Intelligent Fish feeding through Integration of ENabling technologies and Circular principle

Grant Agreement (GA) No: 818036

D15 Demonstration Performance (KPIs) for Recirculating Aquaculture Systems

Version: 2.0

Date: 08th April 2024

Document type:	**Report**
Dissemination level:	**Public**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818036

Project data

Project Title:	Intelligent Fish feeding through Integration of ENabling technologies and Circular principle
Project Grant Agreement (GA) No:	818036
Project Acronym:	iFishIENCi
Duration:	57 months, 1 November 2018 – 31 July 2023
Type of action:	Innovation Action

Deliverable Administration and Summary

Status:	Final	**Due:**	M56	**Date:**	17th July 2023
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Reviewer	Freya Robinson, Giovanni Marco Cusimano (ABT)				
WP	3	**Deliverable Nr.**	3.4	**Relative Nr.**	15
Comments					

Document change history

Version	Date	Author	Description
1.1	15th April 2023	Giovanni Marco Cusimano, Freya Robinson (ABT)	Inclusion of ABT experiments
1.2	20th April 2023	Florian Nagel (AAR)	Inclusion of AAR experiments
1.3	26th April 2023	Pablo Sanchez Cueto (LEITAT)	Inclusion of microbiota results
1.4	25th May 2023	Nicolas Prost (BIO)	Inclusion of behavioural modelling information
1.5	7th June 2023	Franck Le Gall, Luc Gasser (EGM)	Inclusion of iBOSS information
1.6	15th June 2023	Christian Jensen (OXY)	Inclusion of Cobalia information
1.7	14th July 2023	Sergei Budaev (UiB)	Inclusion of FishMet information
1.8	17th July 2023	Freya Robinson, Giovanni Marco Cusimano (ABT)	Final Review
1.9	30th January 2024	Freya Robinson, Giovanni Marco Cusimano (ABT)	Resubmission review
2.0	8th April 2024	Freya Robinson, Giovanni Marco Cusimano (ABT)	Resubmission review

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1 Introduction

The following technologies have been demonstrated in marine and freshwater species such as rainbow trout, hybrid catfish, and Atlantic salmon in recirculating aquaculture systems (RAS) to evaluate the potential use of novel ingredients (yeast and microalgae meal) as a protein source. Additionally, AI tools (Fish-Talk-To-Me, SmartRAS, and iBOSS) have been developed and tested on a pilot scale, which can drive the aquaculture sector towards a new and more technological frontier.

1. Waste2Value: a collection of know-how/guidelines to valorise waste (experimental methodology, protocols etc.) for different aquaculture waste streams (wastewater, sludge, other streams retrieved from the production of ingredients). It will provide specific guidelines and recommendations for aquaculture waste streams, regulatory framework/legislation, technical aspects (characterisation of the substrate obtained for algae/yeast growth), as well as sustainability assessment results (specific recommendations/conclusions from LCA-environmental assessment, and LCC-economic performance, and some insights into infrastructure, when possible). Waste2Value will provide data of waste streams characteristics from RAS, such as dissolved nitrogen, ammonia, and nitrates, which will be linked to fish species and diets through modelling. Waste2Value will include 6 different guidelines:
    - Waste reuse Biogas & Chemicals.
    - Waste reuse as fertilizing products and/or composting.
    - Waste reuse IMTA & Aquaponics.
    - Waste reuse for the production of new feeds – algae and yeast.
2. Fish-Talk-to-Me is an integration of technologies and biology to better understand the state of the fish and to anticipate feeding requirements (e.g., smart feeding). This information could be used for optimizing production site knowledge with early warning information as well as for optimizing AI and machine learning from other group-based sensors or cameras. The know-how of the product includes:
    - Fish tagging technology for collecting fish physiology and movement data;
    - Camera technology for monitoring fish behaviour;
    - Digital twin of fish appetite and feeding (FishMet).
3. iBOSS (iFishIENCi Biology Online Steering System) is a flexible system for monitoring multiple aspects of fish biology and environment. This includes water quality parameters (e.g.,

temperature, dissolved oxygen, pH), characteristics of the microbiota within cultivation systems, fish behaviour, and relevant production metrics such as feed consumption and growth rate measurements. It consists of two main components. iBOSS Cloud: a platform in which data will be stored and processed by analytical algorithms. iBOSS Edge: A local interface that sends and receives data to and from the cloud, and which allows monitoring and control of the farming system. Both iBOSS components can be connected to other farm management cloud systems and data aggregators collecting data from various sensors.

4. SmartRAS is a design concept developed as part of iFishIENCi. In this design, the state-of-the art recirculation aquaculture system (RAS) can be operated by applying the precision fish farming technology principles. The Smart-RAS design also integrates iBOSS, Fish-Talk-To-Me and Waste2Value, which implement the following:
    - Fish feeding behaviour monitored with cameras.
    - Camera data, existing and new, as well as feed intake model data (Fish-Talk-to-Me) collected and evaluated by algorithms in iBOSS cloud.
    - Feeding systems controlled by using data from iBOSS.
    - Waste streams from the system will be analysed to provide data for Waste2Value.

    These integrations of the iFishIENCi innovations will be supported with the following design elements of SmartRAS:

    - Design options to collect nutrient rich water from research and commercial RAS in systems.
    - Design for camera and sensor installations in research and commercial RAS.
    - Design of SmartFeeder integration in research and commercial RAS.
5. Breed4Feed - selecting African catfish specifically for the ability to efficiently utilize lower fish meal-containing feeds or alternative protein sources, such as algae or yeast proteins, for more economical and sustainable production. These ‘Breed4Feed’ catfish were used to test the New Feeds of the project.
6. New Feeds to qualify new functional ingredients obtained from algae and yeast while conserving natural resources and contributing to climate change mitigation.

Apart from the technologies tested, the relationship between bacterial communities present in RAS systems under different diets have been studied. RAS is a system defined by the high level of nutrient input, overcrowding and water recirculation, where small changes, such as new feed ingredient, can lead to a certain imbalance in microbial biodiversity. Understanding the composition of the microbial community and how it interacts with farmed creatures in RAS is crucial for designing management

strategies for the system as the industry does not yet fully understand how to sustain or restore a "healthy" microbial biodiversity. Thus, manipulation of the microbiota stands out as a factor called to increase circularity and sustainability in the aquaculture industry.

In these demonstrations, the potential effects on water and fish microbiota due to application of new feeds has been studied. In addition, the bacterial data generated from healthy RAS systems may be integrated as an additional parameter in the iBOSS technology developed under this project. Although further standardization and exhaustive sampling will be required to validate it as a monitoring tool for the system.

2 Rainbow Trout at Aller Aqua Research

2.1 Key Performance Indicators

Target: Partial fish meal replacement by *Microchloropsis gaditana (former Nannochloropsis gaditana*[1]*)* and Candida meal as well as partial substitution of Astaxanthin by Nanno meal mediated carotenoid pigmentation.

The KPI demonstrated in the pilot scale trial RAS6_275 with rainbow trout were:

1. Rainbow trout grow using novel protein sources with no negative effect on growth performance and body quality traits.
2. Pigmentation of the fillet is the same or better in fish fed novel protein sources with Nanno meal replacing astaxanthin.
3. Rainbow trout sludge and wastewater can be characterized within the framework of the Waste2Value.
4. Novel protein sources do not negatively impact the organoleptic results of the fillet.
5. Microbiota within the system is not significantly affected by fish meal replacement with Candida or Nanno.
6. Diet related changes in the gut microbiota of rainbow trout do no cause either negative or positive effect on fish performance.

2.2 Demonstration Methodology

Rainbow trout of 185g mean weight were stocked in triplicates and adapted to experimental conditions for one week, at optimal and constant temperature of 15°C and DO of 8mg/l. Fish were fed twice a day by hand until apparent satiation. Uneaten pellets were recounted daily for the 8 weeks period of the trial. At the end of the trial the tank biomass was determined, and single fish size was recorded based on random sample of 28 fish per treatment, also used for length-weight detection to calculate the condition factor (K), hepatosomatic index (HIS) and spleen somatic index (SSI). Ten fish per treatment were gutted and weight recorded for slaughter yield (SY), followed by filleting. The left fillet was stored at -23°C and ship to TTZ for organoleptic investigation, while the right filet was used for nutrient analysis. The composition and nutritional profile of the test diets are showed in Table 1 & 2. The three diets consisted of different inclusions of novel protein.

[1]renamed to Microchlopsis gaditana in April 2017 after a taxonomic revision (DOI:10.2216/15-60.1). However, for the purpose of this document, the name will be retained as *Nannochloropsis gaditana* and corresponding diets will be referred to as Nanno.

One test diet incorporated *Candida utilis* meal at 5% inclusion, Nanno 1 test diet incorporated 5% *Nannochloropsis gaditana* meal with a 40mg/kg inclusion of astaxanthin and Nanno 2 test diet incorporated 5% Nanno meal with 20mg/kg of astaxanthin.

Furthermore, samples of inlet, outlet and process water, sludge, faeces and intestinal mucosa as well as fish tissue samples were collected and shipped to Leitat and NORCE for analysis. The samples were taken to support the development of the Waste2Value framework, and the results of the analysis are published in D1.6 – Valorisation of by-products and sludge.

Table 1. Test feed formulation for trial RAS6_275.

Raw material (%)	Control diet	Test diet Candida	Test diet Nanno 1 high pigment	Test diet Nanno 2 low pigment
Fish meal	**15.00**	**10.00**	**10.00**	**10.00**
Sunflower protein	12.00	12.00	12.00	12.00
Soybean protein	10.00	10.00	10.00	10.00
Poultry meal	12.00	12.00	12.00	12.00
Feather meal	8.00	8.00	8.00	8.00
Wheat	15.10	13.35	13.07	13.09
Wheat gluten	4.82	6.14	6.70	6.70
Fish oil	20.31	20.44	19.90	19.90
Vitamins, minerals and additives	1.42	1.45	1.65	1.65
Synthetic amino acids	1.30	1.57	1.63	1.63
Candida meal	**-**	**5.00**	**-**	**-**
Nanno meal	**-**	**-**	**5.00**	**5.00**
Astaxanthin (mg/kg)	**40.0**	**40.0**	**40.0**	**20.0**

Table 2. Nutritional profile of isocaloric and isonitrogenous test feeds of trial RAS6_275.

Parameter (% OM)	Control diet	Test diet Candida	Test diet Nanno 1 high pigment	Test diet Nanno 2 low pigment
Moisture	3.3	3.1	2.7	2.9
Crude protein	**46.7**	**47.3**	**47.6**	**46.6**
Crude fat	22.6	21.4	23.1	23.5
NFE	18.7	19.4	17.9	18.3
Crude fibre	1.2	1.5	1.3	1.4
Crude ash	7.5	7.3	7.4	7.3
P	0.99	1.05	1.00	0.92
Ca	1.31	1.18	1.12	1.08
Gross energy (MJ/kg) calculated	**23.45**	**23.24**	**23.72**	**23.71**
Initial Astaxantin content (mg/kg)	**40**	**40**	**42.6**	**22.6**

2.2.1 Microbiota

The characterization of the microbiota was conducted after the adaptation period of the rainbow trout on the entire gut and on samples of skin and inlet and outlet water of the production system. The sampling was conducted before the commencement of the experiment (T0) and after 12 weeks (T1). For each timepoint, 100ml of inlet and outlet water were collected in triplicate. A total of 9 fish per condition (Diet_1 = Control; Diet_2 = Test candida; Diet_3 =Test nano 1; Diet_4 = Test nano 2) were sampled for skin mucus by swabbing and gut after dissection. The aforementioned water samples were collected and filtered individually with nitrocellulose filters (0.2µm pore size) and the swab and gut tissue stored in DNA/RNA shield buffer (Zymobiomics, Irivine, Canada). A total of 104 samples (46 gut, 46 skin, 6 outlet water and 6 inlet water) were shipped to Leitat Technological Centre for the DNA extraction procedure. Depending on the sample type, different pre-processing methods were carried out (Table 3).

Table 3. DNA extraction protocol.

