Offer for a Thesis Allowance

Subject: Involvement of long-chain omega-3 polyunsaturated fatty acids in the central control of food intake in rainbow trout (Oncorhynchus mykiss)

From February 27, 2020 to June 30, 2020

INRAE is France’s new National Research Institute for Agriculture, Food and Environment. Created on January 1, 2020, it was formed by the merger of INRA, the National Institute for Agricultural Research, and IRSTEA, the National Research Institute of Science and Technology for the Environment and Agriculture.

Researches in the INRA Unit NuMeA (Nutrition, Metabolism, Aquaculture) are conducted in a context of limited marine resources and a strong aquaculture development worldwide. They aim to understand the regulation of the metabolic pathways by nutrients in fish, using an integrative approach (from genes to the animal). The finalized goal is to provide recommendations for innovative strategies in aquaculture feeds in order to optimize growth and feed efficiency in respect of sustainability.

PhD project

In 2014, aquaculture provided for the first time half of the fish that were consumed worldwide. Thus, to cope of growing booming of aquaculture and to fit with environmental and ecological impacts, social and economic sustainability of the aquaculture, the traditional majors ingredients of aquafeed, fishmeal (FM) and fish oil (FO), must be replaced by renewable and alternative sources like terrestrial plants products. However, after twenty years of research, the total replacement of marine products by use of plant products is not achieved and has several disadvantages. In rainbow trout (Oncorhynchus mykiss), a carnivorous species, the concomitant replacement of dietary FM and FO with vegetable ingredients, led to drastically altered fish growth performance and survival rate, poorer reproduction performance, first spawning and offspring survival. In addition, one of the main problems of extensive use of plant-based diet is an unfavorable modification of the fatty-acid composition of farmed fishes particularly of #3 long chain polyunsaturated fatty acids (#3 LC-PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), essential fatty acids known to have beneficial health effects for human and mainly provided by fish consumption. Indeed, modern, sustainable aquafeeds contain increasing levels of terrestrial agriculture alternatives that contain no #3 LC-PUFAs, which has translated into a substantial reduction (by 50% over the last decade) in the content of #3 LC-PUFAs in the flesh farmed fishes. To date, numbers of studies that have investigated the impact of total substitution of FM and FO with alternative terrestrial products have focused their scope on fish performance, metabolism and functional genomic. However, in farmed fishes, qualitative (nutrient type) and quantitative (consumption) feeding is essential for adequate growth, survival and reproduction. In this way, #3 LC-PUFAs
are also known to be essential for fish life cycle, to promote optimal growth and survival, health, reproduction and offspring development. Moreover, alternative ingredients, those containing no #3 LC-PUFAs are known to impact feeding of farmed fishes by reducing swimming activities, feeding rhythms, behavioral development (schooling), and increased abnormal behavior.

In this context, the origin and mechanisms which explained drastic alteration of fish fed with plant-based diet, and the impact of #3 LC-PUFAs absence in the composition of terrestrial products on food intake regulation and their consequence (growth, survival, reproduction) remain elusive. In this context, the research thesis project is hypothesized that the alteration of growth, survival rate and reproduction of rainbow trout fed with total plant-based diets could be explained by the modification of feeding behavior due to the absence of lipids, especially #3 LC-PUFAs.

Objectives:

The principal objective of this thesis project aims to understand the alteration of trout performance regarding total plant-based diet by #3 LC-PUFA by identifying the molecular status and mechanisms of organs known to be involved in the control of feeding behavior in animal: central nervous system and lingual system.

A recent published experiment performed in the unit revealed that #3 LC-PUFA (DHA and EPA) drove the feeding behavior of juvenile rainbow trout. We observed that fish could make a difference between diets containing different levels of #3 LCPUFA and preferred diets highest in #3 LCPUFA. This study highlighted the influence of #3 LCPUFA in the feeding behavior of juvenile rainbow trout. This experiment confirms the initial hypothesis and opens interesting perspectives for studies on feed intake regulation and mechanisms associated with this food preference.

The PhD project hypothesized that the abundance of #3 LC-PUFAs in FO and FM could strongly influence the integrity and function of the fish brain, particularly, that part controlling feed intake. Thus, by suppressing the dietary #3 LC-PUFAs, the mechanisms of feed intake regulation could be severely disrupted and impair growth performances. In mammals, DHA is mainly incorporated into the brain during the first year of life and the most abundant in brain content. On the contrary, EPA is rapidly oxidized and poorly accumulated in the brain. The impact of dietary #3 LC-PUFAs absence on the regulation of feed intake in fishes has never been investigated. Therefore, the main objective of this project is to determine if #3 LC-PUFAs influence the regulation of feed intake in rainbow trout and to characterize the underlying molecular mechanisms. The study will be performed during strategic periods of the rainbow trout life cycle, including juveniles and broodstock, and also progeny, in order to highlight a potential nutritional programming effect linked to maternal feeding. The final issue is to determine if dietary supplementation of DHA of microalgae origin could be a promising solution to restore growth performances of rainbow trout fed a vegetal diet.

To achieve these goals, the PhD project is sub-divided into two objectives which aims to identify and characterize the physiological and molecular status of the central control of feed intake in trout fed from first feeding with a vegetal diet, and to propose new vegetal
alternative diets rich in DHA (from algal origin) in order to restore alteration of growth performance offish fed with a vegetal diet.

