GROWTH HORMONE PRODUCTION BY DNA RECOMBINANT TECHNOLOGY FOR FISH-FARMING APPLICATION: A REVIEW

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ABSTRACT

The use of recombinant DNA technology to produce growth hormone (GH) as strategy for agricultural systems has been widely reported. Face to the necessity of new technological packs for Amazon local development, the aim of this work was to describe the art's state about GH production of amazonian fish species aiming to improve the local fish-farming productivity. An advanced search using the keywords "Growth hormone", "Genetics", "fish-farming" and/or "pisciculture" was performed in different database. Furthermore, a search was performed using the phrase "Heterologous expression of growth hormone in fish", sometimes added of "amazonian". The highest number of articles was 5970, but a thorough analysis of title and abstract resulted in only 23 articles in the last ten years. The results indicate the expression of GH in *Escherichia coli* and *Pichia pastoris*, with weight increasement varying from 21 to 65% for different fish species. The only reported amazonian specie was *Colossoma macropomum* (tambaqui), but an industrial bioprocess for high level expression was not stablished neither an in vivo assay for body weight increasement was described. These results indicate the urgent necessity for the establishment of a high-level expression bioprocess of recombinant tambaqui's GH and validation of effectivity of this hormone to promote improvement of fish-farming productivity.

Keywords: Growth hormone. DNA recombinant technology, Bioprocess, Fish-farming.

1 INTRODUCTION

Fish growth hormone (fGH), a protein hormone with a molecular weight of 22 kDa, is produced by somatotrophic cells in the anterior pituitary gland of fish. It regulates growth and body development in fish[1]. Growth hormones have already been isolated from different vertebrate groups, including: mammals, birds, reptiles, amphibians and fish[2]. cDNA cloning has allowed our structural knowledge of GH in vertebrates to gradually improve.

With the economy in mind, in Brazil the fish produced on a large scale in fish farming is the Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758), a species native to Africa and introduced to South America. However, it is worth recognizing that Brazil has native species with potential for cultivation in the Amazon region, which has approximately 2,500 species of freshwater fish, representing 75% of Brazil's species diversity[3]. Currently, Amazonian species such as tambaqui (*Colossoma macropomum*), matrinxã (*Brycon amazonicus*) and pirarucu (*Arapaima gigas*) are the most cultivated in the northern region, with tambaqui being the most consumed, accounting for around 92% of the volume produced[4]. In addition, these species have aroused interest in the fish industry, but there are no technological packages established for them.

Among the species mentioned, tambaqui is the second most important species cultivated in Brazil in terms of the international market, with a turnover of around US$ 800,000 in 2023. Although the largest domestic consumer market is the municipality of Manaus, with around 40,000 tons per year, local producers continue to face competitiveness problems, requiring the import of approximately 20,000 tons per year to meet this consumer market[5].

In this context, the aim of this work was to describe the art's state in the production of GH from Amazonian fish species with a view to improving the productivity of local fish farming.

2 MATERIAL & METHODS

The research was carried out through a bibliographic survey of the scientific databases Science Direct (www.sciencedirect.com), Scielo ® (www.scielo.br), the National Center for Biotechnology Information - NCBI (www.ncbi.nlm.nih.gov) and Google Scholar (https://scholar.google.pt/schhp?hl=pt-BR&as_sdt=0,5).

To screen bibliographic material relating to genetic engineering and biotechnology/bioproducts, searches were carried out for the keywords "Growth hormone", "Genetics", "fish-farming" and/or "pisciculture". In addition, searches were made using the phrase "Heterologous expression of growth hormone in fish" in the "abstract, title or keywords" fields when available. When compound nouns were used, quotation marks were used and searches were carried out using a terminal connected to RNP (Rede Nacional de Pesquisa - CAPES). Only peer-reviewed material published in indexed journals was considered, excluding preprints or apocryphal material.

3 RESULTS & DISCUSSION

Searching the ScienceDirect database for "heterologous expression of growth hormone in fish" resulted in 3,144 articles, filtering by the last 10 years, by the type of article "research article", subject areas "Biochemistry, Genetics and Molecular Biology", resulted in 162 articles. Screening by reading the title and abstract reduced the number of papers to just four (04) articles. Using the terms "Growth hormone Genetics, "fish-farming" and/or "pisciculture" resulted in "zero results".