Sample	Type	Sample preparation	Lysis tubes	Lysis Reagent	duration lysis	Elution (ul)
Skin	Swab	The entire swab sample was utilized to DNA extraction.	ZR Bashing Bead™ Lysis Tubes	DNA/RNA Shield	40 min 27. 000 rpm Vortex Gen ie 2	50
Gut	Tissue	~400mg (+/-20mg) of Tissue was processed, cutting it in small pieces to facilitate the lysis step.	-	-	-	-
Water	Filter	The entire filter was cut into small pieces to facilitate the lysis step				

Once the DNA was extracted, it was quantified with Qubit 4 Fluorometer (Invitrogen). DNA amplicon libraries were generated targeting the V3-V4 regions (341F/R805) of the 16S rRNA gene and the sequencing was performed by Illumina MiSeq PE300 following the recommendations of Illumina Inc. Sequencing was performed in Genome Québec Inc. (Centre d'expertise et de services Génome Québec, Montréal (Québec), Canada, as was the adapter trimming. The raw sequencing data were processed with the bioinformatic software QIIME2 version 2021.2 28. Briefly, pair-end reads were merged using fastq-join. Chimeric sequences were detected and deleted, meanwhile Amplicon Sequence Variant (ASV) assignment was completed using dada2 plugin. Taxonomy was assigned at a 99% similarity level using the q2-feature-classifier plugin with the SILVA 132 database (version 2019.10.0).

Finally, microbial communities for each sample were analysed using R software (Version 1.4.1717). Alpha diversity was assessed through the Shannon index. Beta-diversity was calculated with the Bray Curtis index. Multivariate statistical significance analysis was carried out using PERMANOVA (Adonis)

and pairwise PERMANOVA (pairwise Adonis) with 999 permutations and adjusted with false discovery rate method (fdr) ($p < 0.1$). Taxonomical differences were computed both at phylum and genus levels. To accomplish this, the ASVs counts were transformed with the centred log-ratio (CLR) approach, to assess the compositionality of the data. Statistical significance was assessed using an ANOVA test (p-value < 0.05).

2.3 Demonstration Results

Fish grew from 185g to an average of 474g (Nanno 1)- 513g (control) over a period of 56 days. No mortality was observed over this time in any of the treatments. Daily feed intake (DFI) was similar across all of the diets with no significant differences observed, but a trend of lower DFI in fish fed the Nanno diets. The feed conversion ratio (FCR) showed the lowest value in fish fed the control diet, followed by the Nanno 2 diet, with both values significantly lower than the fish fed the candida and Nanno 1 diets which showed no significant difference between them. The trend of a lower DFI and higher FCR in the Nanno diets led to a significantly decreased specific growth rate (SGR) in the fish fed the two Nanno diets. Specific growth rate was highest in the fish fed the control diet (Figure 1).

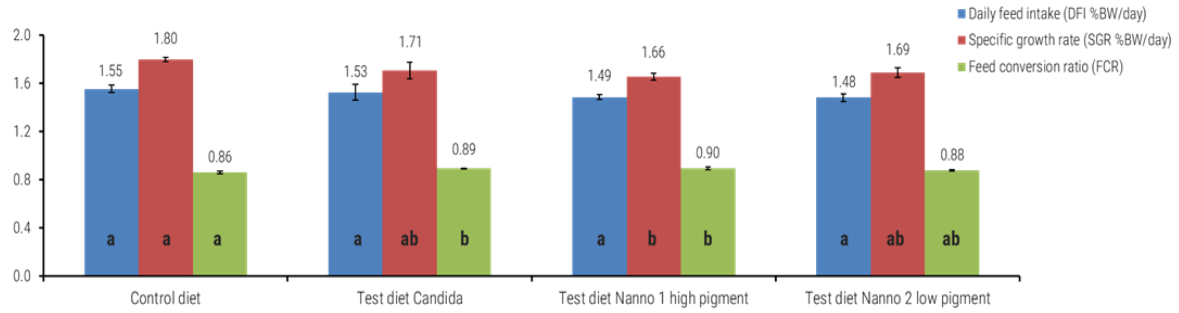

Figure 1. Performance parameters of rainbow trout fed experimental diets for a period of 8 weeks at optimal conditions in RAS. Different letters indicate significant differences (p<0.05).

Slaughter yield (SY) was similar across fish fed all diets with no significant difference being observed between each treatment. The fillet yield (FY) of fish fed the candida meal was significantly higher than those fed control and Nanno 1 diet, which provided a significantly higher filet yields than fish fed the Nanno 2 diet (Figure 2).

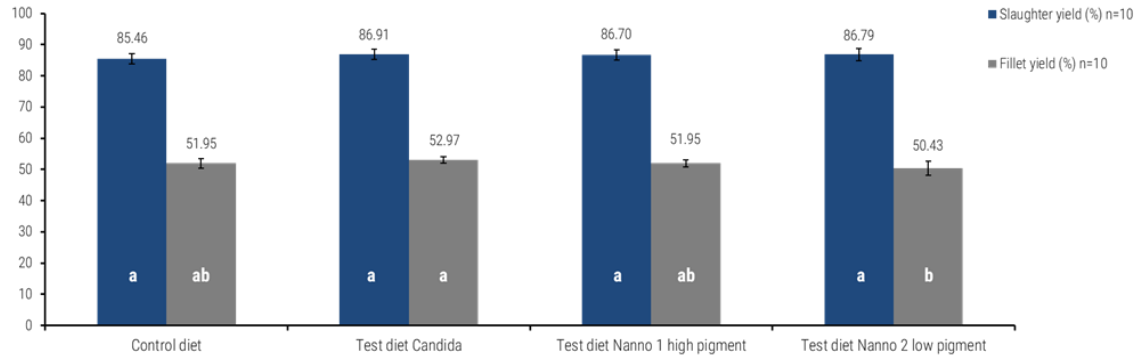

Figure 2.Slaughter and fillet yield of fish of different treatments at the end of trial. Different letters indicate significant differences (p<0.05).

Condition factor (CF), hepatosomatic index (HIS) and spleensomatic (SSI) index ranged at comparable levels among all treatments (Figure 3).

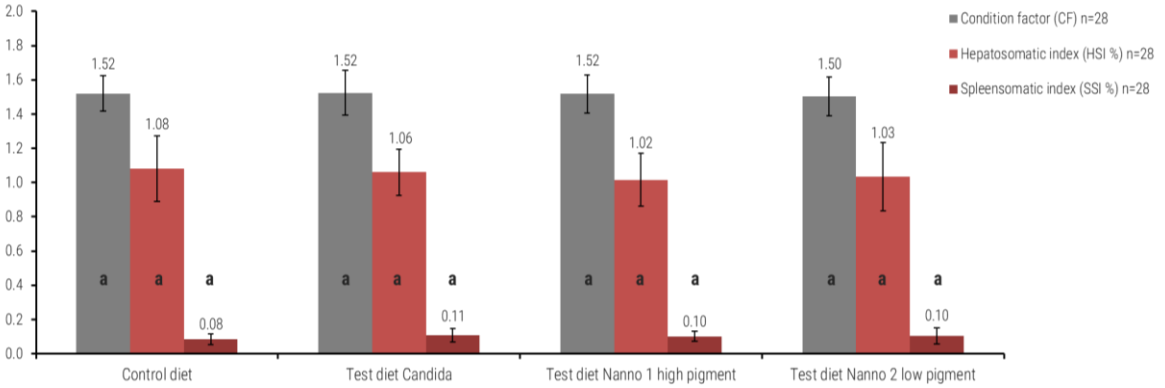

Figure 3. Condition factor, Hepatosomatic Index and Spleensomatic index of 28 fish per treatment at the end of trial. Different letters indicate significant differences (p<0.05).

Fillet pigmentation (DSM SalmoFanTM colour card) reached levels between 28.3 (control diet) and 27.03 (Nanno 2 diet). There was no significant difference between the fillet pigment in the fish fed the control, candida and Nanno 1 diets (Figure 4).

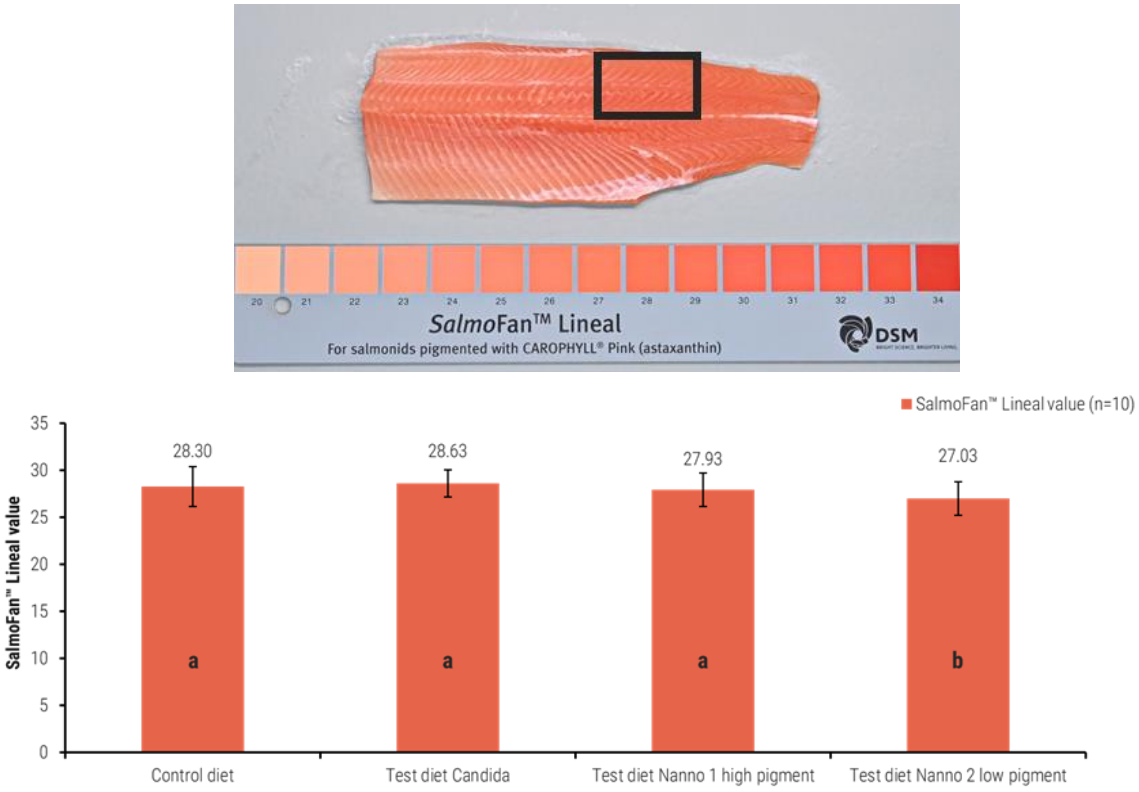

Figure 4. Fillet pigmentation at the end of the experimental trial. Pigmentation was measured with DSM SalmoFan™ colour card at a defined area of the dorsal fillet (Rectangle) utilising a light box with 6500 Kelvin. Different letters indicate significant differences (p<0.5).

Table 4 shows the nutritional profile of the final fillets of the fish fed the different diets. Independent from initial inclusion levels, the astaxanthin degradation in the test feeds ranged between 83.58% (candida diet) and 91.73% (Nanno 2 diet) after ending the experimental trial. Final astaxanthin content

of fillets ranged between 2.3mg/kg (Nanno diet 2) and 3.15mg/kg (Nanno diet 1) without significant differences.

Table 4. Nutritional profile of the final fillet of fish fed different test diets during trial RAS6_275 (n= 10/treatment). No statistical differences have been detected (P<0.05).

Parameter	Control diet	Test diet Candida	Test diet Nanno 1 high pigment	Test diet Nanno 2 low pigment
Moisture (%)	71.19 ± 0.86	71.10 ± 0.63	71.54 ± 0.48	71.80 ± 1.09
Crude ash (%)	1.34 ± 0.05	1.31 ± 0.09	1.34 ± 0.05	1.34 ± 0.07
Crude protein (%)	**20.74 ± 0.47**	**20.43 ± 0.57**	**20.28 ± 0.38**	**20.61 ± 0.45**
Crude fat (%)	6.81 ± 1.29	7.32 ± 1.37	7.00 ± 0.63	6.89 ± 1.41
Astaxanthin (mg/kg)	**3.00 ± 0.92**	**3.11 ± 0.97**	**3.15 ± 1.61**	**2.30 ± 0.92**

Due to the high feed intake and test feed properties, faeces structure was low (particle structure, sinking speed), without significant differences. Organoleptic examination provided no significant differences among the groups, with a trend for consumer preferences for fillets from control group, in terms of appearance, odour, taste, mouthfeel and overall impression. Details of this study can be found in the public Deliverable 3.6 – Assessment of organoleptic and nutritional quality of fish products from the demonstration tests.

