In the context of this invention the term "molecular adjuvant" refers to any molecule of protein nature capable of modulating the immune response against a vaccine antigen, increasing it.

Up to now none research has demonstrated the use of PACAP as a molecular adjuvant. As stated above, it has been proposed to use this peptide in mammals as a therapeutic agent for treating autoimmune diseases, such as septic shock, rheumatoid arthritis and Crohn's disease (Gomariz et al. (2006) Ann. NY Acad. Sci . 1070: 51-74). In this regard, PACAP prevents inflammation in animal models of autoimmune diseases through a proper balance of cytokines and chemokines and their receptors, through the recruitment of immune cells and by regulating the generation and activation of the Th1 cells and the cytokines that these cells secrete. Given these findings, is unexpected the effect of PACAP found as part of our studies, showing that its administration in combination with an antigen increases the immune response against said antigen, through the stimulation of the pro-stimulatory cytokines and other elements of the humoral immune response.

Moreover, it is known that the VIP-PACAP system is involved in the regulation of the expression of genes encoding the "Toll" receptors (Gomariz et al. (2006) Ann. NY Acad. Sci 1070: 51-74). In this sense, both peptides may induce disturbances in the regulation pathways of production of these receptors, which makes non obvious its use as a vaccine adjuvant. Another element contrasting with the new use of PACAP, disclosed in this invention, is the demonstration that this peptide attenuates the circulating levels, and inhibits the release, of cytokines mediated by the endogenous HMGB1 molecule (Tang et al. (2008) International Immunopharmacology 8 (12): 1646-1651).

In the present ~~invention~~ disclosure it is demonstrated, for the first time, that the increase in the immune response specific to the vaccine antigen co-administered with PACAP is observed both at the level of the humoral and the cellular immune response.

In a first aspect, there is provided "pituitary adenylate cyclase activating peptide" (PACAP) for use as a molecular adjuvant for a vaccine antigen in the prevention of diseases in fish caused by infectious agents.

Another object of the present disclosure ~~invention~~ is a vaccine composition comprising PACAP as an adjuvant, at least a vaccine antigen, and pharmaceutically acceptable vehicles or diluents. This vaccine composition containing PACAP as an adjuvant and at least one antigen of interest causes an immune response against the antigen or the antigens that is either local or systemic. The vaccines or vaccine compositions of the present disclosure ~~invention~~ may be based, for example, in recombinant proteins, attenuated

microorganisms or nucleic acids. In one embodiment of the disclosure ~~invention~~ the mentioned vaccine compositions are administered orally, by injection or by immersion baths.

In the context of the present disclosure~~invention~~, in such vaccine compositions, PACAP is administered as a peptide isolated from its natural source, obtained by chemical synthesis or by recombinant DNA technology. PACAP can also be employed as nucleic acids.

In different embodiments of the present disclosure ~~invention~~ the vaccines comprising PACAP in combination with several antigens are administered to mammals, birds and fish, showing, for the first time, an adjuvant effect for PACAP. Unexpectedly, it was observed that the administration of the neuropeptide PACAP combined with an antigen increased the levels of specific antibodies against said antigen in mammals, birds and fish. Additionally, it was demonstrated *in vitro*, for the first time, that PACAP significantly increases the transcripts of interleukin 1 beta (IL 1-β) and interleukin 15 (IL15) in fish leukocytes.

In different embodiments of the present disclosure~~invention~~, that disclose vaccine compositions or combinations comprising PACAP as an adjuvant, diverse antigens are used, such as ovalbumin (OVA), the E2 glycoprotein of the Classical Swine Fever Virus (CSFV), hemagglutinin (HA) of the avian influenza virus, the polypeptide my32 of *Caligus rogercresseyi*, inactivated cells of *Aeromonas hydrophila*, the 106 kDa polyprotein (NH2-VP2-VP4VP3-COOH) of the Infectious Pancreatic Necrosis Virus (IPNV), the G glycoprotein of the Viral Hemorrhagic Septicemia Virus (VHSV) and the ciliate parasitic *Ichthyophthirius multifiliis*.

In a particular embodiment of the disclosure~~invention~~, in the vaccine compositions, PACAP is applied as a formulated feed, at a concentration of 50-750 μg/kg of feed. In a second embodiment of the disclosure~~invention~~, PACAP is applied by injection, at a concentration of 0.1-10 μg per gram of body weight. In another embodiment of the disclosure~~invention~~, PACAP is applied in aquatic organisms by immersion baths, at a concentration of 50-1000 μg per liter of water.