The NCBI database returned 67 articles on the "PubMed Central" database, but after a more refined analysis only one (01) article was specific and actually dealt with the subject. The search for "heterologous expression of growth hormone in fish" resulted in 15 articles, but only three were actually related to the subject. Searching for "heterologous expression of growth hormone in fish" resulted in one article, but it was about the simultaneous overexpression of the GH and STAT5b genes, which inhibits the STAT5 signaling pathway in tilapia embryos (*Oreochromis niloticus*).

For the Google Scholar database, a search for "cloning and heterologous expression of growth hormone in fish", since it is more general, yielded a total of 5,970 articles using data from 2014 to the present day, of which only nine (09) dealt with the aforementioned subject. Restricting to Amazonian fish "cloning and heterologous expression of growth hormone in amazonian fish", only 194 articles were obtained, and in a more precise analysis, only one (01) article dealt with the subject.

All the articles evaluated used submerged fermentation to produce GH, expressing the result as a percentage of GH in the total protein content, percentage yield or final product concentration. Most of these had use *Escherichia coli* as the host microorganism for the recombinant GH expression system, and papers were found on the production of GH from at least 14 commercially important fish species. Remarkable papers with bioprocess results and references are presented at table 1.

Specie	Host Microbe	Bioprocess High-Lights	Reference
Paralichtys olivaceus	*E. coli*	40% of total protein correspond to GH	Jeh et al, 1998 [6]
Plecoglossus altivelise and *Epinephelus awoara*	*E. coli*	96 e 93% of GH yield	Chang et al, 2002 [7]
Pangasianodon hypothalmus	*E. coli BL21*	GH final concentration in 520 µg/mL	Sekar et al, 2014 [8]
Epinephelus lanceolatus	*Pichia pastoris*	GH final concentration in 2,80 mg/L	Hussin et al, 2014 [9]
Misgurnus anguillicaudatus	*Pichia pastoris*	53% of total protein correspond to GH	Yongfang et al, 2014 [10]
Colossoma macropomum	*Pichia pastoris*	NA	Sadalla-Pinto, et al 2014 [11]
Epinephelus lanceolatus	*E. coli*	22% of total protein correspond to GH	Chung et al, 2015 [12]
Oreochromis hornorum	*Bacillus subtilis*	GH final concentration about 100 mg/L	Ng et al, 2016 [13]
Danio rerio and *Oncorhynchus mykiss*	*E. coli MON105*	12 e 18 mg of GH purified in 5L of medium	Ocłoń, et al, 2018 [14]
Paralichtys olivaceus	*E. coli BL21*	GH purified final concentraton in 450 mg/L	Choi e Geletu, 2018 [15]
Epinephelus akaara	*Pichia pastoris*	GH final concentration between 30 and 50 mg/L	Yuan et al, 2020 [16]

Subtitle: NA – data not available.

When it comes to Amazonian fish, there is a lack of research on the subject, with only one study available in the databases[11] on the expression of recombinant GH in tambaqui (*Colossoma macropomum*), a fish species widely distributed in the Amazon basin and promising for fish farming. Silva[17], in her dissertation, details the cloning and expression of tambaqui growth hormone (tGH), using *Escherichia coli* strain BL21 DE3 as a host, data not published in indexed journals.

Studies such as Tsai et al.[18] for the species *Mugil cephalus*, obtained a yield of recombinant GH (rGH) as high as 44 to 47% of the total protein that after renaturation, the rGH was used to inject the fish with 0.1 microgram of rGH per g, once every 2 weeks, and this resulted in increasement in body weight (65%) and length (22%) compared to the control group.

Liu et al.[19] also investigated the effect of feed supplementation with transgenic cyanophycea *Synechocystis sp*. PCC6803 containing the growth hormone (GH) gene from *Paralichthys olivaceus* on growth, feed consumption and feed efficiency ratio, muscle composition, hematology and histology of turbot (*Scoptalmus maximus* L.). At the end of the 40-day feeding trial, the specific growth rate of the fish fed the feed supplemented with 1.0% transgenic *Synechocystis sp*. PCC6803 was 21.67% higher than that of the control fish.