2.3.1 Microbiota analysis

2.3.1.1 Initial bacterial diversity in the water and fish tissues

At T0, bacterial communities of rainbow trout gut were found to be significantly less diverse than the other sample types (Figure 5 top, $p < 0.05$), which is consistent with previous studies in rainbow trout and RAS systems[1]. The bacterial communities associated to skin, outlet and inlet water presented very similar alpha diversity values with no differences among them. In terms of beta diversity, the multivariate analysis of all sample types showed significantly differences between bacterial communities (PERMANOVA, $p < 0.01$). A clear different dispersion and aggrupation by sample type was observed in the PCoA (Figure 5 bottom). In more depth, the bacterial communities were dominated by different genera depending on the sample type. Firstly, the rainbow trout gut presented a very high relative abundance of *Mycoplasma* (Diet 1 T0 = 91% ±7%). The high dominance observed of *Mycoplasma* in this study is largely in accordance with other microbiota studies in salmonids. In terms of skin and outlet water samples, the high alpha diversity is mainly represented by the genera *Flavobacterium, Burkholderia*, *Hydrogenophaga*, *Novosphingobium*, *Flavobacterium*,

[1] Ruixiang Zhao, Jane E. Symonds, Seumas P. Walker, Konstanze Steiner, Chris G. Carter, John P. Bowman, Barbara F. Nowak. Effects of feed ration and temperature on Chinook salmon (Oncorhynchus tshawytscha) microbiota in freshwater recirculating aquaculture systems, Aquaculture, Volume 543,2021, 736965,

Pseudorhodobacter, *Rhodobacter* and *Emticia*. The inlet water encompassed a high relative abundance of microorganisms belonging to Proteobacteria and Patescibacteria phyla that were not possible to identify at genus level, due to limitations of the technology.

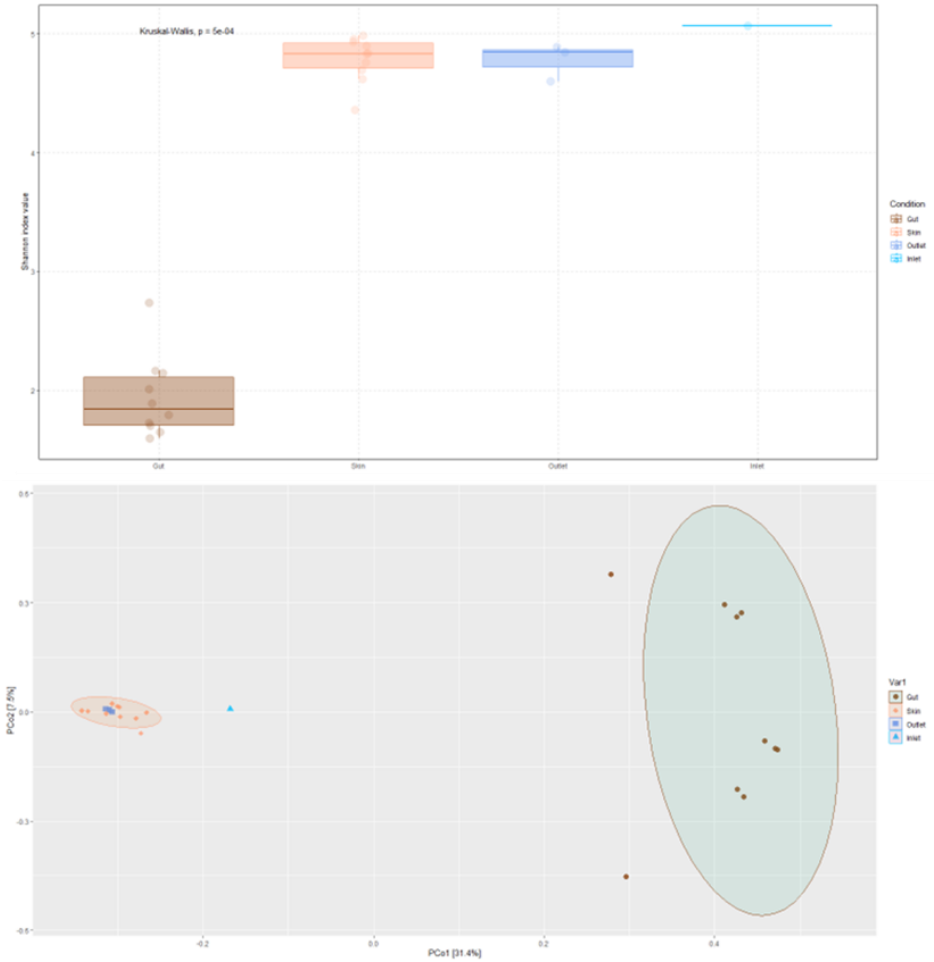

Figure 5. Alpha and beta diversity at T0 of RAS6_275 demonstrating A) Distribution of the Shannon index measurements are presented by sample type Kruskall-Wallis test was performed to assess the statistical significance of the observed distribution differences (p value <0.05)) B) Scores plot for the PCoA performed on the bray curtis beta-diversity index for the different samples studied. Samples are coloured and shaped accordingly to their type. Variance explained by each coordinate is indicated between parentheses in the corresponding axis.

2.3.1.2 Diet impacts on the fish microbiota

After 12 weeks, only the gut bacterial communities of fish fed with Diet 2 showed a significant alpha diversity increase compared to T0. This was not observed in the skin samples, where bacterial diversity decreased significantly and changed at beta diversity level under all diets compared to the T0 (Alpha - Kruskall wallis: $p < 0.05$; beta - pairwise.PERMANOVA: $p < 0.05$). No significant differences at alpha or beta diversity were observed between diets at the end of experiment. At phylum level, the slight decrease of Tenericutes at the end of experiment is concomitant with an increase of Fusobacteria and Proteobacteria, especially from the candida and Nanno diets (Figure 6 top). In terms of skin, a significant increase of the Actinobacteria relative abundance was observed in fish fed Diets 2, 3 and 4 compared to fish fed Diet 1 and the T0 of the trial (Figure 6 top).

Comparing gut bacterial composition at genus level, a decrease in *Mycoplasma* dominance was observed in certain fish fed with Diet 2, 3 and 4 that may be related to the increase of *Cetobacterium* genus. This *Mycoplasma* relative abundance reduction and *Cetobacterium* increment is appreciated when computing the mean values within each diet group (Figure 6 bottom). Nevertheless, due to the high microbiota composition variability between individuals, no significantly differential genera were observed among diets. With regards to the skin, *Paracoccus*, *Micrococcus*, *Candidatus Piscichlamydia* and *Brevudimonas* increased their relative abundance in fish fed all the diets at the end of the trial compared to T0. Comparing between diets, the highest number of differential genera was observed between the fish fed Diet 1 (control) and fish fed Diet 2 (candida inclusion diet). Meanwhile the relative abundance of *Brevudimonas* and *Cetobacterium* decreased in fish fed Diet 2 compared to the control, the genus *Hyphomonas* increased significantly. Albeit the relative abundance of *Hyphomonas* was marginal within the system and does not appear within the 20 most abundant genera of the skin (rel. abundance: 0.1 – 0.5%).

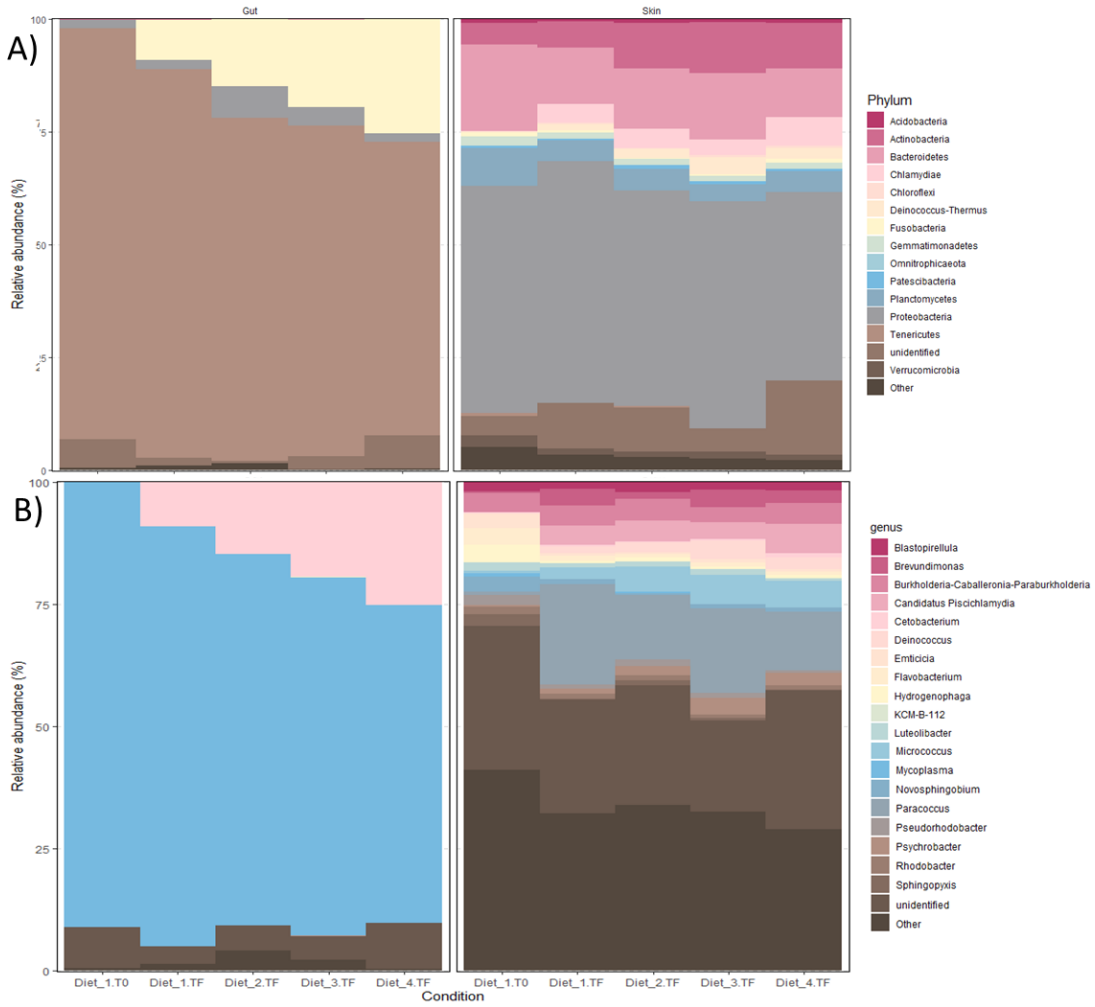

Figure 6. Phylum and genus diversity in rainbow trout gut and skin samples. The microbiota composition is presented in bars displaying the mean relative abundance of each phylum in a different colour presented per condition. A) Distribution of the 15 abundant phyla of the gut and skin microbiota, stratified by dietary regime and sampling timepoint. B) Distribution of the 20 most abundant genera of the gut and skin microbiota, stratified by dietary regime and sampling timepoint.

2.4 Conclusions, Recommendation for Application of the Results in Industry

The results of the trial showed that a replacement of 33% fish meal with candida or Nanno meal resulted in a significant decrease in fish performance. Similarly, a 50% reduction in astaxanthin supplementation in nano 2 diet led to lower pigmentation levels and reduced hedonic quality of final fillets during organoleptic examination. While candida and nano meals did not achieve the nutritional quality of premium fish meal, purified protein and lipid fractions showed potential to match the quality of fish meal or oil. However, further studies are needed to investigate this. Additionally, the study found that a 50% reduction in dietary astaxanthin content could not be fully compensated by carotenoids in the Nanno meal.

2.4.1 Microbiota

The impact of different diets on the bacterial communities of the skin and gut of rainbow trout was investigated, and the findings revealed interesting insights. Firstly, there were no significant differences in the alpha and beta diversity of bacterial communities between the sustainable and control diets, indicating that the dietary composition did not have a substantial effect on the overall bacterial diversity. Albeit the results at phylum or genus level showed no common trends in fish stocks by feeding, some individual-specific changes can be observed. Nevertheless, the effects that these diets may have on the rainbow trout microbiota in a longer-term application remain unclear and the new feeds inclusion should be studied further. Moreover, it is worth noting that the rainbow trout gut exhibited a high dominance of *Mycoplasma*, which aligns with previous research findings. This observation highlights the need for further investigation into the potential synergistic interactions between this genus of microorganism and the fish.

2.5 Dissemination of the Demonstration

Webinars and oral presentations in Germany and Italy.

- Results of the demonstration were presented at AquaFarm – Pordenone Fiere! In Italy to industry and academic stakeholders. The conference was held in May 2022 and the oral presentation was delivered to an audience of fifty - seventy.
- Presented the results of the demonstration at the 3rd Rostock Digital Ocean Convention in Germany in November 2022. The oral presentation targeted industry and academic stakeholders and was delivered to an audience of approximately fifty.