In another aspect, there is provided a vaccine comprising a combination of the "pituitary adenylate cyclase activating peptide" (PACAP) and at least one vaccine antigen for use in the prevention of viral, bacterial or parasite diseases in fish, wherein the PACAP and the antigen are suitable for simultaneous administration, separate administration or sequential administration during the same immunization schedule; and wherein the PACAP and antigen are for use in oral administration, administration by injection, or by immersion baths.

In another embodiment of the disclosure~~invention~~, the antigen and the adjuvant PACAP may be part of a vaccine combination whose elements are administered simultaneously, separately or sequentially during the same immunization protocol. In a particular embodiment of the disclosure~~invention~~, the combination is used in the prevention of viral,

bacterial and parasitic diseases. For this vaccine combination, PACAP and the antigen, or antigens, are administered orally, by injection or by immersion baths.

In a particular embodiment, the PACAP is applied as formulated feed, at a concentration of 50 -750 µg/Kg of feed. In a second embodiment, the PACAP is applied by injection, at a concentration of 0,1-10 µg of peptide per gram of body weight. In another embodiment of the disclosure~~invention~~, PACAP is applied to fish by immersion baths, at a concentration of 50-1000 µg of PACAP per liter of water.

Another ~~aspect~~ embodiment of this disclosure ~~invention~~ is a method for increasing the immune response against an antigen wherein is employed a vaccine composition comprising the "pituitary adenylate cyclase activating peptide" (PACAP) as an adjuvant for said antigen. According to the method disclosed in the disclosure~~invention~~, the PACAP as an adjuvant and the antigen can be administered simultaneously, separately or sequentially during the same immunization schedule. PACAP is used as adjuvant in the method of the disclosure ~~invention~~ as a peptide which has been obtained from its natural source, by chemical synthesis or by the recombinant DNA technology. Also the disclosure ~~invention~~ comprises a method wherein the PACAP used as an adjuvant is administered in the form of nucleic acids. According to the method of the disclosure~~invention~~, the increase in the immune response specific to the ~~the~~ vaccine antigen co-administered with PACAP takes place both in the humoral and the cellular specific immune response.

In another aspect, there is provided use of "pituitary adenylate cyclase activating peptide" (PACAP) as a molecular adjuvant in the manufacture of a vaccine for the prevention of diseases in fish caused by infectious agents.

In another aspect, there is provided use of a combination of "pituitary adenylate cyclase activating peptide" (PACAP) and at least one vaccine antigen in the manufacture of a vaccine for the prevention of viral, bacterial or parasite diseases in fish, wherein the PACAP and the antigen are suitable for simultaneous administration, separate administration or sequential administration during the same immunization schedule; and wherein the PACAP and antigen are for use in oral administration, administration by injection, or by immersion baths.

As demonstrated in several embodiments of the disclosure~~invention~~, the enhancement or increase in the immune response against the antigen of interest is expressed as higher levels of protection against various infectious agents; including viral, bacterial and ectoparasite entities.

Brief description of the Drawings

Figure 1. Titles of total immunoglobulin G (IgG) (A), IgG1 (B) and IgG2a (C) induced in mice by immunization with OVA co-administered with the neuropeptide PACAP38. Two experimental groups of 6 animals each one were used. The negative control group (phosphate buffered saline (PBS)/OVA) was inoculated intraperitoneally on day 0 and 7 with a dose of 6 μg of OVA in 0.2 mL of PBS. The group that also received PACAP38 (PBS/OVA+peptide) was inoculated intraperitoneally on day 0