Another relevant finding was for recombinant GH (r-fGH) from sole (*Paralichtys olivaceus*), which resulted in expression corresponding to more than 40% of the *E. coli* cellular protein produced during fermentation. Oral administration of purified r-fGH to juvenile sole, once a week for four (04) weeks, at a dosage of 40 µg r-fGH/g body weight of the fish, resulted in animals weighing 24% more than those in the control group[6].

The rapid growth of rainbow trout (*Salmo gairdneri*) was also promoted by the administration of recombinant growth hormone in *E. coli* [20] via intraperitoneal injection. The animals that received the medium treatment (1µg of GH per g of biomass) jumped from an initial 60g to 240g in 8 weeks, 57% more weight than the control group.

These results indicate that recombinant GH has effective growth-promoting activity and that treatment with this hormone results not only in greater weight gain, but also in accelerated growth capable of reducing time to harvest. Thinking about the national or regional economy, almost four decades have passed after the first report of this technology cited in this work (1988) and so far no large-scale biotechnological product has been developed to increase the mass of Amazonian fish, which indicates a niche market with potential to be developed and exploited.

4 CONCLUSION

With a view to producing tambaqui or other important species for regional and national aquaculture, there is an urgent need to develop the production of recombinant GH on an expanded scale, as well as evaluating its effectiveness in promoting biomass gain in fish.

Further efforts will be made to reactivate recombinant *E. coli* strains containing the tGH gene, developing the bioprocess for high-yield expression on an expanded scale and analyzing their efficiency in accelerating the growth of juvenile *C. macropomum.*