Research and Product Development at Customer Receptions for Aller Aqua

- Oct 2022 - **Web Conference - EAFP Hannover (Germany)**: Functional feeds against bacteria diseases. Audience: scientists, pathologists, trout farmers.

- Jan 2023 - **Visit Danforel (Denmark):** Discussion and training for AA clients, which feeds which feeding programme, which new raw materials and feed developments.
- Mar 2023 - **Meeting with Politicians (Germany):** from District Dithmarschen/district assembly in Heide - Tour through AAR facilities, discussion how to develop the facility further.
- Mar 2023 – **Webinar (Chile):** Salmon feed development and solutions. Audience: salmon farmers.
- Mar 2023 - **Meeting in Ustka (Poland):** Meeting with trout famers, effect of high temperature of farming and how functions feeds and carefully selected raw materials could improve fish health and performance.
- Feb 2023 - **Client Seminar (Serbia)**: Feed developments and improvements, feeding programmes and recommendation for trout and carp.

3 Catfish at AquaBioTech Group

3.1 Introduction

Catfish are an expanding sub-sector for aquaculture with production growing exponentially over the past decade, reaching 7.6% of the global inland aquaculture production in 2020. Although taxonomists recognise >3000 species of catfish, only some of these contribute to the global harvest. The genus Clarias has been widely introduced to many countries beyond its native range, including Brazil, Cuba, Bangladesh, China, Philippines, Belgium, Netherlands, Germany, Czech Republic, Poland, Romania and Hungary (who lead the European production of African catfish with over 3000 tons/year). Although the use of the pure strain is widespread, the hybridisation process can offer several advantages over pure lines, including faster growth, increased disease resistance, improved feed conversion, better fillet quality, and reduced inbreeding. Testing of hybrid catfish, which were obtained from the breeding of *Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂ was performed in two main trials at ABT that aimed to demonstrate: 1) the inclusion of a novel ingredients from the project as protein sources; 2) to compare two genetic lines of the breeding, respectively. The demonstrations were conducted in two recirculating aquaculture systems at AquaBioTech Group in Malta, equipped with a state-of-the-art technology in confined aquaculture (RAS). Demonstrating in RAS has the advantage of a stable environment with physiochemical parameters that can be maintained within specific ranges. However, the water quality management is of general concern due to the limited exchange of water that can be performed.

Catfish are widely farmed in pond and flow-through systems around the globe, where water is continuously pumped into the system from a natural source, such as a river or lake, and then released back into the environment. This allows for a constant flow of fresh water through the system, which is important for maintaining water quality and ensuring the health of the aquatic organisms being raised. While flow-through systems can be more environmentally sustainable than RAS, they are typically less efficient in terms of water usage and require careful management to avoid negative impacts on the surrounding ecosystem (Singh et al., 2020). However, the use of RAS in commercial farming across Europe (the Netherlands and Belgium) allow the achievement of higher stocking densities (700-1000kg/m^3) using extruded balanced feed. Based on these commercial stocking densities, ABT aimed to demonstrate that hybrid catfish could be successfully grown in a pilot scale RAS at a stocking density of approximately 100kg/m^3.

3.2 Key Performance Indicators (Catfish Expt. 1)

The reduction of reliance on traditional raw materials like fishmeal and soybean meal in aquafeed is of paramount importance to promote sustainability and mitigate negative environmental impacts.

Recent research has demonstrated the promising potential of microbial products, particularly yeast, as a sustainable protein source for food or feed (Yadav et al., 2022; Liu et al., 2021; Jones et al., 2020; El-Sayed, & Mahmoud 2019; Øverland & Skrede, 2016). Yeast has the ability to convert non-food waste biomass from agriculture and forestry into high-protein feed with minimal dependence on arable land and water (Khambhaty & Mody 2020; Ge et al., 2019; Kuhad et al., 2017)

The demonstration at ABT aimed to test a *Candida utilis* meal (commonly referred as yeast meal), produced within the project, as a raw ingredient in aquafeed to replace plant-based proteins in a low fishmeal content diet. The water quality management and the potential reuse of the sludge produced were also assessed. The partial or total valorisation of the waste is used to develop Waste2Value - a set of recommendations/guidelines to valorise different aquaculture waste streams (wastewater, sludge, waste from the production of ingredients) and to contribute to the generation of more circular value-chains within the aquaculture economy and beyond.

The key performance indicators (KPIs) for this demonstration were as follows:

- Growth performance: hybrid catfish to reach an average size of 400g in approximately nine weeks of feeding trial.
- Catfish stocked at a stocking density of approximately 100kg/m^3 with minimal impact on water quality management.
- Catfish grow using candida meal as novel protein source with no negative effect on growth performance and body quality traits.
- Microbiota within the system is not significantly affected by fish meal replacement with Candida.
- Diet related changes in the gut microbiota of African catfish do no cause either negative or positive effect on fish performance.
- Catfish sludge can be characterized within the framework of the Waste2Value.

3.3 Key Performance Indicators (Catfish Expt. 2)

The aquaculture industry incurs a significant economic impact from fish feed, which comprises 50 - 70% of its overall production cost (Gong et al., 2019; Llagostera et al., 2019). A balanced feed that meets all the nutritional requirements of the target species is crucial for their energy, movement, normal function, disease resistance, maintenance, and metabolic growth (Ansari et al., 2021). However, the reduction of the overall production cost through either the use of alternative

ingredients, or the improvement of feed utilisation and efficiency through genetic improvement programmes are considered a key step in the growth of fish farming, especially in developing countries.

Breeding programs for major farmed carnivorous fish species have been successful in enhancing growth, feed efficiency, disease resistance, and product quality (Quinton et al., 2021). At ABT, a demonstration was conducted to evaluate the performance and survival of two hybrid catfish lines (*Clarias gariepinus* ♀ x *Heterobranchus longifilis* ♂) over an 8-week period. They were fed a commercial feed with low fishmeal content.

The key performance indicators (KPIs) for this demonstration were as follows:

- Selected line showed enhanced growth performance compared to the control line.
- Fish stocked at a stocking density of approximately 100kg/m^3 with mild water quality deterioration.
- Average weight of approximately 400g reached in 8 weeks of trial.

3.4 Demonstration Methodology (Catfish Expt. 1)

The trial was conducted in a recirculating aquaculture system of 12x 650L circular tanks equipped with UV disinfection (UltraAqua MR1-220PP UV), drum filter (Faivre DF 2-60 @40 microns Drum filter), protein skimmer (AquaCirc CL10) and Biosystems (former TMC) LED lights and continuously monitored through an OxyGuard Pacific unit (DO, T, pH, ORP). Each of the experimental tanks was provided with a swirl separator, which was used to collect raw sludge to be used for the aforementioned characterization of the waste.

As a pilot-scale system, the demonstration aimed for a target stocking density of approximately 100kg/m3 and a final average fish weight of 400g. The formulation of the extruded diets was based on the latest data from scientific and commercial recipes where the inclusion of fishmeal in catfish feed has been drastically reduced and replaced with plant-based ingredients, mainly represented by soybean meal (Table 5).

A control diet and two test diets were formulated with 0, 10 and 20% of candida meal respectively. The diets were tested in triplicate groups of hybrid catfish (140 fish per tank, 77.7±0.25 g mean weight) for nine weeks.

Table 5. Formulation of the experimental diets and proximate composition.

Ingredients (%)	CTRL	CN10	CN20
Candida meal	0	10.0	20.0
Wheat meal	28.6	28.6	30.6

Soyabean meal	26.0	20.0	10.0
Wheat gluten	17.0	13.0	14.0
Soybean protein concentrate	9.0	7.0	3.0
Hydrolyzed feather meal	6.0	8.0	8.0
Fish meal (70% protein)	5.0	5.0	5.0
Poultry oil	3.0	3.0	4.0
Fish oil	3.0	3.0	3.0
Vitamin & Mineral premix	1.0	1.0	1.0
Monocalcium phosphate	1.4	1.4	1.4
Proximate composition (% as fed)			
Moisture	7.7	7.5	7.8
Crude protein	44.5	44.9	43.4
Crude fat	8.6	9.5	11.1
Crude fiber	1.9	1.7	1.2
Crude ash	5.1	6.3	6.6
NFE	32.2	30.1	29.9
Gross energy (MJ/kg)	19.8	20.0	20.2

Table 6. Amino acids composition of the experimental diets.

Amino acids (g/kg)	CTRL	CN10	CN20
Alanine	17.6	18.8	18.9
Arginine	25.7	25.4	23.2
Aspartic acid	34.6	34.5	31.0
Cysteine	8.7	9.0	8.6
Glutamic acid	106.3	96.0	93.1
Glycine	20.5	21.4	20.7
Histidine	8.9	8.2	7.6
Iso-Leucine	18.8	19.1	18.3
Leucine	33.9	33.6	32.2
Lysine	18.6	18.7	18.3
Methionine	6.9	6.7	6.5
Phenylalanine	22.4	21.5	20.3
Serine	25.7	26.8	26.0
Threonine	15.5	16.6	16.6
Valine	21.5	23.0	22.9

Fish were fed three times a day until apparent satiation and the growth performance was checked at the end of the trial. As a part of the experimental design, a multidisciplinary pool of analysis was performed to assess if the inclusion of the *C. utilis* meal in the feed, might influence feed utilization

and colour intensity of the flesh, which was measured with a Chroma-Meter (Minolta CR-400 cat. 1878-209). Additionally, multiple samples of faeces, mucus, water from the system and biofilter were collected for the characterization of the microbiota at the beginning of the trial as a screening of the stock population, and at the end of the growth phase to evaluate any potential influence of the test ingredient.

The water quality represented a challenge during the trial because of the high feed demand of this species. Daily water quality tests of ammonia, nitrite, and nitrate as well as physicochemical parameters such us temperature, dissolved oxygen, pH, ORP, salinity and water hardness and routinary veterinary checks were performed to ensure a stable and liveable environment. The collection and characterization of the sludge as part of the Waste2Value product was performed following the procedure described in D1.6 – Valorisation of by-product and sludge.

3.4.1 Microbiota analysis

The microbiota analysis was conducted on the distal part of the gut and on skin samples as well as sludge, water tank, inlet water and biofilter of the system. Samples were collected at the beginning (T0) and at the end (TF) of the experiment. For each timepoint, 150ml of water from the inlet tank and raw sludge were collected in triplicate from each tank. A total of 18 fish at T0, which represented the baseline and 9 fish per diet at TF (CTRL = Control; CN10 = 10% candida inclusion; CN20 = 20% candida inclusion) were sampled to collect skin mucus by swabbing and then dissected to obtain samples of the distal gut. The water samples were filtered individually with nitrocellulose filters (0.2µm pore size) and the swab and gut tissue stored in DNA/RNA shield buffer (Zymobiomics, Irivine, Canada). A total of 141 samples (45 gut, 45 skin, 12 sludge, 12 water tank, 12 inlet water, 6 biofilter and 3 water biofilter) were shipped to Leitat Technological Center for the DNA extraction. Further processing methods and bioinformatics analysis are provided in section 2.2.1.

3.5 Demonstration Methodology (Catfish Expt. 2)

The trial was conducted in a recirculating aquaculture system of 12x 500L rectangular tanks (AquaCirc GRP Fiberglass) equipped with UV disinfection (TMC Pro Pond 110), drum filter (BaseDrum 15, Ratz), protein skimmer (Tornado, Aquosis) and continuously monitored through an OxyGuard Pacific unit (DO, T, pH, ORP).

The KPIs description stated a target stocking density of approximately 100kg/m3 and a final fish size of 400g mean weight for the demonstration. A commercial feed provided by AAR (Aller Claria float) was tested in triplicate groups of selected and control line of hybrid catfish (110 fish per tank, 73.47 ± 0.07 g mean weight) for 8 weeks. The declared nutrient composition of the diet is showed in Table 7.

Table 7. Declared composition of the test diet.

Declared nutrient composition (%)	Aller Claria float, 3mm
Crude protein	45.0
Crude fat	12.0
NFE	26.4
Ash	6.0
Fibre	2.6
Phosphorus	1.0
Gross energy (MJ/kg)	20.3
Digestible energy (MJ/kg)	17.0

Fish were fed by hand to apparent satiation three times a day and growth performance was compared at the end of the trial. The water quality profile represented one of the KPIs, therefore daily checks of major parameters (T°, pH, DO, S, ORP, water hardness, NH_4, NO_2, NO_3) were performed in order to maintain the environmental condition within ranges. Additionally, multiple health screenings were conducted to guarantee optimal fish welfare.

