The interaction between immune competence and stress responses in relation to fish health problems in aquaculture

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Projects

- *Effects of genotype and genotype-environment interactions on the stress responses in common carp exposed to chronic stress*

Projecr: 806.46.031
Projectleader: Prof. dr. C.J.J. Richter (WUR)
PhD-student: Drs. N.M. Ruane (1998 – 2002)

- *Modulatory effects of stress on the genetically determined disease resistance of common carp*

Project: 806.46.032
Projectleader: Prof. dr. W.B. van Muiswinkel (WUR)
PhD-student: Ir. J.P.J. Saeij (1998 – 2002)

- *The immuno-neuroendocrine interactions in carp strains selected for stress responses or immune capacity*

Project: 806.46.033
Projectleader: Prof. dr. S.E. Wendelaar Bonga (KUN)
PhD-student: Drs. J.R. Metz (1998 - 2002)

Summary
It is known, that severe and chronic stress is unavoidable during standard procedures in aquaculture. These conditions may lead to acute mortalities or losses caused by diseases. Improving the resistance to disease and/or stress by genetic means is an attractive approach to reduce these widespread welfare problems in aquaculture. However, genetic selection for one type of response may affect the other, due to the delicate balance between the neuroendocrine system and the immune system. To our knowledge these possible negative effects of selection have not been investigated in fish.

This research programme is a multidisciplinary approach to investigate the genetic and physiological relationship between the stress response and the immune response in carp. The programme consists of three closely related and interdependent projects aimed at the analysis of specific carp strains selected for either stress response or immune competence for the following aspects: (1) the physiological responses to a stressor (crowding), which is typical for an aquaculture situation; (2) the modulating effect of a stressor on the genetically determined differences in immune responsiveness; (3) the quantitative analysis of the stress response and of immuno-neuro-endocrine interactions.

Results
Project 806.46.031
Effects of genotype and genotype-environment interactions on the stress responses in common carp exposed to chronic stress

The objective of this program is to investigate the genetic and physiological relationship between disease resistance and stress response in inbred carp strains, selected for immune competence or for stress response. The main hypothesis is that both types of selection may modify or even disturb the intricate balance between both systems. No major changes in the original workplan were required (see for details the projects below).

Project 805-46.031-P

Project leader Dr. J. Komen, Fish Culture and Fisheries Group, Wageningen University.

The general aim of investigating the influence of environment and genetic factors on the stress response of carp has been reached. Experiments have been conducted determining the effects of environmental factors (such as prior exposure to high densities, periods of optimal/maintenance feeding levels) on an all male isogenic strain of carp. This strain was used to allow for a proper comparison of results between experiments, as the strain is made in the Wageningen University facilities and reared under controlled conditions. Similarly, in order to investigate genetic effects on the stress response, different genetic strains of carp were tested. All carp strains were reared under identical conditions to reduce any environmental differences. Experiments have shown that genetically different carp strains can exhibit different cortisol stress responses. Carp previous selected for a high or low cortisol response to a cold shock, were also shown to respond with the same high/low response to confinement. Differences were also found to exist between some unselected strains of carp.

The research focussed mainly on describing the physiological basis for the difference in cortisol stress response between two carp strains, the normal responding standard strain (STD) and a low responding strain (E5). Results have shown that the low responding E5 fish also show a lower sensitivity to ACTH in vitro which suggested that the cause of the low cortisol response was to be found in the steroid-producing interrenal cells of these fish. Indeed, E5 fish were found to have enlarged head-kidney tissues (within which are found the interrenal cells) and a higher number of larger interrenal cells. The reason for the lower cortisol production was found to be due to a dysfunction of one of the main steroid synthesizing enzymes (P450c17), resulting in a low rate of cortisol synthesis and thus the regulation of the HPI axis in these fish is compromised. The larger head-kidneys of these fish may be a physiological adaptation to increase cortisol synthesis, and be due to a higher stimulation by ACTH due to the reduced negative feedback. Unfortunately measurement of ACTH levels in carp have proved troublesome (see project 3).

In addition to the above mentioned goals, the project also looked at the difficulty of measuring chronic stress levels in carp through measurements of blood indicators. A technique to measure cortisol (the stress indicator) in water was established and levels in tanks with a high density of fish were found to be higher than the lower control tanks.

