

**O-090.****Yeast as a protein source with health beneficial properties in diets for smoltifying Atlantic salmon (*Salmo salar* L.)**

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**Abstract**

Atlantic salmon (*Salmo salar* L.) as an anadromous fish undergoes a series of physiological, structural and functional changes in transition from freshwater (FW) to seawater (SW). Fish are more susceptible to stress, physical damage and infectious diseases leading to high mortality and significant economic loss for the aquaculture industry. Yeast produced from lignocellulosic biomass has the potential to serve as a high-quality protein source with health beneficial properties, especially during seawater transfer (SWT) period. In this study, we evaluated the effect of adding 25% *Candida utilis* (LYCC7549) yeast in diets for smoltifying salmon, on growth performance and overall health by using morphometry, immunohistochemistry, cytokine enzyme-linked immunosorbent assays (ELISA) and gene expression analysis during and after SWT. A control diet (Control) and a test diet containing 25% yeast (Yeast) were fed to Atlantic salmon for 4 weeks in FW and 4 weeks after transfer to SW. Our results showed that fish fed the Yeast diet during the whole period or during the FW or SW period achieved higher feed intake and higher growth rate than fish fed the Control diet. Morphometry and immunochemistry analysis of distal intestine (DI) showed that yeast modified immunosuppressive responses related to SW acclimation. A decrease in length of simple folds and in number of CD3 labelled cells in the simple folds of DI in fish fed Control diet was observed, while changes were not present in fish fed the Yeast diet. Yeast significantly decreased the secretion of protein level of cytokines in DI (IFN $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-8) and tended to decrease the expression of pro-inflammatory cytokines interleukin 1 beta (*Il1b*) and interleukin 8 (*Il8*) on transcriptomic level. Yeast also modulated the gene expression of aquaporin 8 (*aqp8ab*), superoxide dismutase (*sod1*) and major histocompatibility complex 1 (*mhc1*) in DI, suggesting immunomodulation in Yeast fed fish. These findings suggest that *Candida utilis* yeast is a promising protein source with functional properties in diets for smoltifying Atlantic salmon before and after SWT.

**Keywords:** Atlantic salmon, yeast, smoltification, immune response, histology

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**O-091.****Bee pollen addition in zebrafish diets and its effect on immune response and microbiota**

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**Abstract**

Apitherapy is recognized from conventional until contemporary treatment methods. Bee pollen, a natural product with high nutritional properties is

recommended as dietary supplement due to many immunostimulating functions as antioxidant, antimicrobial and anti-inflammatory potential. Zebrafish, *Danio rerio*, a widely used species as an experimental model for humans and animals research provides advances in many issues, including immunological investigations. The identification of mechanisms involved in fish immunological activity subjected to bee pollen supplementary treatment can provide important unknown information for the recommendation of this product in diets. Also, zebrafish larval stage is a critical moment due to high mortality rates related to immune challenges. Thus, the objective of our study is to better understand how bee pollen addition in zebrafish diets can influence adults and their offspring immunity. Wild-type zebrafish experiments and procedures were performed as approved by the Consejería de Agua, Agricultura, Ganadería y Pesca de la CARM (authorization number #A13170801). Fish diets containing flakes (3% body weight - BW) and live food, *Artemia* nauplii, were used as control and compared to fish receiving the same diet supplemented with bee pollen (3% BW) coming from northeastern Brazil. Fish diets were administered during 70 days and the animals were spawned weekly. At 3th and 5th week after diet started, zebrafish offspring with 72 hours post fertilization (hpf) were tested for neutrophil migration after tail wounding. Neutrophils were counted at site of injury at 15, 90 and 360 minutes post wounding (mpw). After diet administration period, intestines from a zebrafish group were collected to metagenomics analysis to compare microbiota between both feeding regime. Total RNA extraction from kidney and abdominal organs of another fish group were quantified for immune-related gene expression after *Mucormycosis* (*Mucor circinelloides*) intraperitoneal infection. Offspring of zebrafish supplemented with bee pollen presented higher ( $P < 0.05$ ) neutrophil migration at 360 mpw compared to offspring of fish fed with standard diets in both weeks' analysis. No changes were observed at initial times after larvae tail wound. Neutrophils are pivotal effector cells of innate immunity and can play an essential role in fish against immunological challenges. Bee pollen dietary supplementation may influence recruitment of these cells in zebrafish offspring. Data regarding metagenomics and gene expression analyzes are still being processed.

**Keywords:** Fish immunology; Natural products; Nutrition; Neutrophil; Metagenomics

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**O-092.****Impact of down-stream processing of bakers yeast (*Saccharomyces cerevisiae*) on immune responses in Atlantic salmon (*Salmo salar*)**

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**Abstract**

The increased demand for high-quality feed ingredients for the aquaculture industry have turned microbial ingredients as viable sources of protein and lipids. However, limited information exist on the effect of different down-stream processing of microbial ingredients on digestibility and immune responses in fish. In the present study, a feeding trial was performed for 30 days using three different down-stream processing for yeast: spray drying, autolysis or cell crushing and the effect of these treatments on digestibility, immune stimulation and microbiota was evaluated.

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 $\alpha$  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 µg/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<sup>-1</sup> 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