REFERENCES

1. Silverstein, J. T., Wolters, W. R., Shimizu, M., Dickhoff, W. W. 2000. Bovine growth hormone treatment of channel catfish: Strain and temperature effects on growth, plasma IGF-I levels, feed intake and efficiency and body composition. Aquaculture, 190 (1-2): 77–88.
2. Tae-Jin Choi, Geletu, T. T. 2018. High level expression and purification of recombinant flounder growth hormone in *E. Coli*. Journal of Genetic Engineering and Biotechnology, 16 (2): 347-355, DOI https://doi.org/10.1016/j.jgeb.2018.03.006.
3. Cohen, D.M. 1970. How many recent fishes are there? Proc. Calif Acad. Sciences, Ser. 4, 38:341-346.
4. Pantoja-Lima, J.; Santos, S.M.; Oliveira, A.T.; Araujo, R.L.; Silva-Junior, J.A.L.; Aride, P.H.R. 2015. Pró-rural aquicultura: relatos das principais ações de extensão tecnológica e um panorama do setor aquícola do Estado do Amazonas, Brasil. Nexus - Revista de Extensão do IFAM, 1(1): 36-46.
5. Associação Brasileira de Piscicultura. 2024. Anuário Brasileiro da Piscicultura 2024 - Peixe BR. Ed. Peixe BR: São Paulo. 63 pp.
6. Jeh, H.S., Kim, C.H., Lee, H. K., Han, K. 1998. Recombinant flounder growth hormone from *Escherichia coli*: overexpression, efficient recovery, and growth-promoting effect on juvenile flounder by oral administration. Journal of Biotechnology. 60 (3): 183-193. DOI https://doi.org/10.1016/S0168-1656(98)00002-9.
7. Chang, C. C., Tsai, C. T., Chang, C. Y. 2002. Structural restoration of inactive recombinant fish growth hormones by chemical chaperonin and solvent restraint approaches. Protein Eng. 15(5):437-41. DOI 10.1093/protein/15.5.437. PMID: 12034864.
8. Sekar, M., Singh, S. D,. Gupta, S. 2014. Cloning and Characterization of *Pangasianodon hypophthalmus* Growth Hormone Gene and its Heterologous Expression. Appl Biochem Biotechnol. 173: 1446–1468. DOI https://doi.org/10.1007/s12010-014-0946-7
9. Hussin A., Rothan, T. S. H., Zulqarnain, M. 2014. Effect of Codon Optimization on the Production of Recombinant Fish Growth Hormone in *Pichia pastoris*. The Scientific World Journal. Article ID 514835, 6 pages DOI http://dx.doi.org/10.1155/2014/514835
10. Yongfang, J., Xiaolin, J., Qiongqiong, W., Lifang, W., Ningning, L., Qiyan, D., Zhongjie, C. 2014. *Pichia pastoris* Expressing Recombinant *Misgurnus anguillicaudatus* Growth Hormone Promotes the Growth of *M. anguillicaudatus* Larvae. Pakistan Journal of Zoology.46(4).
11. Sadalla-Pinto, E. A, Porto, J. I., Silva, S. D., Carmo, E. J., Andrade, E. V, Astolfi-Filho, S. 2014. Cloning and expression of cDNA encoding growth hormone tambaqui (*Colossoma macropomum*) in the yeast *Pichia pastoris*. BMC Proceedings, 8 (4): 146. DOI https://doi.org/10.1186/1753-6561-8-S4-P146
12. Chung, W. J., Huang, C. L., Gong., H. Y., Ou, T. Y., Hsu, J. L., Hu, S.Y. 2015. Recombinant production of biologically active giant grouper (*Epinephelus lanceolatus*) growth hormone from inclusion bodies of *Escherichia coli* by fed-batch culture. Protein Expression and Purification. 110: 79-88. DOI https://doi.org/10.1016/j.pep.2015.02.012.
13. Ng, A. K. L., Lam, G. C. C., Yeung, K., Dik, D.W.N., Lai, C. Y., Yong, J. L. W., Qian, P.Y., Wong, W. K. R. 2016. Enhancement of fish growth employing feed supplemented with recombinant fish growth hormone expressed in *Bacillus subtilis*. Research Journal of Biotechnology. 11: 1-11.
14. Ocłoń, E., Solomon, G., Hayouka, Z., Gertler, A**.** 2018. Preparation of biologically active monomeric recombinant zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*) recombinant growth hormones. Fish Physiol Biochem**.** 44: 1215–1222. DOI https://doi.org/10.1007/s10695-018-0512-2
15. Choi, T. J., Geletu, T. T. 2018. High level expression and purification of recombinant flounder growth hormone in *E. Coli*. Journal of Genetic Engineering and Biotechnology. 16(2): 347-355. DOI https://doi.org/10.1016/j.jgeb.2018.03.006.
16. Yuan, X., Yuxin, L., Jingkai, Q., Zhang, Y., Guokun, Y., Ruijian, Zongzhen, L., Caiyun, S., Wensheng, L. 2020. Molecular identification, tissue distribution and in vitro functional analysis of growth hormone and its receptors in red-spotted grouper (*Epinephelus akaara*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 250, 110488. DOI https://doi.org/10.1016/j.cbpb.2020.110488.
17. Silva, S. D. 2012. Clonagem e expressão do hormônio de crescimento de Colossoma macropomum em Escherichia coli. Dissertação de mestrado: Genética, Conservação e Biologia Evolutiva. INPA. Disponível em <https://repositorio.inpa.gov.br/handle/1/37540>
18. Tsai H. J., Lin, K. L, Kuo, J. C., Chen, S. W. 1995. Highly efficient expression of fish growth hormone by *Escherichia coli* cells. Appl Environ Microbiol. 61: 4116-4119. DOI https://doi.org/10.1128/aem.61.11.4116-4119.1995
19. Liu, S., Zang, X., Liu, B., Zhang, X., K K I U, A., Zhang, X., Liang, B. 2007. Effect of growth hormone transgenic *Synechocystis* on growth, feed efficiency, muscle composition, haematology and histology of turbot (*Scophthalmus maximus* L.). Aquaculture Research, 38: 1283-1292. https://doi.org/10.1111/j.13652109.2007.01796.x
20. Agellon, L. B.; Emery, C. J.; Jones, J. M.; Davies, S. L.; Dingle, A. D.; Chen, T. T. 1988. Promotion of Rapid Growth of Rainbow Trout (*Salmo gairdneri*) by a Recombinant Fish Growth Hormone. Canadian Journal of Fisheries and Aquatic Sciences. 45(1): 146-151. DOI https://doi.org/10.1139/f88-016

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