3.6 Demonstration Results (Catfish Expt. 1)

The demonstration was conducted for a total of nine weeks, at the end of which fish were sampled to assess growth performance, fillet colour intensity, characterization of microbiota of the gut, skin, water from the system and biofilter. The demonstration KPI´s listed in chapter 3.2 were partially achieved. Specifically, the experimental diets were well accepted by the fish and no sign of low palatability was recorded. End of trial growth performance, biometric and morphometric indexes and body colour intensity are shown in Table 8 and Figure 7.

Table 8. Growth performance and bio-morphometric indexes of hybrid catfish after nine weeks of trial.

Growth performance	CTRL	CN10	CN20
Final body weight (g)	418.10 ± 8.29	421.42 ± 9.83	423.91 ± 10.68
Weight gain (g)	340.35 ± 8.10	343.73 ± 9.95	346.16 ± 10.33
Feed conversion ratio (FCR)	0.90 ± 0.01	0.89 ± 0.02	0.90 ± 0.01
Specific growth rate (SGR %)	2.59 ± 0.03	2.60 ± 0.04	2.61 ± 0.03
Protein efficiency ratio (PER)	2.64 ± 0.04	2.67 ± 0.01	2.67 ± 0.01
Specific feeding rate (SFR %)	2.34 ± 0.05	2.31 ± 0.02	2.34 ± 0.02
Survival rate (%)	99.0 ± 1.09	98.8 ± 1.49	98.3 ± 0.41
Biometric and Morphometric indexes			
Condition factor, K (g/cm^3)%	0.79 ± 0.01	0.75 ± 0.08	0.79 ± 0.03
Viscerosomatic index (VSI%)	7.68 ± 0.98	8.38 ± 0.71	8.90 ± 0.06
Hepatosomatic index (HSI%)	0.95 ± 0.03	0.96 ± 0.14	0.94 ± 0.04

Spleen somatic index (SSI%)	0.08 ± 0.02	0.09 ± 0.01	0.07 ± 0.01
Fillet Yield (%)	46.17 ± 1.77	43.44 ± 3.15	42.73 ± 0.88
Lightness (L*)	46.78 ± 0.80	46.79 ± 1.74	44.54 ± 0.89
Red/green coordinate (a*)	0.17^{a} ± 0.17	1.51^{ab} ± 1.47	3.00^{b} ± 1.17
Yellow/blue coordinate (b*)	6.82 ± 0.29	7.00 ± 1.18	7.33 ± 0.09

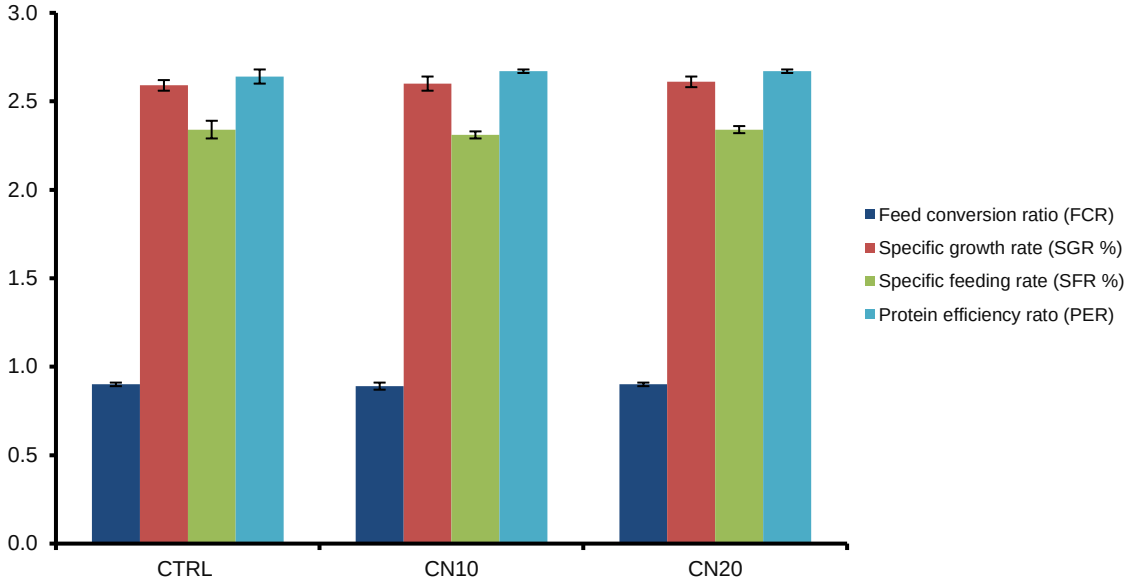

Figure 7. Growth performance of hybrid catfish after nine weeks of trial

The fish fed the experimental diets showed comparable performance ($p>0.05$) to the fish fed the control diet, and there is no evidence that candida meal inclusion reduces performance. Fish fed 20% inclusion of candida meal showed slightly higher specific growth rates (2.61 ± 0.03%) compared to the fish fed the control diet (2.61 ± 0.03%) and 10% inclusion diet (2.60 ± 0.04%). However, the FCR was lower in fish fed the 10% inclusion of candida meal (0.89 ± 0.02) than the control diet and 20% inclusion of candida meal (0.90 ± 0.01).

The overall survival rate was >98% indicating an absence of negative effect of the treatments. Finally, the biometric and morphometric indexes revealed no significant difference among the treatments, while the body colour intensity showed a significant higher value of a* (red/green coordinate) of the fish fed the 20% inclusion compared to the fish fed the control diet.

The target stocking density was not fully achieved in any of the treatments as the average value ranged between 89.2 ± 0.8kg/m^3. The water quality management even if in a pilot scale system was challenging due to the target stocking density of approximately 100kg/m^3 and the feeding method adopted. The amount of nutrients generated due to feeding until apparent satiation is showed in Figure 8. Despite several spikes of nitrogenous compounds (NH_4 up to 8mg/l; NO_2 up to 40mg/l and

NO_3 up to 1000mg/l), the fish showed no sign of stress, and the feeding behaviour was not negatively affected. As reported in literature, the tolerated ammonium (NH_4) level in RAS is between 15 and 19.7mg/l (Bovendeur et al 1987; Haylor 1989; Peteri et al 1989; Pruszyñski & Pistelok 1999). However, to maintain an adequate environment, several water exchanges were performed in order to reduce the concentration of such chemicals and as such the KPI was only partially met.

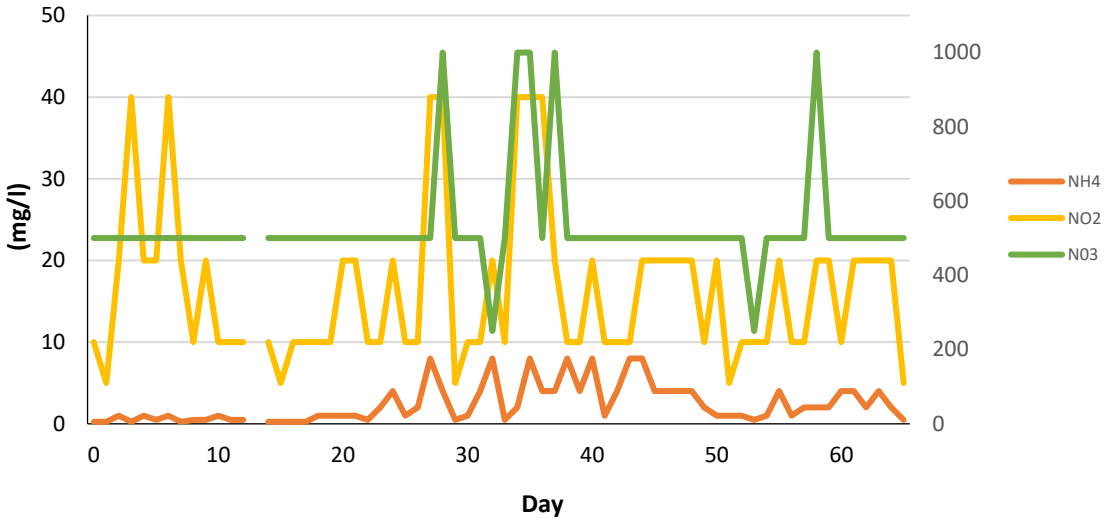

Figure 8. Water quality screening during the growth phase.

The results of the sludge and water characterization of the treatment with 10% and 20% of inclusion of candida meal are showed in Table 9.

Table 9. Characterization of the waste produced for each experimental treatment.

Parameter	CN10		CN20	
	Concentrated sludge	Water	Concentrated sludge	Water
pH [(1)]	5.02±0.0	6.2	6.66±0.0	5.7
Salinity (ppt)	n.a.	2.2	n.a.	2.3
Moisture (%) (gravimetry)	83.29±0.13	n.a.	84.46±0.30	n.a.
Dry matter (%) (gravimetry)	16.71±0.13	n.a.	15.54±0.30	n.a.
TKN (mg/kg or L) (kjeldahl method)	5794±199	n.a.	6286±226	n.a.
NH_4^+-N (mg/kg or L) (Ion chromatography)	520	n.a.	450	n.a.
NO_2^- – N (mg/kg or L) (Ion chromatography)	< 0.3	n.a.	< 0.3	n.a.
NO_3^- – N (mg/kg or L) (Ion chromatography)	< 3.5	117[(6)]	6.5	136[(6)]
Total N (mg/kg or L)	5794[(2)]	106[(5)]	6293[(2)]	128[(5)]
Organic N (mg/kg or L) [(3)]	5274	n.a.	5836	n.a.
Inorganic N (mg/kg or L) [(4)]	520	n.a.	456	n.a.
PO_4^{3-}-P (mg/kg or L) [(6)]	n.a.	25	n.a.	33

(1) pH, sample preparation: the solid sample was mixed with distilled water (1:2.5), centrifuged at 10.000 rpm for 15 min. Next, the supernatant was analysed.

(2) Total Nitrogen calculated as the sum of TKN + NO_2^- -N + NO_3^--N.
(3) Organic Nitrogen calculated as the difference between TKN and NH_4^+-N.
(4) Inorganic Nitrogen calculated as the sum of NH_4^+-N + NO_2^- -N + NO_3^--N.
(5) Analysed with Photometer PF-12^{Plus} and NANNOCOLOR ® total Nitrogen TN_b kit
(6) Analysed with Photometer PF-12^{Plus} and VISOCOLOR ECO Nitrate test kit.
(7) Analysed with Photometer PF-12^{Plus} and VISOCOLOR ECO Phosphate test kit.

3.6.1 Microbiota analysis

3.6.1.1 General analysis

At T0, the lowest alpha diversity values within the system were observed in the bacterial communities of gut and sludge of African catfish, with no statistical differences among them ($p = 0.6$) (Figure 9 top). Samples of skin and water from the tank showed significantly higher alpha diversity values of the bacterial communities compared to samples of sludge and gut, but lower than what was found in samples of water from the inlet ($p < 0.05$) and biofilter ($p < 0.05$). The characterization of the biofilter samples showed the highest bacterial diversity values. At beta diversity, the multivariate analysis of bacterial communities within the system, showed statistical differences between the gut and the rest of the samples (pairwise.PERMANOVA, $p < 0.05$). Albeit the skin showed a less clear aggrupation of samples than the ones from the gut (Figure 9 bottom), statistical differences were observed between the bacterial community of the skin and the rest of the samples, except for the water sampled from the tank (pairwise.PERMANOVA, $p = 0.16$). Finally, sludge, water from the tank and water from the inlet showed no statistical differences (pairwise.PERMANOVA, $p = 0.1$), being all statistically different compared to the biofilter. At genus level, the predominant group was represented by Cetobacterium in gut, skin, sludge, water from the tank and from the inlet (mean abundance: Gut = 75% ±10%, Skin = 58% ± 11%, Sludge = 87% ±2%, Water from tank = 64% ±3%, Inlet = 54% ±2%). Additionally, the genus *Romboustia,* which showed its highest relative abundance in water from the tank, represented the second most abundant genus. Regarding the samples of the biofilter, *Nitrospira*, *Luteolibacter*, *Rhodobacter* and *Epulopiscium* were the most dominant genera.

