Hormone, Secretagogues, Peptides, Larvae, growth, Innate Immune state

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Introduction

Aquaculture is part of the solution for meeting the growing world demand for food, mainly as an animal protein source. However, current yields are insufficient for aquaculture to play this crucial role. Growth-rate enhancement is one of the approaches that have been exploited in this regard. Also, losses caused by bacteria, viruses and parasites remain a significant problem. It has been demonstrated that growth stimulants contribute not only to growth enhancement but also to fish health improvement.

In teleosts fish, secretion of GH is regulated by several hypothalamic factors that are influenced by the physiological state of the animal. There is an interaction between immune and endocrine systems through hormones and cytokines. GH in fish is involved in many physiological processes that are not overtly growth related, such as saltwater osmoregulation, antifreeze synthesis, and the regulation of sexual maturation and immune functions. (Devlin *et al.* 2009)

The synthetic growth hormone (GH) secretagogues (GHSs) consist of a family of ligands, first described by Momany *et al.*, (1981). They were initially termed GH-releasing peptides (GHRPs). These synthetic compounds were developed to release GH *in vitro.* They were predicted to mimic the effect of an endogenous factor that would activate a specific receptor in the pituitary and the hypothalamus. The cloning of the receptor for these non-classical GH-releasing compounds, together with the more recent characterization of the endogenous ligands, ghrelin and des-Gln14-ghrelin, has unambiguously demonstrated the existence of a physiological system that regulates GH secretion along with GHRH and somatostatin.

Methodology

In this work we characterized for first time the GHRP-6 action over fish and crustacean growth and immunity. GHRP-6 is one of the earliest-developed, synthetic, peptidyl growth hormone secretagogue receptor agonists. It was described by Bowers *et al.* 1984.

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Our research group demonstrated that GHRP-6 increased pituitary GH secretion *in vitro* (Lugo *et al*. 2008; Martinez *et al*. 2012). In addition, food intake increased when GHRP-6 was administered by intraperitoneal injection to juvenile tilapia (Lugo *et al*. 2010), and body weight gain when given by immersion baths to tilapia larvae (Martinez *et al*. 2012). We also demonstrated that intraperitoneal administration of GHRP-6 induces liver insulin growth factor-I messenger RNA, and increases growth hormone levels in juvenile tilapia (*Oreochromis* sp.), in a time-course experiment. GHRP-6 also stimulates growth using non-encapsulated and encapsulated peptide administered by a plastic tube to the pharyngeal cavity to juveniles. In addition, administration of GHRP-6 in formulated feed to tilapia larvae was assessed and growth stimulation by this administration route demonstrated.

Results and Discussions

Growth and immune parameters such as lectin titers and intestinal intraepithelial lymphocyte numbers were increased in treated larvae.

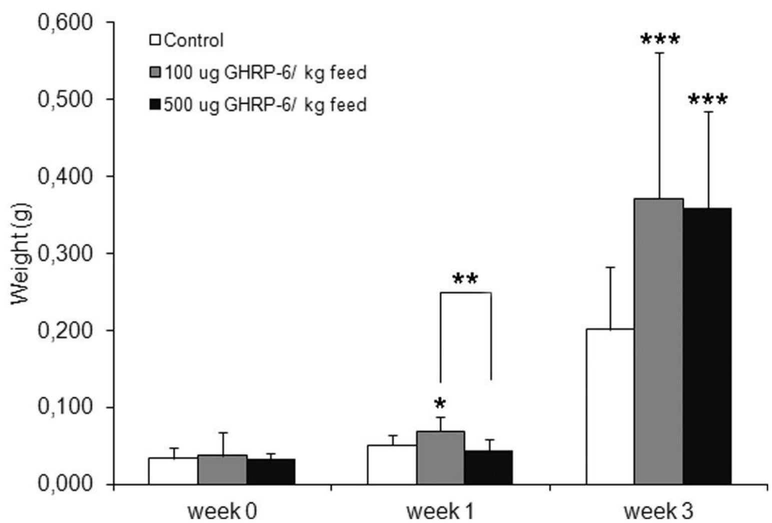

Figure 1.Growth experiment in tilapia larvae fed with GHRP-6. Two different doses were assayed: 100 µg of GHRP-6/kg feed and 500 µg of GHRP-6/kg feed. A control group was fed with the same feed but without the peptide. GHRP-6 growth-promoting effects were evaluated by measuring the body weight increase at week 1 and 3. Data are represented as mean + standard deviation. Data were analyzed by Kruskal–Wallis test followed by Dunn's Multiple Comparison Test. Asterisks represent statistically significant differences: (*) p b 0.05; (**) p b 0.01; (***) p b 0.001.