Project 805-46.032-P
Project leaders Prof. dr. W.B. van Muiswinkel, Dr. ir. G.F. Wiegertjes, Cell Biology and Immunology Group, Wageningen University

The aims of this particular project were defined as follows:
1) Investigation of the modulating effects of the standard stressor on the disease resistance of genetically different carp strains
2) Investigation of the role of immunomodulating genes as markers for improved disease resistance and welfare of fish.

Ad 1). We have continued using a parasite disease model (Trypanoplasma borreli: a haemoflagellate parasite) to study the aims defined above. To do so, we have applied the read-out characters developed during the first three years to evaluate disease resistance. Different from the end-point ‘death’, the use of these parameters allowed us to minimize the degree of discomfort for those experimental animals used for challenge experiments.

Previously we found that extracellular parasites such as T. borreli can induce fish host phagocytes to produce large amounts of nitric oxide (NO). In vivo, NO produced during infection with T. borreli seemed counter-productive and lowered disease resistance. Further, ex vivo analysis indicated that parasite-induced NO induced immunosuppresion in host lymphocytes. We now found that NO lowers the capacity of phagocytes to counteract nitrosative and oxidative stress by lowering their glutathione level, sending the cells in apoptosis (programmed cell death).

In agreement with the initial workplan an in vivo challenge at the facilities of ID-Lelystad was performed. The role of macrophages in the defense against the parasite was investigated using injections with liposomes filled with clodronate. Liposomes specifically target macrophages, while clodronate induces apoptosis (programmed cell death) of these cells. Further work on the liposome-clodronate protocol was undertaken in the last year of the project. Using flow cytometric analysis of leukocyte sub-populations we could show that in the peritoneal cavity, where the liposomes were administered, neutrophilic granulocytes rapidly accumulate. These cell types were not affected by clodronate treatment. In contrast, macrophages are affected and send in apoptosis. The effect on parasitaemia was marginal, indicating a minimal effector role for macrophages in the immune response against fish tryps.

Ad 2). As a general rule, non-MHC genes appear to regulate the early phase of the immune response to pathogenic organisms, for which reason we have developed primers specific for a number of immunomodulating genes. Using these primers as a read-out system (RT-PCR) we previously found that IL-1, TNF and NO are uregulated by trys infections. When phagocytes are exposed to cortisol, we find that these genes are downregulated. This could explain the negative effect of stress on resistance to fish tryps, observed previously.

The later phase of pathogen elimination often depends on the generation of MHC-restricted T cell-mediated immunity. Both class I as well as class II A and B genes have been identified in carp. An F1 cross between homozygous carp strains bearing different MHC class II B genes has been made. These fish have been used to produce an F2 generation and backcrosses to the original carp strains homozygous for their MHC. The immune response and defense against T. borreli was studied in these MHC-typed carp strains. It was found that MHC genes can be correlated with the immune response against defined hapten-carrier antigens but do not explain genetic differences in resistance to fish tryps.

Deelproject 3 (805.46.033P)
Project leaders Prof. dr. S.E. Wendelaar Bonga, Prof. dr. G. Flik, Dept. of Animal Physiology, University of Nijmegen

This project is concentrating on the physiological mechanisms behind the stress response.
a. The aims as formulated in the original research proposal have been partly modified. Fish showing high- and low cortisol response to stress were insufficiently available for experiments due to slow production progress. Research was directed towards basics of (i) the physiology of stress in fish in general and (ii) the interaction between the immune system and the neuroendocrine (stress-)system.
b. The dynamic interaction of a fish with its environment poses demands on its physiology to meet requirements for homeostasis. Homeostasis and its adjustments require communication systems within the body. Environmental changes (e.g. alterations in temperature, crowding) evoke a stress reponse in the body that aims to reallocate energy flows to overcome the stressor. Energy may for instance be withdrawn from processes like growth, reproduction and defence.

In stressed fish, the hypothalamic-pituitary-interrenal axis (HPI-axis) is activated. Hypothalamic CRH (corticotropin-releasing hormone) and TRH (thyrotropin-releasing hormone) directly innervate cells from the pituitary gland to release corticotropes like ACTH (adrenocorticotropic hormone), □-MSH (□-melanocyte-stimulating hormone) and □-endorphins to the blood. These hormones activate i.a. the steroid-producing interrenal cells in the head kidney to produce and secrete cortisol.

Signals of the immune system (e.g. interleukins) affect the neuroendocrine system. We have strong experimental evidence now that physiological doses of recombinant carp interleukin-1 (rcIL-1) stimulate release of hormones from the pituitary gland (Figure 1). This is the first study to demonstrate such a direct activation of the HPI-axis by an immune cell signal.