3.6.1.2 Diet impacts on the fish microbiota

After 9 weeks of experiment (TF), several differences were observed in the bacterial communities isolated from each tissue, when compared either to the beginning of the experiment and between diets. Alpha diversity in gut bacterial communities was significantly increased in all diets comparing with the findings of T0, but no differences were observed between diets at TF (mean values: T0 = 1.3, CTRL.TF = 1.8, CN10.TF = 1.5, CN20.TF = 1.6). Regarding the skin samples, the bacterial communities showed a significant increase only in fish fed CN20 compared to the T0 (mean values: T0 = 2.1, CTRL.TF = 2.2, CN10.TF = 2.1, CN20.TF = 2.5). At beta diversity, no statistical differences were observed in the gut bacterial communities among diets and when compared with T0 (pairwise.PERMANOVA, $p > 0.05$). However, the skin bacterial communities of fish fed CN20 evolved significantly during the

experiment, showing significant differences when compared to the T0 and the rest of diets. (pairwise.PERMANOVA, $p < 0.05$)

Analysing the samples at phylum level, the significant higher alpha diversity values in bacterial communities at the end of experiment may be mediated by a slight mean dominance decrease of Fusobacteria in the CTRL or Firmicutes and Bacteroidetes in CN20 (Figure 9 top). Nevertheless, Fusobacteria, Firmicutes and Bacteroidetes are the most dominant phyla in the African catfish gut regardless the diet used. In terms of fish skin, similar distribution to the gut can be observed but with a more relevant presence of Proteobacteria. This phylum increased significantly in African catfish fed with CN20, compared to the other diets.

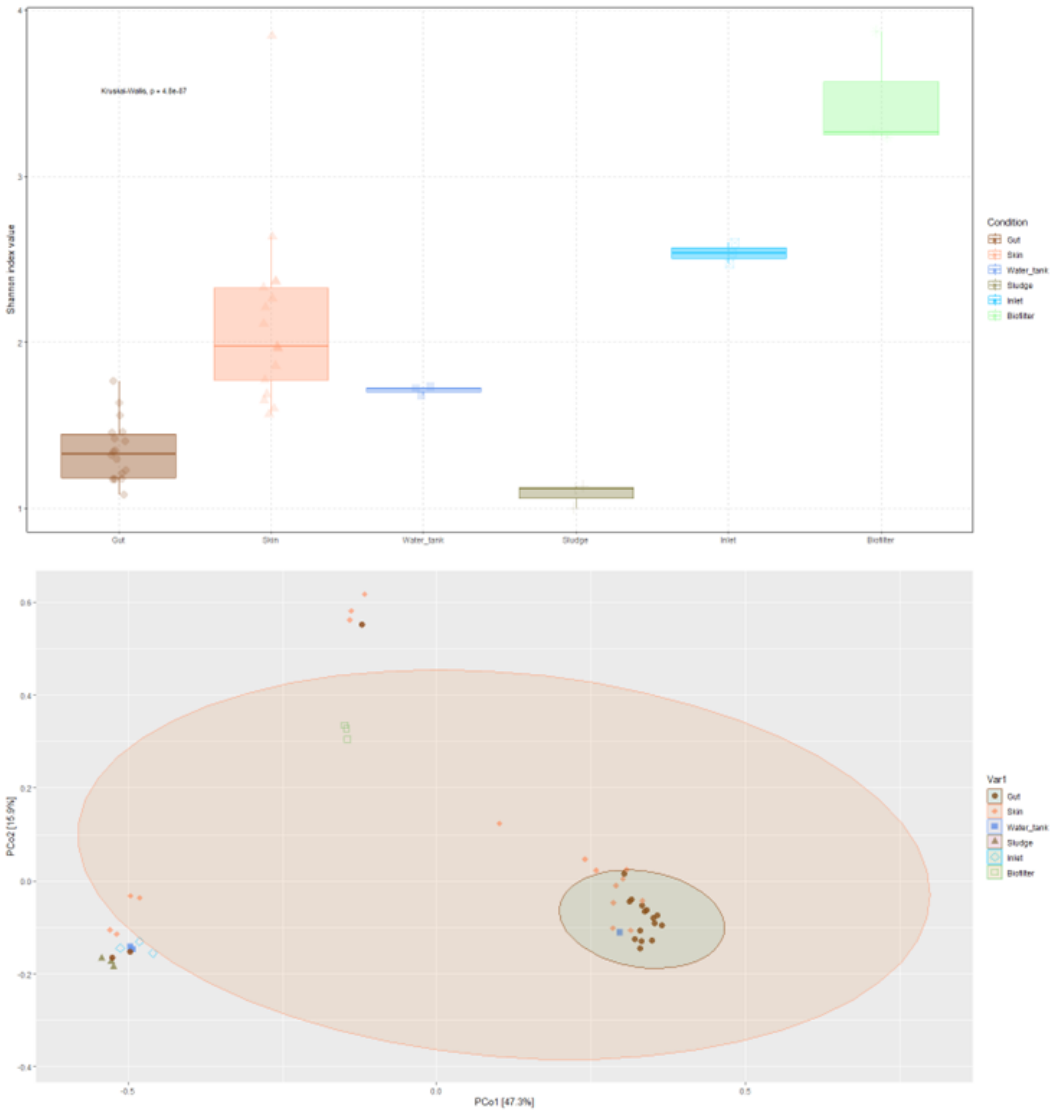

Figure 9. Alpha and beta diversity at the T0 of IFN03. A) Distribution of the Shannon index measurements is presented by sample type Kruskall-Wallis test was performed to assess the statistical significance of the observed distribution differences (p value <0.05)) B) Scores plot for the PCoA performed on the bray curtis beta-diversity index for the different samples studied at T0. Variance explained by each coordinate is indicated between parentheses in the corresponding axis.

At genus level, a significant increase of *Clostridium sensu stricto 1* was observed within the bacterial communities in both skin and gut samples of fish fed CTRL diet, compared to T0 and CN20 (Figure 10 bottom). Besides, a significantly higher relative abundance of *Cetobacterium* was observed in the gut of CN10 and CN20 diets compared to the CTRL at the end of the experiment. In terms of skin, the bacterial communities of fish under CN20 diet experienced a significantly increased of *Methylibum* dominance and a decreased of *Cetobacterium*, *Roumboustia*, *Epulopiscium* and unidentified genera form *Barnesiellaceae* family compared to the other diets and the T0.

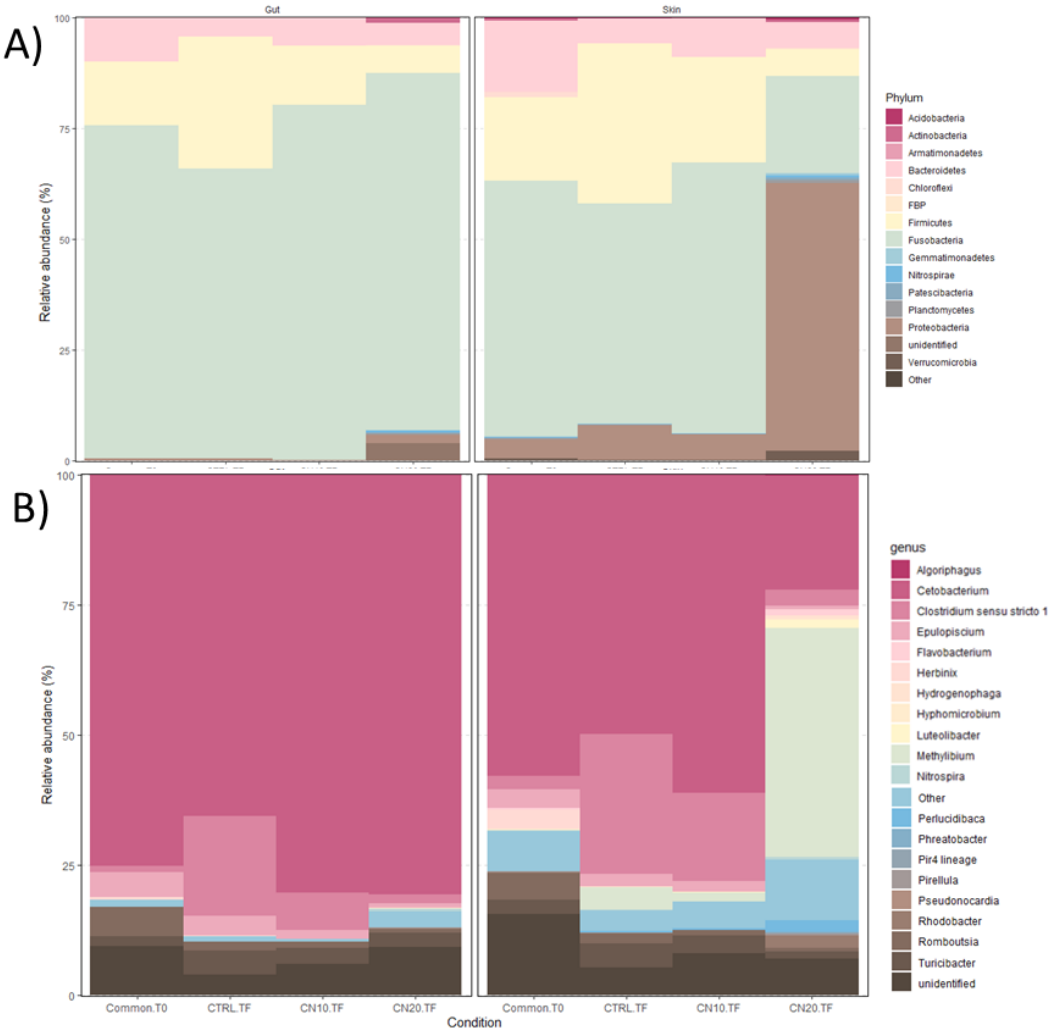

Figure 10. Phylum and genus diversity in African catfish gut and skin samples. The microbiota composition is presented in bars displaying the mean relative abundance of each phylum in a different colour presented per condition. A) Distribution of the 15 most abundant phyla of the gut and skin microbiota, stratified by dietary regime and sampling timepoint. B) Distribution of the 20 most abundant genera of the gut and skin microbiota, stratified by dietary regime and sampling timepoint.

3.6.2 Growth performance

The demonstration lasted for eight weeks, at the end of which tank biomass was determined and growth performance (FCR, SGR, SFR, and survival) were calculated. The demonstration KPIs listed in chapter 3.3 were partially achieved. Specifically, the comparison of growth performance and survival

between the selected and control line did not show a significant difference as shown in Table 10. The final mean weight and SGR were slightly higher in the control line (400.84 ± 19.21g and 3.27 ± 0.07%) than in the selected line (460.19 ±11.25g and 3.21 ± 0.04%), while the SFR and survival rate were higher in the selected line (2.74 ± 0.04% and 98.18 ± 0.91%) compared to the control line (2.71 ± 0.04% and 95.45 ± 2.41%). Both treatments achieved the stocking density of 100kg/m^3 fully, with an average value ranging between 101.94 ± 2.59kg/m^3.

Table 10. Growth performance of hybrid catfish after eight weeks of trial.

Growth performance	CTRL	Select
Final body weight (g)	474.33 ± 19.21	460.19 ± 11.25
Weight gain (g)	400.84 ± 19.21	386.75 ± 11.23
Feed conversion ratio (FCR)	0.83 ± 0.03	0.85 ± 0.03
Specific growth rate (SGR %)	3.27 ± 0.07	3.21 ± 0.04
Specific feeding rate (SFR %)	2.71 ± 0.04	2.74 ± 0.04
Survival rate (%)	95.45 ± 2.41	98.18 ± 0.91

The water quality profile reflected the first experiment where the high feed demand caused a deterioration of the environmental parameters.

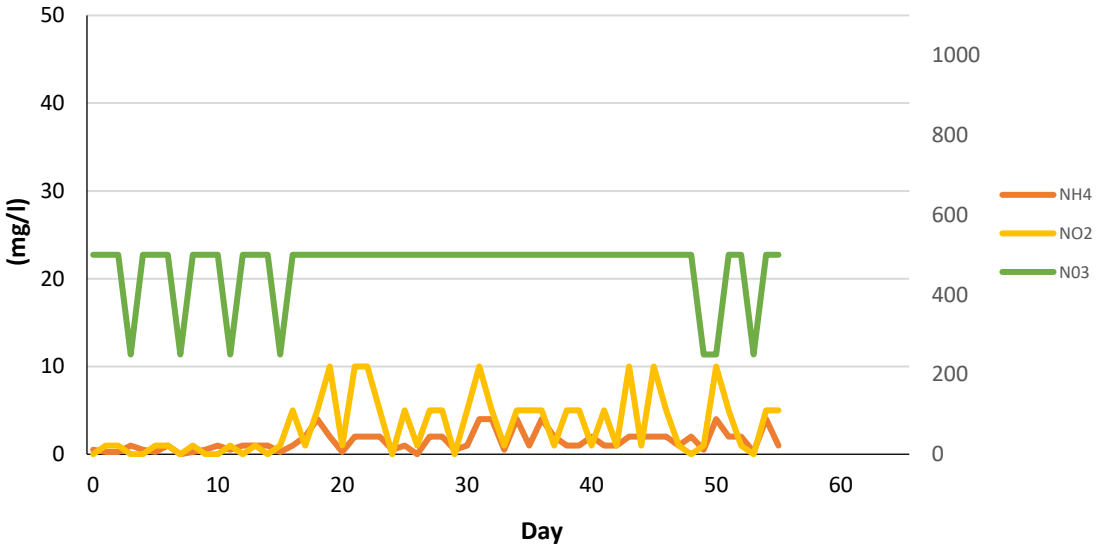

Figure 11. Water quality screening during the growth phase.