Work plan:

A recent experiment performed in our laboratory revealed that #3 LC-PUFA (DHA and EPA) drove the feeding behaviour of juvenile rainbow trout. Fish could distinguish between diets containing different levels of #3 LC-PUFA and exhibit preference for diets containing high levels of EPA and DHA. In this PhD project, the student will characterize the physiological and molecular mechanisms regulating feeding behaviour in fish fed a vegetal diet devoid of DHA compared to fish fed a commercial-like diet containing FM and FO and high levels of DHA. He/She will evaluate the impact of a dietary DHA supplementation to a total vegetal diet on these mechanisms. For this purpose, female rainbow trout will be fed from first-feeding on one of the following diets: a commercial-like diet (C) based on FM and FO (35%) and containing 25% of DHA as % of total fatty acid content, a vegetal diet(V1) totally devoid of DHA or a vegetal diet supplemented with DHA (V2) (ultra purified oils from microalgae, strain Schizochytrium sp). The zootechnical parameters (e.g. survival rates, growth, feed intake and feed efficiency) will be recorded during 12 months. The student will investigate the molecular mechanisms shown to be involved in feeding behaviour regulation in humans and fishes. This will be done using the central nervous system by differentiating five areas of the brain: telencephalon, mesencephalon, myelencephalon, cerebellum and hypothalamus, and the lingual system known as the fatty acid tasting centre and involved in the regulation of dietary fat intake. Analyses will be performed after 3, 6 and 9 months. We will analyse at the transcriptional level (micro-array and target gene by qPCR), protein level (western blot, ELISA and immunohistochemistry) and, if relevant, at the enzymatic level, using specific markers of the brain areas concerned with the preferential pathways known to be affected by DHA. Markers of neurogenesis, synaptogenesis and neuropeptides, indicators of cellular type, animal stress and oxidant status, inflammation and genes regulating animal behaviour (anxiety, depression, reward system) will be assessed at the level of the brain. At the lingual level, PhD student will investigate markers of sensorial information and tasting involved in the palatability modulating feeding behaviour. These markers include nutrient sensing receptors, neurotransmitters, enzymes and hormones. As physiological effects of #3 LC-PUFAs could be attributed to their oxidation byproducts, fatty acid and by-product profiles will be determined in whole body and brain tissues using targeted biochemical analyses performed in collaboration with Dr T. Durand (UMR 5247, IBMM-CNRS) for non-enzymatic oxygenated by-products of fatty acids (e.g. isoprostanes and neuroprostanes) and Dr. J. Bertrand-Michel (UMR1048 INSERM) for enzymatic oxygenated byproducts of fatty acids (e.g. prostaglandins, eicosanoids and leukotrienes). In addition, PhD student will investigate the impact of DHA supplementation in the vegetal diet on feed preference of juveniles fed after 3 months with a commercial-like diet. To do this, He/She will evaluate, using self-feeders, the voluntary feed intake of juveniles by testing their preference between diets used in first feeding experiment : C vs V1, C vs V2 and V1 vs V2. Intentional feed intake (trigger activation by fish) will allow us to measure feed intake variables (feed preference, feed demand, feed consumption and feed waste). Altogether, this project, which will be the subject of three peer-reviewed articles, will decipher for the first time, the physiological and molecular mechanisms
regulating feeding behavior of farmed fish fed total vegetal diet from first feeding and determine the role of DHA in these mechanisms.

**Literature References:**


* Lazzarotto., V, Médale, F., Larroquet, L., Corraze, G. Long-term Dietary Replacement of Fishmeal and Fish Oil in Diets for Rainbow Trout (Oncorhynchus Mykiss): Effects on Growth, Whole Body Fatty Acids and Intestinal and Hepatic Gene Expression. Plos one13 (1), e0190730 DOI:10.1371/journal.pone.0190730

**Hosting laboratories:** UMR1419 INRAE-UPPA NuMeA (Nutrition, Metabolism, Aquaculture), Aquapôle, 64310 StPee-sur-Nivelle, France

**PhD supervisor:** Jérôme Roy

**PhD co-supervisor:** Sandrine Skiba-Cassy

**Starting date:** October 2020

**Length:** 3 years

**Employer:** UPPA, doctoral school ED 211 Doctoral School of Exact Sciences and their Applications.

**Gross monthly salary:** 1878 € for a PhD (UPPA doctoral contract, according to E2S UPPA project, including 96h of teaching during the three years)

**Co-funding 50% E2S(Energy, Environment, Solutions)UPPA(University Pau et Pays de l'Adour)and 50% INRA departmentPhysiologie animale et systèmes d'élevage (PHASE).**
Required skills and competences - Who can apply?

The candidate should have a strong predilection for laboratory work.

He/She is rigorous, highly motivated, with the capacity to work autonomously.

Team working abilities are highly appreciated.

Good knowledge in English and good writing skills are required.

Application - Evaluation criteria

Application files will be evaluated based on the following criteria:

* Candidate’s motivation, scientific maturity and curiosity
* Grades and ranking in Licence/undergraduate, M1 and M2
* English language proficiency
* Oral and written communication skills
* Candidate’s ability to present her/his work and results
* Professional experience (e.g. internships in laboratory or other, any research work previously carried out, reports, publications ...)

Application file composition and submission deadline

Please send an email to jerome.roy@inrae.fr and complete candidature containing the following documents:

* CV
* Cover letter detailing candidate's motivations
* Copy of the diploma
* Candidate's MSc or equivalent: marks and ranking
* 2 letters of recommendation with contact details of the referees

Submission deadline: June 30th, 2020