CLAIMS

USE OF THE PACAP AS A MOLECULAR ADJUVANT FOR VACCINES

1. ~~Use of the "p~~ "Pituitary adenylate cyclase activating peptide" (PACAP) for use as a molecular adjuvant for a vaccine antigens in the prevention of diseases in fish caused by infectious agents.
2. ~~The~~ PACAP for use ~~of~~ according to claim 1 wherein the ~~peptide~~ PACAP is for ~~administered~~ administration as a peptide or in the form of a nucleic acid.
3. ~~The~~ PACAP for use ~~of~~ according to claim 1, wherein the PACAP peptide is obtained a) by isolation from its natural source, b) by chemical synthesis, or c) by recombinant DNA technology.
4. ~~The use of claim 1 wherein said vaccine antigens are used in the prevention of diseases caused by infectious agents.~~
5. ~~The use of claim 4 wherein the diseases occurs in mammals, fish or birds.~~
6. ~~4.~~ ~~The~~ PACAP for use ~~of~~ according to claim ~~4~~ 1 wherein the infectious agent is a virus, a bacterium or an ectoparasite.
7. ~~Vaccine composition comprising the "pituitary adenylate cyclase activating peptide" (PACAP), at least one vaccine antigen and pharmaceutically acceptable vehicles or diluents.~~
8. ~~The composition of claim 7 which is used for the prevention of diseases caused by infectious agents.~~
9. ~~The composition of claim 7 which is administered orally, by injection, or by immersion baths.~~
10. ~~The composition of claim 7 wherein the PACAP is administered as a peptide obtained) by isolation from its natural source, b) by chemical synthesis, or c) by recombinant DNA technology; or as a nucleic acid.~~
11. ~~The composition of claims 9 and 10 wherein the PACAP is used in a concentration of 50 to 750 µg/Kg of feed if applied as a formulated feed, in a concentration of 0.1-10 µg per gram of body weight if it is applied by injection, or in a concentration of 50-1000 µg per liter of water if it is applied by immersion baths.~~
12. ~~Vaccine combination comprising the "pituitary adenylate cyclase activating peptide" (PACAP) and at least one vaccine antigen.~~

~~13.~~5. ~~The combination of claim 12 wherein the~~Vaccine comprising a combination of the "pituitary adenylate cyclase activating peptide" (PACAP) and ~~the~~ at least one vaccine antigen for use in the prevention of viral, bacterial or parasite diseases in fish, wherein the PACAP and the antigen are suitable for simultaneous administration, separate administration or sequential administration ~~are administered simultaneously, separately or sequentially~~ during the same immunization schedule; and wherein the PACAP and antigen are for use in oral administration, administration by injection, or by immersion baths.

~~14.~~6. The ~~combination~~ vaccine for use of claim ~~13~~-5 wherein the PACAP is ~~used~~ formulated in a concentration of 50 to 750 µg/Kg of feed if it is ~~applied~~ for admininstration as a formulated feed, in a concentration of 0.1-10 µg per gram of body weight ~~when applied~~if it is for administration by injection, or in a concentration of 50-1000 µg per liter of water if it is ~~applied~~ for administration by immersion baths.

~~15. Method for increasing the immune response to a vaccine antigen characterized by comprising the use of a composition that comprise the "pituitary adenylate cyclase activating peptide" (PACAP) as an adjuvant for said antigen.~~

~~16. The method of claim 15 wherein the composition comprising the PACAP as an adjuvant and the antigen is administered simultaneously, separately or sequentially during the same immunization schedule.~~

~~17. The method of claim 15 wherein the PACAP peptide is obtained by a) isolation from its natural source, b) by chemical synthesis, or c) by recombinant DNA technology; or as a nucleic acid.~~

7. Use of "pituitary adenylate cyclase activating peptide" (PACAP) as a molecular adjuvant in the manufacture of a vaccine for the prevention of diseases in fish caused by infectious agents.

8. The use according to claim 7, wherein the PACAP is for administration as a peptide or in the form of a nucleic acid.

9. The use according to claim 7, wherein the PACAP peptide is obtained a) by isolation from its natural source, b) by chemical synthesis, or c) by recombinant DNA technology.

10. The use according to claim 7, wherein the infectious agent is a virus, a bacterium or an ectoparasite.

11. Use of a combination of "pituitary adenylate cyclase activating peptide" (PACAP) and at least one vaccine antigen in the manufacture of a vaccine for the prevention of viral, bacterial or parasite diseases in fish, wherein the PACAP and the antigen are suitable for simultaneous administration, separate administration or sequential administration during the same immunization schedule; and wherein the PACAP and antigen are for use in oral administration, administration by injection, or by immersion baths.

12. The use of claim 11 wherein the PACAP is formulated in a concentration of 50 to 750 µg/Kg of feed if it is for admininstration as a formulated feed, in a concentration of 0.1-10 µg per gram of body weight if it is for administration by injection, or in a concentration of 50-1000 µg per liter of water if it is for administration by immersion baths.