Despite several spikes of nitrogenous compounds (NH_4 up to 4mg/l; NO_2 up to 10mg/l and NO_3 stable at 500mg/l for a relatively long period), the fish showed no signs of stress, and the feeding behaviour was not negatively affected. However, to maintain an adequate environment, several water exchanges were performed to lower the concentration of such chemicals, hence, the KPI was to a certain extent not achieved.

3.7 Conclusions, Recommendation for Application of the Results in Industry

The aquaculture industry is continuously evolving, and as demonstrated in both experiments performed at ABT, reducing dependence on conventional raw materials such as fishmeal and plant-based products, as well as enhancing feed efficiency and utilization through genetic selection, are of paramount importance. Specifically, the first trial demonstrates an opportunity to provide a novel and sustainable protein alternative to the industry, which could help to reduce environmental pressure on both marine and land-based stocks. *C. utilis*, which has a high protein content (55%) and a favourable amino acid profile (including lysine, threonine, histidine, and arginine) as well as B-complex vitamins, could represent a viable alternative. Although not statistically significant, the results showed that reducing plant-based ingredients in test diets by approximately 14% and 28% resulted in similar or slightly improved growth rates compared to the control diet. Specifically, the FCR of fish fed the control diet was 0.90 ± 0.01, the same as fish fed at 20% inclusion of candida meal, while fish fed at 10% inclusion of candida meal showed a lower FCR, 0.89 ± 0.02. Concerning the specific growth rate, fish fed the control diet showed the lowest value among the treatments (2.59 ± 0.03%) compared to 2.60 ± 0.04% and 2.61 ± 0.03% of fish fed CN10 and CN20 respectively. The good palatability of the yeast-based diets tested contrasts with previous research on African catfish fingerlings, where 30% yeast inclusion worsened feed intake due to low diet palatability (Elesho et al., 2021). Further investigation is necessary to determine the potential use of *C. utilis* as a sustainable and cost-effective protein source in the aquaculture industry. However, the results from this preliminary study suggest that *C. utilis* has the potential to partially replace plant-based ingredients in catfish diets without compromising growth performance. This could lead to a reduction in the demand for traditional raw materials such as fishmeal and soybean meal, which could help to alleviate the pressure on marine and land-based stocks and promote a more sustainable aquaculture industry.

The multidisciplinary holistic approach has provided valuable information about the physiology, feed utilization, and microbial community of hybrid catfish fed with different inclusions of *C. utilis*.

In terms of the microbiota analysis, the inclusion of candida at 10% and 20% did not significantly change the bacterial diversity within the gut. However, it is recommended to keep exploring the "healthy" status of the African catfish microbiota in both gut and skin, when raw materials substitution is carried out. The significant decrease of *Clostridium senso stricto 1* in the gut and the significant increase of *Methylibium* in the skin of fish fed CN20 diet, calls out for further studies on their role in fish performance.

In the second trial, the two hybrid catfish lines supplied by a project partner (GYE and MATE University, Hungary) were produced as follow:

- Selected line: eggs of *Clarias gariepinus* females selected for higher growth using a low fishmeal content diet, fertilized with non-selecte *Heterobrancus longiphilis* sperm
- Control line: eggs of *Clarias gariepinus* females non-selected for higher growth fed with normal diet, fertilized with non-selected *Heterobrancus longiphilis* sperm

The hybridization of these two species, as confirmed by the supplier, improves the growth rate, reduces cannibalism through more homogeneous growth, and delays sexual maturation over the production cycle, allowing the fish to utilise energy solely for growth. However, the genetic selection of the female did not result in the enhancement of growth performance and feed utilisation as predicted.

Additionally, as stated in both experiments, the water quality management must be considered of paramount importance, because the accumulation of nitrogenous compounds in the water might have a negative long-term effect on general health status of the fish. From an industrial perspective, the accumulation of such pollutants could be mitigated with a more appropriate design and sizing of the biofilter and the use of innovative nanomaterial to enhance the bacterial colonisation. Moreover, the results of the waste characterisation of the first experiment, showed the potential for the re-use and valorisation under a circular approach and towards zero waste, which could also reduce the nutrient load of the system and represent a secondary profit for the industry if properly managed.

Further research is needed to define the optimal inclusion level of Candida meal, the replacement of plant-based ingredients, and a market analysis of the production costs of such ingredients at an industrial scale to ensure the sustainability of yeast production as part of aquafeed, not only for catfish but for many high-value farmed species. With regard to the genetic selection, the evaluation of performance should be tested in a grow out trial up to commercial size, where the possibility of a higher growth might be demonstrated. Finally, the growing interest in catfish farming worldwide and the development of related technology make it a valuable candidate for further investigation.

3.8 Dissemination of the Demonstration

- Webinar event: African catfish in SmartRAS virtual demo event. A virtual tour of the facility and the SmartRAS technology used to perform the catfish trial was showed in a hybrid event hosted at the ABT facility in Malta on the 5th of December 2022. The virtual demonstration explained how their demonstration trials are helping to advance SmartRAS, and the New Feeds and Breeding program of the project https://ifishienci.eu/media/events/.
- Hungarian (MATE) event: The results of the demonstration were presented at the Fisheries and Angler Specialists Meeting in Gödöllő, Hungary on 26th. Of January 2023. Over 200 participants from aquaculture companies attended the event. Six lectures during the meeting

were dedicated to presenting and discussing the potential applications of the iFishIENCi project's results. Informative talks were delivered by iFishIENCi partners, including NORCE, HCMR, ABT, MATE-AKI, GYE (Bajcshal), and VT. The lectures covered a wide range of topics, such as the examination of feed additives, the African catfish selection breeding program, and tests of hybrid African catfish conducted by ABT in RAS (Recirculating Aquaculture Systems). All the presentations emphasized the practical applications of the project's findings in the industry and highlighted the available support for local farmers to effectively utilize these results.

https://uni-mate.hu/en/h%C3%ADr/-/content-viewer/a-mate-n-tal%C3%A1lkoztak-az-akvakult%C3%BAra-hazai-szakemberei/20123

https://szakmainap.e-lapozo.hu/lapozhato/6/

- Editorial (international Aquafeed): the catfish trial was advertised in the April 2023 edition of International Aquafeed magazine “Catfish, a more sustainable approach to feeding” where ABT published the latest results of the demonstration, which dwells into more sustainable aquaculture practise on feeding catfish.

  Link: https://issuu.com/international_aquafeed/docs/iaf2304_web
- Oral presentation: G.M. Cusimano, JC. Chiang, N.E. Panasiak, F.M. Robinson, D. O´Brien, T. Bardócz*. (2023, September 18-21). iFishIENCi Feed and Feeding technology development in RAS. [Conference presentation]. Aquaculture Europe conference, Vienna, Austria.
- In the summer of 2022, nine Aquaculture Master level students and their 3 supervisors from the University of MATE (Hungary) participated in a professional workshop and training course at ABT, Malta. The agenda included project presentations, lecture on freshwater intensive technologies, an introduction to SmartRAS engineering, onsite training on monitoring and control systems, presentation of iFishIENCi RAS research trials on trout, presentation of Maltese Aquaculture with insights on circularity and digitalisation elements from iFishIENCi. Since this program, several of these students have graduated and are now working in the Hungarian industry where they are applying their knowledge to ensure the digital shift of the industry.

4 Atlantic Salmon at AquaBioTech Group

4.1 Key Performance Indicators

The development of new technologies has transformed aquaculture from a traditional labour-intensive farming to a mechanized production with a gradual evolvement to automated systems. The labour-intensive model mainly relies on human experience, with high labour cost. RAS, on the other hand, has greatly reduced but at the same time changed the labour demands, and production is often increased. However, automated production requires high skilled workers, affecting cost-effectiveness and resources such as water and feed are still impacted (Engle et al., 2019). The results are the decreasing labour availability and increasing demand of aquaculture products which necessitate of a new intelligent aquaculture model. The development of big data, AI, 5G networks and robot technologies makes intelligent aquaculture possible. AI applied to aquaculture employs remote control or robotics control of the aquaculture facilities, equipment, and machinery to complete all production and management operations. The integration of modern information technology and the whole industrial chain of aquaculture production, operation, management, and service represents a new business form of modern aquaculture development. Intelligent equipment driven by AI could solve labour force limitations and mitigate environmental and resource problems caused by aquaculture. AI in aquaculture involves aspects like:

- Monitoring and control: AI can be used to develop advanced monitoring systems that employ computer vision and machine learning algorithms to track and analyse fish behaviour, growth rates, feeding patterns, and health conditions. This data can help farmers optimize feeding regimes, detect diseases or stress early, and make informed decisions about water quality and environmental conditions.
- Feeding Optimization: AI algorithms can optimize feed formulation by considering multiple variables such as fish species, growth stage, environmental factors, and nutritional requirements. AI can analyse data from various sources, including genetic information, water quality parameters, and historical growth data, to create personalized feeding strategies that improve fish growth, reduce waste, and minimize environmental impact.
- Disease Detection and Management: AI-powered image recognition and machine learning models can identify specific diseases or abnormalities in fish by analysing images or videos. Early detection of diseases allows farmers to take prompt action, reducing the spread of infections and minimizing losses. AI can also assist in predicting disease outbreaks based on environmental conditions and historical data.

- Water Quality Management: AI can analyse real-time data from sensors and monitoring systems to predict and optimize water quality parameters such as dissolved oxygen levels, temperature, pH, and ammonia concentration. By continuously monitoring and adjusting these parameters, AI helps maintain optimal conditions for fish growth and health.
- Predictive Analytics: AI can analyse historical data, environmental factors, and other relevant variables to provide predictive insights. This can help farmers make informed decisions on stocking densities, production planning, and market trends, ultimately improving profitability and sustainability.

In this context, the iFishIENCi project has been targeting the improvement of production control and management for all fish aquaculture systems, aiming to maximise feed utilisation through smart feeding, providing continuous monitoring of fish behaviour, health, and welfare towards a Biology Online Steering System (iBOSS). The iBOSS deployment in RAS called Smart-RAS has been demonstrated at the ABT facility in Malta. The demonstration targeted Atlantic salmon of approximately 14g for a period of 16 weeks.

The key performance indicators (KPIs) for this demonstration were as follow:

- Detection and tracking of fish at hatchery size.
- Feed behaviour algorithms can evaluate fish activity at different feeding regimes using a scoring system of 0 to 2.
- Feed behaviour algorithms can evaluate different feeding regimes using behaviour of Atlantic salmon.
- Smart feeders can be regulated through iBOSS.
- iBOSS was successfully used to display data from SmartRAS and FishMet.
- FishMET can predict body mass based on feed intake and temperature.

4.2 Demonstration Methodology

The demonstration was divided into three phases, each serving a specific purpose.

- Phase 1: development and calibration of equipment for real-time detection, tracking, and monitoring of fish.
- Phase 2: development and calibration of a scoring system to assess fish activity during feeding events.
- Phase 3: comparison of the growth performance of fish subjected to two different automatic feeding strategies.

The trial was conducted in a recirculating aquaculture system consisting of 12 circular tanks, each with a capacity of 650 liters. The system was equipped with UV disinfection (UltraAqua MR1-220PP UV), a drum filter (Faivre DF 2-60 @40 microns Drum filter), a protein skimmer (AquaCirc CL10) and Biosystems (former TMC) LED lights and continuously monitored through an OxyGuard Pacific unit for measuring dissolved oxygen (DO), temperature, pH, and oxidation-reduction potential (ORP) and data was pushed to the iBOSS cloud. Due to the system design and positioning of the cameras, only two tanks were used during the different phases of the experiment. However, in phases 1 and 2, real-time fish detection and tracking, along with activity assessment under various feeding regimes, did not require specific replication due to ample data collected to calibrate the algorithm. On the contrary, phase 3 faced challenges with real time data analysis, hindering proper sample sizing and replication, resulting in weak statistical significance. This has been taken into consideration when interpreting the results of this trial and while further replication is needed to provide a complete demonstration of the technology, the initial findings show promise.

