

Results showed that dry matter digestibility ranged from 42.9 to 63.6% on ingredient level, with the lowest level for the spray dried yeast (250 °C) and the highest level for the 16 h autolyzed yeast. Digestibility of protein ranged from 49.5 to 89.7%. Interestingly, 16 h autolyzed yeast induced the secretion of IL-8, while cell crushed yeast induced the secretion of TNF α in distal intestine, analyzed by ELISA. Further, these diets also increased the number of cells expressing IgM in head kidney, suggesting mobilization of immune cells. In parallel, using high-resolution flow cytometry of bacterial shape (forward scatter) and DNA content (DAPI staining) we were able to quantify the number of bacteria forming the microbiota in the gastrointestinal tract. Individual populations of bacteria were phylogenetically homogeneous and their frequencies changed due to the different diets. The results showed highest number of bacteria in fish fed spray dried yeast, and number of bacteria was correlating with digestibility values having increased number of bacteria in DI of fish having decreased digestibility. This innovative method provides a fast and inexpensive tool to interrogate the microbiota on the single-cell level and offer a unique opportunity to isolate and define bacterial populations for further molecular and functional analysis.

In conclusion, different down-stream processing of yeast do have an impact on protein availability and immune response when used as a protein source in fish feed. Furthermore, its impact on microbiota can be monitored using high resolution flow cytometry.

Keywords: Yeast, down-stream processing, immune effect, flow cytometry, microbiota

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O-093.

A combined in vivo – in vitro approach to evaluate the influence of dietary plant oils on innate immune competence and eicosanoid metabolism process in common carp *Cyprinus carpio*

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Abstract

The study aimed to evaluate the influence of dietary pure linseed oil or sesame oil and their mixture in replacing fish oil on innate immune competence and eicosanoid metabolism in common carp (*Cyprinus carpio*) through a combined in vivo – in vitro approach. A batch of 168 carp juveniles with an initial body weight of 100.4 ± 4.7 g were randomly allocated into 12 tanks of 100 L at a density of 14 juveniles per tank. Four iso-nitrogenous (crude protein = 36.9%) and iso-lipidic (10%) diets were prepared from three lipid sources (cod liver oil, CLO as fish oil source; linseed oil, LO; sesame oil, SO and a blend of linseed oil and sesame oil – SLO, v/v, 1/1 as plant oils). Fish were fed to apparent satiation twice a day for 6 weeks. At the end of the feeding period, on day 42, blood plasma was sampled for lysozyme, complement (ACH50) and peroxidase activity analyses whereas head kidney and liver were dissected for analysing the expression of candidate genes involved in immune competence (*lys*, *nkef*, *b/c2*); pro-inflammatory response (*cxcl8*); fatty acid biosynthesis (*elovl5*, *fads*) and eicosanoid metabolism process (*pla*, *pge2*, *lox5*). On day 45, head kidney leukocytes (HKL) and peripheral blood mononuclear cells (PBMC) were isolated and exposed to *Escherichia coli* lipopolysaccharide (LPS) at the dose of 10 μ g/mL for 24h. Then the culture medium was collected for peroxidase activity assay while cells were used for gene expression. Results showed that SLO diet enhanced the feed utilization in common carp but no differences of survival and growth were found between diets. No differences were found for genes involved in FA biosynthesis on day 42. Plant oil

diets did neither alter lysozyme and peroxidase activities nor gene expression levels (*lys*, *b/c2*, *cxcl8* and *nkef*), except for a lower ACH50 for fish fed SO diet. Moreover, they did not affect the expression levels of some genes (*pla*, *pge2*, and *lox5*) involved in the eicosanoid metabolism process. However, when head kidney leukocytes were exposed to LPS, *lys* expression was up-regulated in LO-fed fish ($P < .05$) while the expression level of *pge2* in SLO groups was higher than in other groups. Peroxidase activity of HKL exposed to LPS was higher than in control and the highest value was also found in SLO-fed group. In conclusion, our results confirmed that the utilization of plant oil in common carp diets did not induce any negative effect in fish growth, survival and immune competence status even if some humoral compounds seemed less effective in SO than in LO fed fish. Moreover, a dietary combination of SO and LO improved the feed utilization efficiency and seemed more effective in inducing a better immunomodulatory response to LPS through a more active eicosanoid metabolism process.

Keywords: Plant oil, eicosanoid metabolism, immunocompetence, common carp, pro-inflammatory response

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O-094.

Responses in post-prandial patterns of plasma macro-minerals and immune functions of juvenile rainbow trout, *Oncorhynchus mykiss* fed different monophosphate supplements

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Abstract

Application of feed phosphates (mon/di/tri basic) in high plant protein based diet is a common strategy to accurately cover mineral requirements, especially phosphorous (P), for better growth and health of fish. Considering bioavailability of minerals, monobasic form is more available than di or tri basic ones. A small difference in the P bioavailability may have significant effects on the fish performance and the amount of P discharge to the water. So, the evaluation of different monophosphate supplements is very important to develop eco-friendly aquafeeds. Post-prandial plasma mineral levels and measurement of immune responses can serve as an indicator of mineral bio-availability and health status of fish, respectively. A 60 day's feeding trial was conducted to evaluate the effects of different monophosphates on post-prandial pattern of plasma macro-minerals and immune functions in rainbow trout (*Oncorhynchus mykiss*). Four isoproteic (460 g kg⁻¹ protein) experimental diets were formulated by supplementing monocalcium phosphate, monoammonium phosphate, monopotassium phosphate and monosodium phosphate @ 1.14, 1.0, 1.15 and 1.0 % for diet groups MCP, MAP, MKP and MSP respectively. Triplicate groups of fish (18.5 g) were randomly stocked in 100-L fiberglass aquarium at a rate of 26 fish per aquarium and fed to apparent satiation twice daily. The post prandial plasma P levels were significantly influenced by the dietary treatments and the period of time after a single meal. Significant interaction effects were also observed between the dietary treatment and time period. The baseline concentration of P was significantly higher in MAP, MKP and MSP diet groups compared to the MCP group. The other macro minerals (Ca, Mg, K and Na) were only significantly influenced by the postprandial time interval. Significant positive peak of postprandial P absorption in fish fed diet groups MCP and MAP was observed at 1.5 h while MKP and MSP diet groups showed non-significant increment until 6 h. In all dietary groups significant peak concentration of post prandial plasma macro minerals Ca, Mg, K and Na was observed in 1.5, 4.5, 4.5 and 6 h

