THE IMPACT OF STRESS ON THE BARRIER FUNCTION OF THE SKIN IN ATLANTIC SALMON (*SALMO SALAR*) REARED IN CLOSED CONTAINMENT SYSTEMS.

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Introduction

The risks for escapees, infection of micro- and macroparasites and excess leakage of nutrients to the recipient are strong drivers for the development of closed-containment systems on land and semi-closed containment systems in sea. Due to a higher production cost in such systems compared to traditional open cages, the production intensity is predicted to increase (Terjesen et al., 2013). This may include utilization of higher fish densities, higher water temperatures and elevated inlet O_2 concentrations, which in some cases may lead to deterioration in water quality (Thorarensen and Farrell, 2011). Under such conditions the fish may experience a mild, chronic stress that can affect their performance, health and welfare and the ability to respond to new acute stressors (Fridell et al 2007, Sundh et al 2009, Sundh and Sundell, 2015).

Due to the intimate contact between the fish and their environment, they rely on well-functioning physical barriers across all epithelia, gills, gastro-intestinal tract and skin. In closed containment systems a reduced skin health in combination with increased pathogen transmission rates due to high densities, represents potential disease challenges. Skin wounds constitute damage to the barrier against the pathogenic bacteria potentially present, which may give rise to increased mortality and reduced fish welfare. An estimate is that between 1.1 and 2.5% of farmed salmon are lost due to wounds (Takle et al., 2015). Not only mechanical damage, but also chronic stress has been demonstrated to reduce the barrier functions of epithelia such as the intestine (Sundh and Sundell, 2015). However, the consequences of chronic stress on the barrier function of the skin are poorly investigated.

The aim of the current experiment was to elucidate the effect of mild chronic stress, inflicted by high fish density and reduced water quality, on the barrier function of the skin.

Methods

Atlantic salmon smolts (66 g) were stocked in triplicate 3.3 m^3 tanks at initial densities of 50 and 14 kg m^{-3} in two production systems, brackish water (BW)-RAS or seawater (SW)-flow through (FT). The biomass was maintained at approximately 80 kg m^{-3} for the high density (HD)/mild chronic stress group and 30 kg m^{-3} for the low density (LD) group, respectively, by regular biomass adjustments during the six month long experiment. The average temperature in BW-RAS (12 ppt) during the study was 13.1 ± 0.5ºC and the temperature in the SW-FT was matched to the BW-RAS using a heat-exchange system (Terjesen et al., 2013) averaging 13.1 ± 0.4ºC. The oxygen saturation levels in all tanks were maintained above 85% (independent of other tanks). The average daily system exchange rate in BW-RAS was 37 ± 19% with an average of 0.9 ± 0.5 m^3 kg^{-1} feed of make-up water. The specific water flow rate for the low density group was maintained between 0.7-0.8 l min^{-1} kg^{-1} and between 0.3-0.4 l min^{-1} kg^{-1} for the high density group. Water quality (temperature, pH, conductivity, salinity, CO_2, TAN and turbidity) were measured at the tank outlet on four occasions during the experiment and additional CO_2 measurements at the tank outlet were done in connection to the regular biomass reductions. The skin from RAS fish were sampled at 560 g and 900 g and FTS fish at 900 g for

assessment of barrier function in an Ussing chamber set-up (Sundell and Sundh, 2012). Main parameters studied were the permeability of a radiolabeled, inert marker molecule, ^{14}C-mannitol, and transepithelial electrical resistance (TER). Skin tissue was also fixed on formalin for general histology and freshly frozen for mRNA extraction and qPCR measurements of tight junction protein genes.

Results and discussion

The skin permeability (leakiness) was similar between BW-RAS and SW-FT at both sampling points. In SW-FT, chronic stress (HD) increased skin leakiness compared to control (LD). In BW-RAS the skin leakiness assessed as Papp, but not TER, increased with time. Further, similar to FTS fish, chronic stressed fish in BW-RAS displayed increased skin leakiness. Skin permeability could not be correlated to fish size or thickness of skin epidermis. In conclusion, these results demonstrate that the skin barrier function is reduced by chronic mild stress in Atlantic salmon post smolts reared in both BW-RAS and SW-FT, indicating increased disease susceptibility.

Acknowledgements

This study was funded by The Research Council of Norway (project #233870/E40: "Welfare of Atlantic salmon post-smolts in closed-containment production systems, using a function-based approach (SalmoFutura)" and 237856/O30 CtrlAQUA SFI, Centre for Closed-Containment Aquaculture.

References

Fridell, F., Gadan, K., Sundh, H., Taranger, G. L., Glette, J., Olsen, R. E., Sundell, K. & Evensen, Ø. (2007) Effect of hyperoxygenation and low water flow on the primary stress response and susceptibility of Atlantic salmon Salmo salar L. to experimental challenge with IPN virus. *Aquaculture*, 270**,** 23-35.

Sundell KS and Sundh H (2012) Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. Front. Physio. 3:388. doi: 10.3389/fphys.2012.00388

Sundh, H., Olsen, R E., Fridell, F., Gadan, K., Evensen, Ø., Glette, J., Taranger, G L., Myklebust, R and Sundell, K (2009) The effect of hyperoxygenation and reduced flow in fresh water and subsequent infectious pancreatic necrosis virus challenge in sea water, on the intestinal barrier integrity in Atlantic salmon, *Salmo salar* L. J Fish Dis 32:8 687-698

Sundh, H and Sundell, K (2015) Environmental impacts on fish mucosa, *In:* Mucosal health in aquaculture, Editors Benjamin H. Beck and Eric Peatman, Elsevier.

Terjesen, B., Rosten, T., Ulgenes, Y., Henriksen, K., Aarhus, I., Winther, U., 2013a. Betydning av vannmiljøet ved produksjon av laksefisk i lukkede systemer i sjø. Water quality requirements for efficient farming of Atlantic salmon in closed systems. In Norwegian, English abstract. VANN 48, 14-27.

Thorarensen, H., Farrell, A., 2011. The biological requirements for post-smolt Atlantic salmon in closed-containment systems. Aquaculture 312, 1-14.