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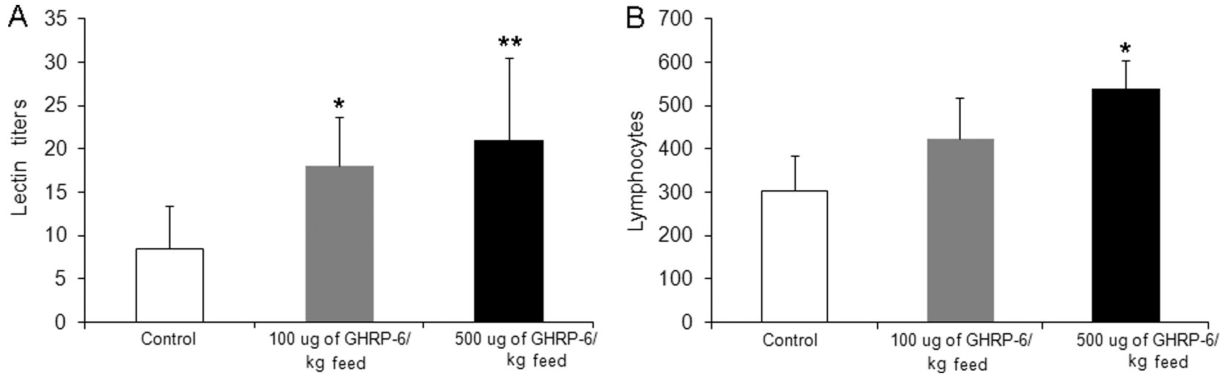

Figure 2: Immune parameters in larval homogenates at 21 days from the beginning of the experiment: Hemagglutination titers for lectins (A) and intraepithelial lymphocytes (B). Two different doses were assayed: 100 µg of GHRP-6/kg feed and 500 µg of GHRP-6/kg feed. A control group was fed with the same feed but without the peptide and treated under similar conditions. Data are represented as mean + standard deviation. Data were analyzed by Kruskal–Wallis test followed by Dunn's Multiple Comparison Test. Asterisks represent statistical significant differences: (*) p b 0.05; (**) p b 0.01

GHRP-6 has also a capacity of to boost the immune response to co-injected antigens in tilapia and catfish. Modern subunit vaccines have excellent safety profiles and improved tolerability, but do not elicit strong immune responses without the addition of adjuvants. Adjuvants are substances added to vaccine formulation that are intended to enhance the humoral and/or cell-mediated immune response to the antigen. They can be used to enhance the magnitude and the type of the antigen –specific immune response As far as we know our results demonstrate for first time the adjuvant effect caused by GHRP-6 to enhance the immune response in fish .

Ghrelin has been studied mainly in vertebrates; thus, little is known about its role in invertebrates, including crustaceans. We first evaluated the effect of GHRP-6 injection over feed intake in shrimp and its effects on shrimp growth when the peptide was administrated by successive immersion baths. GHRP-6 increased feed intake, body weight and size, the number of rostral spines and gill branches, protein concentration and haemocyte number in treated shrimps.