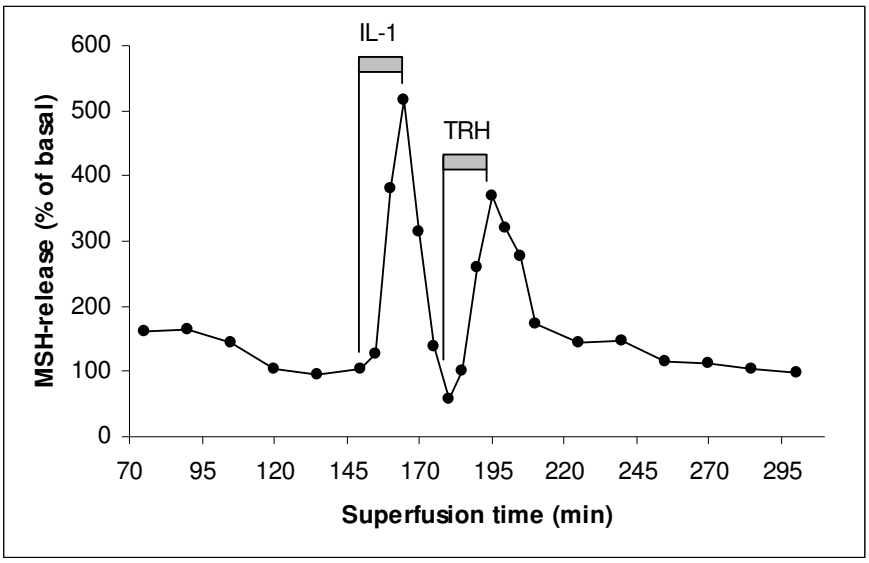

Figure 1. Stimulation of □-MSH release from the pituitary gland by rcIL-1. These results were obtained by in vitro superfusion, in which isolated pituitary glands were overflowed with physiological medium supplemented with IL-1 and/or TRH.

Studies in our laboratories in Nijmegen and Wageningen continue to broaden and refine the central model of the stress paradigm in fish. Although we meet similarities with the mammalian model, also striking differences are found. For example, in fish ACTH appears to be a far less potent corticotropic signal and there is evidence for another factor from the pituitary pars intermedia next to ACTH, supporting the cortisol surges during stress. We presently focus on a characterisation of the family of melanocortin receptors (specific for ACTH, □-MSH and other POMC-derivatives) to unravel the targets of these hormones in the head kidney and the immune system. So far we elucidated a partial sequence of the carp melanocortin-5 receptor, with extremely high homology to its mammalian counterpart. Future studies will expand our knowledge on signalling during stress.

Future research

The results of the 3 projects did raise many more new questions than that can be solved in the projects themselves. For example, project 1 has led to the description of a strain of carp which suffers from an Addison's Disease-like syndrome, resulting in an insufficient production of corticosteroid hormones. It is the first time such a disease has been reported in a non-mammalian vertebrate and therefore may provide us with a unique model for investigating comparative aspects of the disease, its treatment and endocrinology of teleost fish in general. This important new model will be incorporated in the future research of the Fish Culture and Fisheries group at Wageningen. Other important questions are dealing with the applications of our data in adult carp on young and developing aninmals or in other species.

The extensive studies at Nijmegen and Wageningen provide us with a broad and refined model for the relation between stress, welfare and health in fish. The results did improve our insight in the physiological and genetic mechanisms behind the interaction between the brain-pituitary-interrenal (BPI) axis and the immune system in fish. The expression of immune/stress modulating genes and the subsequent release of their products during immune or stress responses can be regarded as important tools for the evaluation of health and welfare in animals . It is also expected that our results can be used for improving routine procedures in aquaculture as well as the pet fish industry (e.g. stocking, rating, harvest, transport). Comparable circumstances may also occur in the commercial and sport fisheries, where live fish are sometimes stocked or transported during longer periods.

The practical applications of our work can be expected, when authorities are able to enforce a "health- and welfare-passport" for animals in aquaculture systems. Such a passport can be based upon stress

and immune parameters as discovered in our program. However, simple and fast tests have be developed which can be applied in field situations. We regard this as a challenge for the future.

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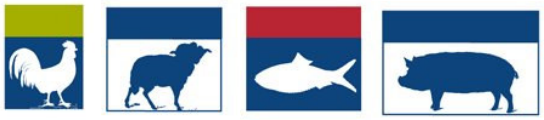

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