Formas FRESH projektrapport

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Projekttitel: Djurskydd i moderna produktionssystem för fisk

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Scientific report

Introduction, aim and hypothesis

Fish welfare is an area of research until recent years largely neglected. In modern fish farms, tens of thousands of fish are held together in large cages making it difficult for the human caretaker to visually evaluate the welfare of an individual fish. It is difficult to assess the stress, suffering and welfare of fish by visual evaluation as they lack many of the facial expressions and behaviors that, to humans, signal negative stress, disease, pain and discomfort. A fish may appear to respond in a similar manner independent of strength or type of stressor, i.e. by flight in direct conjugation with the stressor, and in some individuals with reduced feed intake over days and even weeks after a stressful event, while reduced growth and increased mortality and/or increased disease occurrence is a more long term and multi-cause response. Highly stressful events such as crowding, netting, sorting and transports are today 'part of life' in commercial fish farms. There has been a rapid development of alternative feed ingredients replacing the traditional marine ones for farmed fish. This development is expected to continue to be of central importance regarding fish welfare as the present plant based alternatives as e.g. soy and rape seed are increasingly replaced by protein and oils of microbial, insect and alternative marine origin.

In our project FRESH ('Fish REaring and Stress Hazards') we aimed to establish a highly qualified fish welfare platform and combine the expertise of the group members from the two universities so that we could thoroughly map a variety of physiological stress indicators in fish species that are commonly farmed in Sweden. We took an integrative approach in order to identify the mechanisms of specific physiological responses which commonly cause detrimental tertiary stress responses (i.e. decreased growth, diseases resistance, and swimming capacity) in farmed fish. Furthermore, we aimed to use the obtained results to create recommendations to ensure the protection of fish in aquacultural settings and improve future production/management systems.

Materials and methods

The laboratory based studies were carried out mainly in the animal facility at the Department of Biological and Environmental Sciences, University of Gothenburg (GU), as well as at Aquaculture Centre North, and Ultuna, Swedish University of Agricultural Sciences (SLU). The on-site commercial scenarios were carried out on established collaborating farms in Sweden and Åland. We focused on rainbow trout (*Oncorhynchus mykiss*) and Arctic char (*Salvelinus alpinus*), the two main aquaculture

species in Sweden. We investigated how the cardiovascular and gastrointestinal systems as well as barrier functions are affected by different stressors induced by common farming practices. To understand the causality and severity of different farming practices, we investigated a range of physiological responses *in vivo* and *in vitro* both in the lab and out at the fish farms. The studies included in the project were divided into the following three work packages; 1) The effects of alternative sources of proteins in fish feed, 2) Cardiovascular and gastrointestinal effects of acute and chronic stress, and 3) Continuous physiological welfare evaluation in farmed fish.

The laboratory based studies covered a wide variety of physiological variables associated with stress including; stress hormones, blood chemistry, gut microbiota, primary barrier functions, metabolism, nutrient uptake, heart disease, circulatory and respiratory effects.

In the on-site studies we used completely novel systems capable of measuring stress responses in free swimming fish: surgically implanted bio-loggers in a few selected focal fish, to monitor physiological variables and temperature for three weeks prior to harvest. This information in combination with other known indicators of stress were then used to assess the severity of the stress response induced by a range of farming practices.

Results

The effects alternative sources of proteins in fish feed
Data on post-prandial plasma AA profile, haematological parameters and erythrocyte indices will be presented together with data concerning the interaction between diet and stress in fish where fishmeal has been replaced by yeast up to 60%. Also, data concerning changes of the microbiota of fish given different types of yeast *Saccharomyces* and *wickerhamomyes* show dramatic differences. Fish fed saccharomyces had higher abundance of lactic acid producing bacteria's than fish meal fed fish. With 60% inclusion of *Wickerhamomyes* increased the abundance of the pathogenic yeast *candida albicans.* Furthermore, live yeast can replace 40% of fishmeal without disrupting bacteria communities in the gut of rainbow trout.

Cardiovascular and gastrointestinal effect of acute and chronic stress
Rainbow trout exposed to long-term stress showed elevated levels of the stress hormone cortisol and develop pathological heart growth and cardiovascular disease. Furthermore, we showed that the pathological heart morphology resulted in a higher mortality when faced with an acute stressor such as transport. In a different work package we investigated how gastrointestinal blood flow was affected by different stressors we revealed a bivalent stress response in gastrointestinal blood flow depending of the type and duration of a stressor is in rainbow trout. A reduced gastrointestinal blood flow does not explain the intestinal barrier dysfunctions occurring in rainbow trout after acute stress.