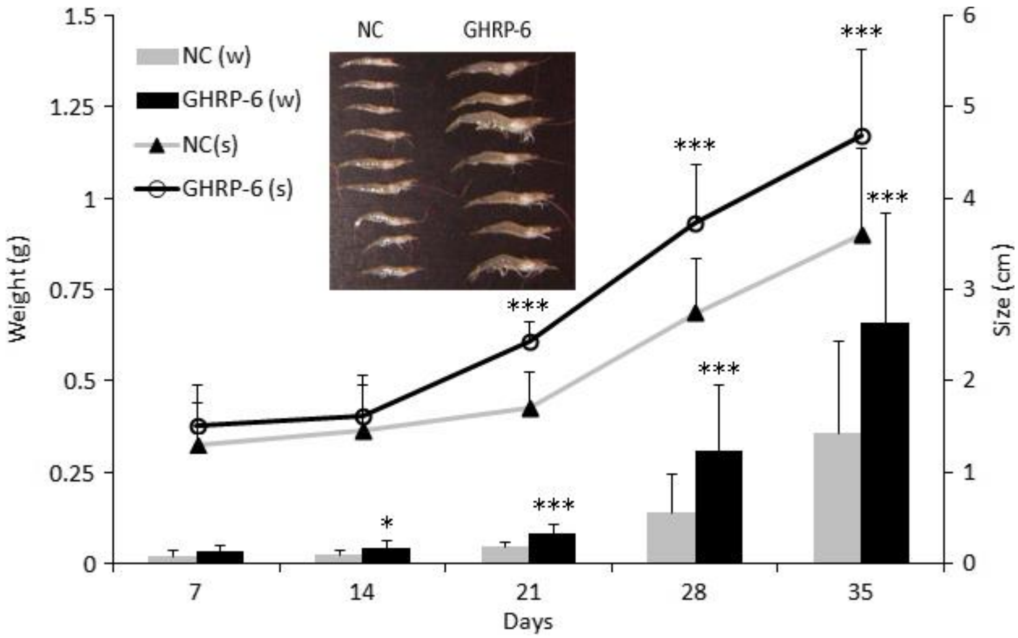

Figure 3.Growth experiment in stage 15 *Litopenaeus vannamei* postlarvae immersed in the ghrelin analogue GHRP- 6 over a 35-day period. Left axis: body weight (w in the legend) in grams (g). Right axis: size (s in the legend) in centimetres (cm). The picture on top corresponds to samples at 35 days of treatment. Samples of 50 animals were weighed and sized at 0, 7, 14, 21, 28 and 35 days. Data are represented as mean + standard deviation. GHRP-6- treated group received 200 lg of GHRP-6 per litre of water (200 lg L 1), and non-treated group received only the reduction in water volume similar to the treated group. Asterisks represent statistical significant differences: *(P < 0.05); ***(P < 0.001). A Mann–Whitney test was performed.

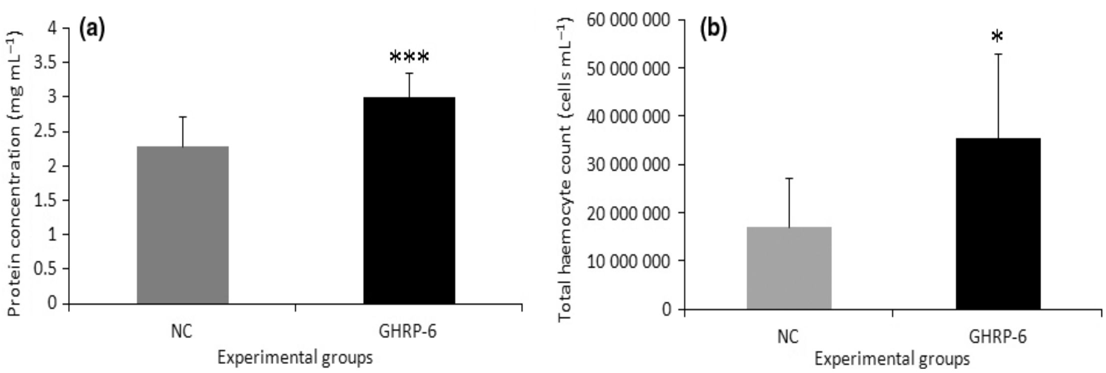

Figure 4.(a) Total protein concentration in shrimp homogenates. (b) Total haemocyte number in the shrimp haemolymph at 35 days of the *Litopenaeus vannamei* growth experiment. GHRP-6-treated group received 200 lg of GHRP-6 per litre of water (200 lg L 1), and non-treated group received only the reduction in water volume similar to the treated group. Data are represented as mean + standard deviation, N = 10. Asterisks represent statistical significant differences: *(P < 0.05), ***(P < 0.001). An unpaired t-test was performed.

We also evaluated the peptide uptake and clearance in a pharmacokinetics, using [H3] GHRP-6 administered to postlarvae. Given a limited exposure and efficient clearance of the peptide-associated radioactivity from larvae, our findings suggested that GHRP-6-treated *Litopenaeus vannamei* can be consumed safely by humans after aquaculture applications.
These results propose that GHRP-6 could be an additional tool to study growth physiology in crustaceans and also a promising candidate for development into a new biotechnology product for improving shrimp and fish growth and quality.

Scientific Support:

Article 1

Oral administration of the growth hormone secretagogue-6 (GHRP-6) enhances growth and non-specific immune responses in tilapia (Oreochromis sp.). Rebeca Martinez, Yamila Carpio, Antonio Morales, Juana María Lugo, Fidel Herrera, Claudina Zaldívar, Olimpia Carrillo, Amílcar Arenal, Eulogio Pimentel, Mario Pablo Estrada. **Aquaculture** 452 (2016) 304–310.

Article 2

Significant improvement of shrimp growth performance by growth hormone-releasing peptide-6 immersion treatments. Rebeca Martínez*, Yamila Carpio*, Amílcar Arenal, Juana María Lugo, Reynold Morales, Leonardo Martín, Ramón Franco Rodríguez, Jannel Acosta, Antonio Morales, Jorge Duconge, Mario Pablo Estrada. (*) Both authors contribute equally to this work. **Aquaculture Research**. (2017)

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