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ABSTRACT BOOK

T46

NEW IN VITRO ASSAYS FOR THE STUDY OF INTERACTIONS BETWEEN VIRUS INFECTIONS AND FUNCTIONAL FEED INGREDIENTS IN THE SALMONID INTESTINE

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Within aquaculture, the link between nutrition, immune responses and infection diseases is still unclear, partly due to the lack of targeted research tools. The aim of the present study was to establish in vitro infection models using an intestinal epithelial cell line from rainbow trout, the RTgutGC, and important viral pathogens challenging the salmon industry. This cell line, well established in our laboratory, has been applied for evaluation of effects of functional feed ingredients on gut health. By establishing infection models with the RTgutGC cells, we will be able expand our in vitro studies to comprise studies of interaction mechanisms between feed ingredients, pathogen infection and gut health in the salmonid intestine.
Infection models for the three important salmon pathogens; infectious pancreatic necrosis virus (IPNV), infectious salmon anemia virus (ISAV) and salmonid alphavirus 3 (SAV3) were established based on the RTgutGC cell line. While IPNV is known to enter the host through the intestinal epithelial barrier, it is not clear if ISAV and SAV3 infect the host through the gut mucosa. Here, we demonstrated that all three viruses infected and replicated within the RTgutGC cells, although with different infection profiles. Cytopathic effects (CPE) and cell death in RTgutGC cells after virus exposure showed that the cells were permissive to virus infection, and immune staining visualized that virus had entered the cells. Gene expression analyses demonstrated that virus replicated within the cells and that anti-viral cell processes were initiated by the infections. Results will also be presented concerning effects on the integrity of the intestinal epithelial barrier in the early stages of infection.

T47

EFFECTS OF DIETARY SILKWORM (BOMBYX MORI) PUPAE, MEALWORM (TENEBRIO MOLITOR),SUPERWORM (ZOPHOBASMORIO) AND BLACK SOLDIER FLY (HERMETIA ILLUCENS) LARVAE ON THE HAEMATOLOGY, IMMUNITY AND RESISTANCE TO STRESS OF GILTHEAD SEABREAM (SPARUS AURATA).

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Overfishing with the subsequent depletion of fish stocks and increase of fishmeal costs call for the urgent finding of alternatives to fishmeal for use in animal feeds. Insect meals may not only be such an alternative, they may also have some immunostimulating activity and may increase animal resistance to diseases as reviewed recently (Gasco et al., 2021). The present experiment investigated the effect of 4 defatted insect larval meals (silkworm pupae, Bombyx mori (BM), mealworm, Tenebrio molitor (TM), superworm, Zophobas morio, (ZM) or black soldier fly, Hermetia illucens, (HM)) on juvenile gilthead seabream, Sparus aurata. Fish were kept in 21 aquaria (125L) within a closed recirculation seawater system. Seven isonitrogenous and isoenergetic diets were formulated so that 10% of the dietary proteins originated from FM in the control diet (C0 diet), from one of the 4 defatted insect meals (8.4% BM, B10 diet, 8.9% TM, T10 diet, 9.2% ZM, Z10 diet or 10.3% HM, H10 diet) or from a mixture of them (MIX diet). A low dose of BM meal (0.8%) was also tested representing 1% of dietary proteins (B1 diet). Fish were fed to satiation for 11 weeks before hematological and humoral immune parameters were determined. The experiment lasted for a further 5 weeks-period at the end of which fish were marked individually with barcode chips. Fish fed different diets were redistributed in the 21 aquaria and subjected or not to a chasing stress twice daily for 5 days before intraperitoneal (i.p.) injection with lipopolysaccharides (LPS) in order to mimic a bacterial infection or with phosphate buffer saline (PBS) to assess the injection stress. In total, 5 types of stress/infection combinations were applied to different aquaria: 1) chased/LPS, 2) chased/PBS, 3) not chased/LPS, 4) not chased/PBS or 5) not chased/not injected. One day after the i.p. injection, blood was sampled and humoral immune parameters were determined to assess if some of the experimental diets protected the fish against the applied stress. Results will be discussed.
Reference:

Gasco, L., Józefiak, A., Henry, M. 2021. Beyond the protein concept: Health aspects of using edible insects on animals. Journal of Insects as Food and Feed, 7 (5): 715 - 741. https://doi.org/10.3920/JIFF2020.0077

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SOFT BONE DUE TO LOW DIETARY PHOSPHORUS INTAKE: THE RESPONSE TO EXERCISE

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Dietary phosphorus (P) is essential for teleost bone health. Zebrafish and Atlantic salmon fed with low-P diets develop large amounts of non-mineralized, albeit healthy, bone. In zebrafish a low-P diet even expands the bone volume due to increased non-mineralized bone matrix secretion (Witten et al. 2016; Cotti et al., 2020). Taking advantage of the zebrafish 'soft bone' model, we explored the response of bone to physical exercise regarding bone formation, mineralization, and malformations.
1-month old zebrafish were fed with a regular- (RP) or low-phosphorus (LP) diet for 3 months. Diet groups were split into control (C) and an exercised (E) group: RPC, RPE, LPC, LPE. RPE and LPE individuals were trained against a laminar flow, 6h/day and 5 d/week (speed: 10 SL/s). Calcein live staining labelled bone mineralisation at the start and the end of the experiment. Specimens were analysed for bone cells, bone microstructure and to quantify bone formation and mineralization (distance between the Calcein lines, extent of non-mineralized area).
Exercise in combination with the low P-diet did not elicit the development of deformities and had no impact on growth. Exercise increases bone formation in RPE animals compared to RPC animals. The low P diet increased bone volume in LPC and LPE animals independent from exercise. Strikingly, exercised LPE animals increased mineralization not only of vertebral centra but also of scales, indicating a systemic response. The increase of mineralization in LPE animals was surprising. We currently investigate the possible P source. The lack of increased scale resorption rules out the scales.
Cotti S, Huysseune A, Koppe W, Rücklin M, Marone F, Wölfel EM, Fiedler IAK, Busse B, Forlino A, Witten PE (2020) More Bone with Less Minerals? The Effects of Dietary Phosphorus on the Post-Cranial Skeleton in Zebrafish. Int J Mol