Continuous physiological welfare evaluation in farmed fish
Our findings demonstrate that resting heart rate is significantly correlated to plasma levels of the stress hormone cortisol. Following surgery, transportation and reintroduction with conspecifics in the sea cage, it took ~4 days for heart rate to recover and for a clear circadian heart rate rhythm to emerge. Stressful farming practises or events (indicated by elevated plasma cortisol levels) such as

air exposure during brailing and aquatic hypoxia triggered a hypoxic bradycardia until fish were released back into oxygenated water whereupon heart rate significantly increased to repay the accumulated oxygen debt. Repeated stress induced by multiple farming practises clearly had a cumulative and long-lasting effect and therfore the results suggest that stressed fish are unlikely to respond to a new challenge in the same manner as an unstressed animal would.

Discussion, conclusion

The welfare of farmed fish has become of increasing concern for consumers, producers, interest groups and authorities. To improve the welfare of farmed fish it is necessary to identify the underlying causes for husbandry induced stress so that various alternatives for farming practices can be considered and risks of poor welfare better can be assessed allowing for interventions to take place. Our results demonstrates that the use of implantable bio-loggers opens up a broad range of possible applications that will allow researchers to investigate the effects of environmental and/or anthropogenic stressors on the welfare of fish in scenarios more realistic to the aquaculture industry. New analytic and experimental tools, evaluating metabolic and microbiota changes also open up new possibilities to understand nutritionally induced factors and their importance for the welfare of the fish. However, our results also clearly illustrate the urgent need for better understanding of the complexity of the stress response in fish. With all different studies associated with this project we have only started to unravel the combined roles of e.g., changes in stress hormones, blood chemistry, gut microbiota, primary barrier functions, metabolism, nutrient uptake, osmoregulation, circulation, heart disease, in the welfare of farmed fish.

Members of the research group

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Scientific papers published in international reviewed journals

- Brijs J, Gräns A, Ekström A, Olsson C, Axelsson M & Sandblom E (2016), Cardiorespiratory up-regulation during seawater acclimation in rainbow trout: Effects on gastrointestinal perfusion and post-prandial responses. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* 310, R858-R865

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The following original articles have been submitted to peer reviewed journals, awaiting decision.

- Brijs J, Sandblom E, Axelsson M, Sundell K, Sundh H, Huyben D, Broström R, Kiessling A, Berg C, & Gräns A,. The final countdown: continuous physiological welfare evaluation of farmed fish during common aquaculture practices before and during harvest. (*Submitted*)

- Gräns A, Brijs J, Sandblom E, Rosengren M, Sundell K, Berg C, Bjarnason A, & Axelsson M,. Prospects and pit-falls of using heart rate to assess performance and stress levels of freely swimming fish. *(submitted)*

- Sundh H, Gräns A, Brijs J, Sandblom E, Axelsson M, Berg C, & Sundell K,. Effects of coeliacomesenteric blood flow reduction on intestinal barrier function in rainbow trout Oncohynchus mykiss. *(submitted)*

- Vidakovic A., Huyben D., Nyman A., Vielma J., Olstorpe M., Passoth V., Kiessling A, Lundh T. Growth, digestibility and nutrient retention of rainbow trout (*Onchorhynchus mykiss*) fed graded levels of *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus. Aquaculture Research (revision)*

PhD / Licenciate theses

- Vidaković A (2015). Fungal and Mussel Protein Sources in Fish Feed: Nutritional and Physiological aspects. Doctoral/PhD Thesis, Faculty of Veterinary Medicine and Animal Science, Acta Universitatis Agriculturae Sueciae 2015:90; SLU Uppsala. ISSN 1652-6880, ISBN (print version) 978-91-576-8378-6, ISBN (electronic version) 978-91-576-8379-3.

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- Ekström, A (2017), Thermal tolerance in teleost fish –importance of cardiac oxygen supply, ATP production and autonomic control, Department of biological and environmental sciences, University of Gothenburg, ISBN 978-91-629-0087-8

- Brijs, J (2017), Gastrointestinal Motility and Blood Flow in Teleosts during Digestion and Osmoregulation, Department of biological and environmental sciences, University of Gothenburg, ISBN 978-91-629-0043-4

In addition, at least 18 conference abstracts have been published.