respectively. In terms of immune functions, respiratory burst and complement activities, total serum protein, peroxidase activity and catalase activity, one phosphate supplement could not prove significant relative advantage over others. However, fish fed dietary group MSP showed significantly higher lysozyme activity and it was not significantly different with MKP and MAP diet groups. MCP diet showed significantly lower lysozyme activity. The results of different immune gene expressions will be discussed in the conference.

Keywords: Monophosphates, Post-prandial absorption, macro minerals, immunity, rainbow trout

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O-095.

Searching integrated strategies for the evaluation of the physiological status in fish fed functional diets: The example of SDPP in gilthead sea bream (*Sparus aurata*)

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Abstract

The sustained development and intensification of production systems may negatively affect the health of fish, compromising the industry productivity. One of the most used strategies for the sustained improvement in production efficiency is the generation of functional diets. In this study, we evaluated the effect of spray-dried porcine plasma (SDPP) as dietary supplement and its impact upon the physiological and defense status in the skin-associated lymphoid tissue (SALT) of gilthead sea bream. To do it, fish were fed a high-content fish meal diet supplemented with 3% SDPP. After 95 days of feeding, the SDPP-supplemented diet showed a higher somatic growth performance compared to those fish fed basal diet. A higher viscerosomatic index and lower perivisceral fat index were also registered in SDPPfed fish. Fillet proximate composition showed no changes in the fatty acid profile neither the lipid peroxidation nor the activity of oxidative stress enzymes. The skin transcriptomics showed differential expression of 194 genes (DEGs) involved in different processes including cell metabolism, gene expression, protein transport, and protein localization to membrane. The skin mucus proteome identified 35 differential synthesized proteins associated to different processes including epidermis & skin development, epidermal cell differentiation, and metabolism. Importantly, when an integrated multi-omics analysis was carried out, other biological processes were identified, including innate immune response and response to stimuli (organic substances, external stimuli). Taken together, our results suggest that (1) the SDPP is a promising feed additive for sea bream giving a beneficial impact on its growth performance; and (2) the multi-omics analysis is an interesting initial strategy for aquatic organisms that greatly helps to understand the biological processes in an integrated physiological context.

Keywords: Animal nutrition, dietary supplements, mucosal-associated lymphoid tissue (MALT), innate immunity.

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O-096.

Feeding stress due to soy bean meal as a model for the development of molecular immune markers in rainbow trout

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Abstract

The increasing intensification of aquaculture practices has led to public debate about the welfare status of cultured fish.

In order to verify the immune status of trout in context of husbandry stress and to investigate the relationship between chronic stress, immunosuppression, husbandry and feeding we established molecular stress markers using a feeding stress experiment with soybean meal. The welfare status of trout was verified by investigation of mRNA expression of different potential stress regulated genes in whole blood to establish a minimal invasive method. A 56-day feeding experiment was carried out. The triplicate fish groups were fed isoenergetic and isonitrogenic feed mixtures in which the fish meal (50 % of the total diet) was replaced by 0 %, 33 %, 66 % and 100 % soybean meal. EDTA blood was collected from the caudal vein of immobilized trout. In total 88 different genes were tested for their suitability in stress detection using a Fluidigm Biomark HD and a Light Cycler System. Specific primers were designed. Regulated genes belonging to the superior signal transduction pathways such as SERPIN G superfamily, intracellular PI3K/actin, Toll-like receptor, NF-κB, MAP kinase and JAK-STAT signal transduction or intracellular pathogen recognition receptors were tested. The mRNA expression of blood cells was tested for different pro- and anti-inflammatory cytokines, chemokines, substances involved in the acute phase reaction, complement cascade or inflammatory reactions, and heat shock proteins. Finally, different marker genes for specific cell populations were investigated. The housekeeping genes β-Actin, EF1 and RPS5 served as internal standards.

Different genes (e.g. SAA, MPO, NOS2, UCP2) emerged as suitable stress and immune markers and therefore as welfare indicators on a molecular level, while some genes (e.g. IL10, IFN, HSP47) revealed no correlation to feeding stress.

The results represent an important basis for a better assessment of animal welfare in trout farming. They are an important first step towards making well-founded, early assessments of chronic feeding stress of trout in the future, which are minimally invasive. So far, the parameters have often been based on observations such as behavior, color changes and such aspects that are difficult to standardize. These results provide a basis for the development of practical detection systems - comparable to a diabetes test.

Keywords: Stress, inflammation, soy bean meal, feeding stress, molecular marker

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O-097.

Modulation of the immune condition in European seabass (*Dicentrarchus labrax*) juveniles through long-term methionine supplementation

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