Phase 1 involved 400 juvenile Atlantic salmon (with a mean weight of 13.6g) allocated to one experimental tank for 10 weeks. The purpose was to calibrate the tools used for real-time fish detection, tracking, and monitoring. The fish were fed four times a day (at 9:00, 11:30, 14:00, and 16:00) with commercial feed until apparent satiation. The focus was on developing feeding habits in Atlantic salmon in response to reduced human activity.

In Phase 2, the same group of fish (N=400, with a mean weight of 39.88g) were automatically fed four times a day (as previously described) with a fixed feeding rate using commercial feed. The experiment lasted for three weeks, and the feeding rate was adjusted weekly as follows:

- Week 1: Feeding rate of 1.5% based on commercial guidelines from the feed supplier.
- Week 2: Feeding rate of 0.75% to stimulate fish activity under conditions of limited feed availability.
- Week 3: Feeding rate of 3% to achieve satiation.

The objective of this phase was to assess the activity of the fish under different feeding regimes and develop a scoring system ranging from 0 to 2 to measure it.

During phase 3 of the demonstration, the focus was on comparing two feeding strategies. The first strategy was based on a percentage of body weight, while the second strategy utilized the scoring system developed in phase 2. The data collected during this phase were also used to evaluate the growth performance. To conduct the experiment, we randomly allocated 200 fish with a mean weight of 60.58 ± 0.92g into two experimental tanks. The fish were fed four times a day using automatic feeders, as previously described.

In one of the tank, the automatic feeder was regulated by an algorithm that detected fish activity during feeding events, based on the scoring system developed in Phase 2. The quantity of feed delivered was estimated based on the recorded behaviour during the previous feeding, and a corresponding score was assigned. For example, if the computed feeding score during the first feeding was 1.4, it indicated that the fish were 1.4 times more active than usual, suggesting increased hunger. Consequently, the automatic feeder would deliver 1.4 times the quantity of feed during the next feeding event.

In the other experimental tank, feed was delivered based on 1.5% of the live biomass. The underlying principle behind the automatic regulation of feeding was that the more active the fish were during feeding, the hungrier they were. To convert the feeding score into the amount of feed, the automatic feeder was connected to OxyGuards Cobália Farm Management cloud for communication. Cobália was able to send statistics (such as the actual amount of feed delivered) from the Feeder controller to iBOSS and receive the new feeding score from iBOSS. The feeding score was then transmitted back to the Feeder controller.

An interface between iBOSS and the FishMet model was developed over internet using a secure web-based API. Starting from week 16, 2023, daily simulations were conducted using the actual (changeable) feeding schedule and following main simulation parameters: initial fish mass, temperature, simulation duration, food input rate, and feed pellet mass. The feeding schedule data represented the timestamps for the start and end of the feeder operation. The FishMet model produced output data including feed intake pattern, stomach and gut fullness, energy balance of the fish as well as instantaneous growth estimate. For the current demonstration, we focus on the fish growth prediction. Each week, the results of the fish growth predictions provided by the FishMet with the actual fish measurement data was compared. At the end of the trial period, a single simulation using the archived feeder data was conducted, temperatures, pellet mass and the fish weight at the start of the simulation period was used. This was done to check the overall longer-term reliability of the fish growth prediction by the FishMet model without regular weekly adjustment of the fish mass. The observed dynamics of the average fish mass over the weekly measurement are presented in Table 11.

Table 11. Average body mass over weekly measurements.

Measurement date	Average weight, g	Pellet mass, g
18/04/2023	38.71	0.006
26/04/2023	43.42	0.006
03/05/2023	45.06	0.025
10/05/2023	50.56	0.025
23/05/2023	60.58	0.025
30/05/2023	66.31	0.025

06/06/2023	72.37	0.025

4.3 Demonstration Results

The listed demonstration Key Performance Indicators (KPIs) in chapter 4.1 were only partially achieved. In phase 1, the fish detection and tracking were carried out using YOLOv5, a deep learning object detection model implemented in Python, and Norfair, a Python library that implements a tracker using Kalman filters. During phase 1, around 100 out of 400 fish were detected while at rest, and between 20 and 40 fish were detected during feeding. However, since the goal was to estimate the behaviour of the entire fish group, the percentage of undetected fish did not impose a limitation on the technology's development.

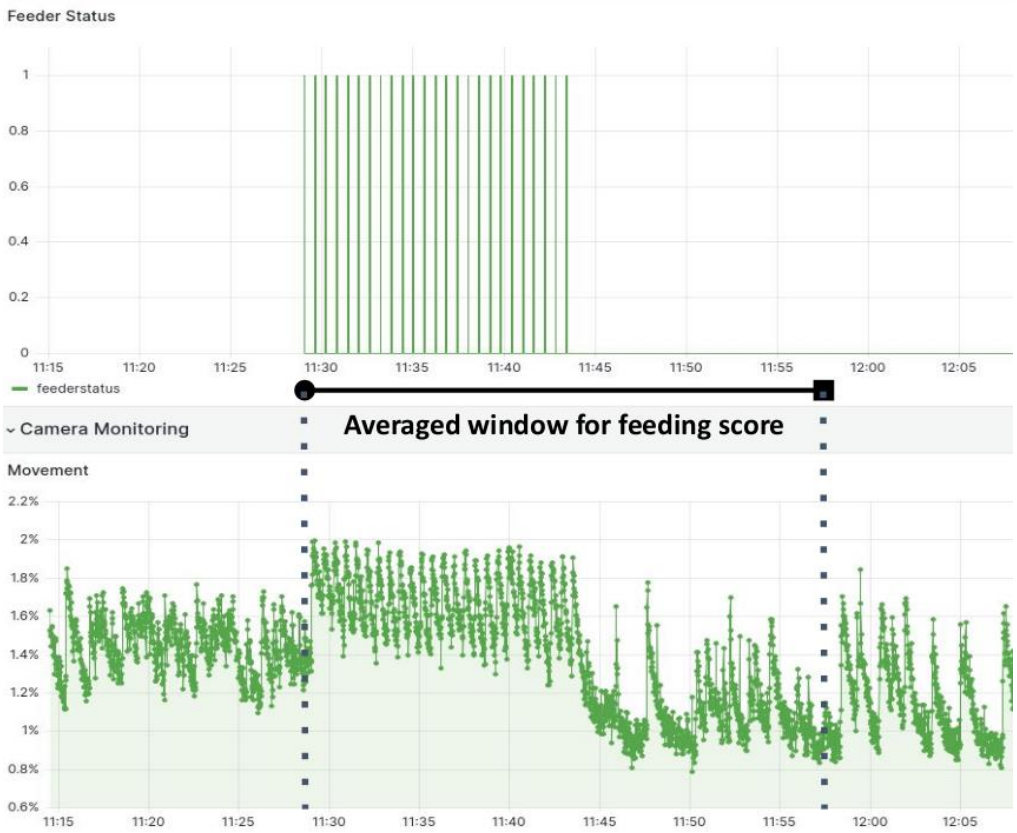

Figure 12. Feeder status and camera monitoring of the fish throughout the day.

Tracking the fish was crucial due to their high speed, reaching up to 3-5 times their body length per second. As a result, processing the tracking required a minimum of 12 frames per second (every other frame) to effectively track them, which posed a computational constraint. Real-time tracking of the fish throughout the day was not feasible due to the heavy computational load, and it would have been wasteful in terms of computational resources and power consumption. Therefore, the recording was set to begin 10 minutes prior to the feeding event and end 10 minutes after it.

In Phase 2, fish movement was estimated globally by comparing two successive frames of the video. The difference between the frames indicated the level of activity exhibited by the fish. The algorithm used for this purpose was relatively lightweight and capable of running in real-time throughout the day. The raw difference between successive frames was calibrated during the preliminary trial to ensure its value could be centred around 1. The measured movement was then averaged from the start of feeding until 15 minutes after it ended, taking into account the resting time after feeding. The resulting average value represented the feeding score.

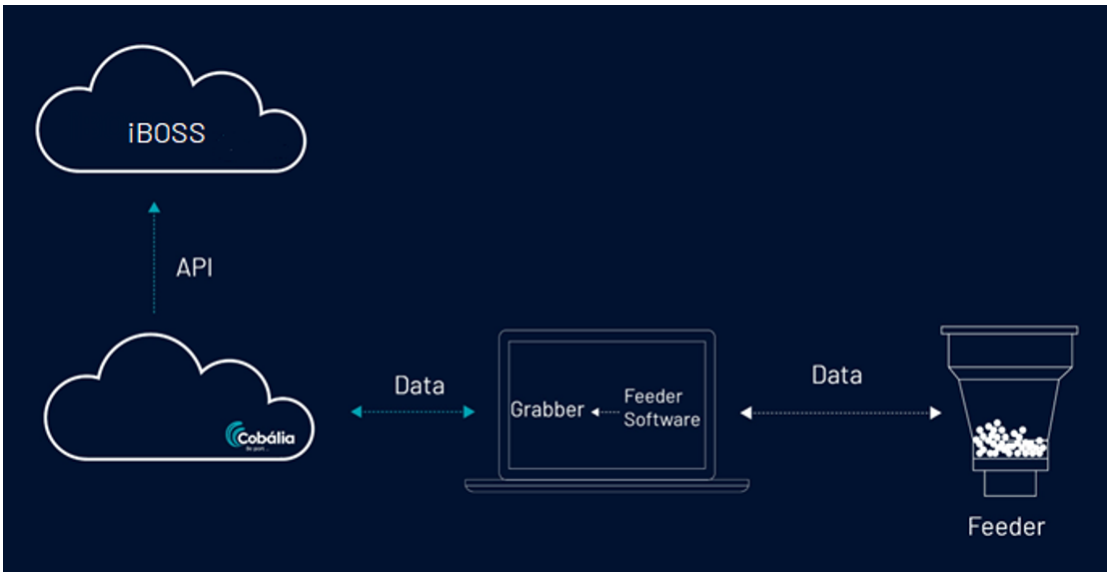

Figure 13. Automatic feeder installation and control access in the Smart RAS configuration.

Figure 12 displays the feeding pulses in the first image, while the movement curve is shown in the second image. This information is available in real-time, and the graph includes the time window used to define the feeding score. It is evident that the fish exhibit a peak of activity during each feeding pulse immediately following a feeding event, which concluded at 11:43. It took approximately five minutes for the fish to settle down after that initial peak. Subsequently, they displayed additional but less intense pulses, spaced out over time. Please note that the graph and real-time data provide a visual representation of the feeding pulses and fish movement patterns observed during the demonstration.

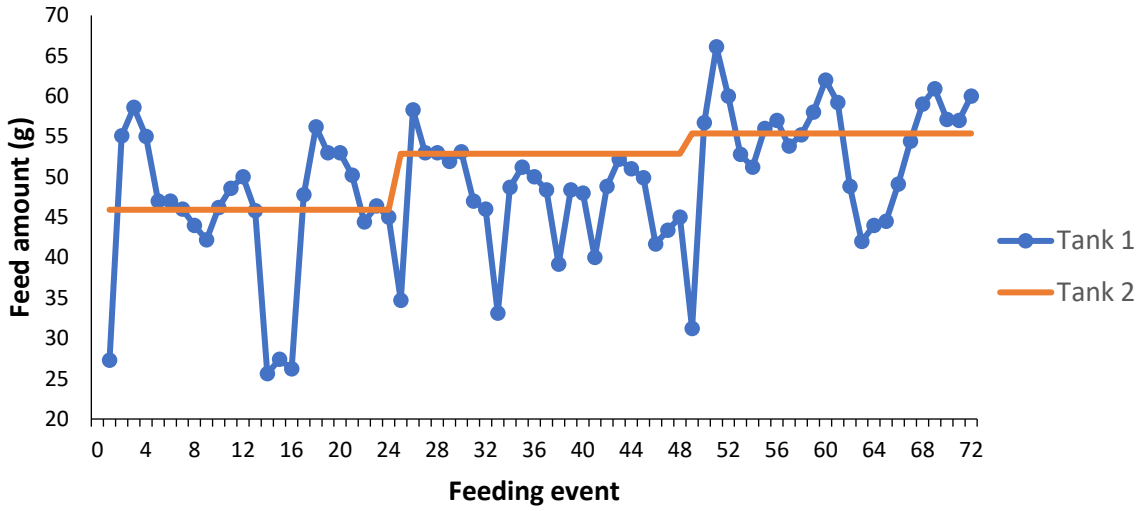

Figure 15. Feed amount provided per feeding event during the demonstration trial.

The implementation of the smart feeding system, based on the technology developed during the demonstration, was controlled using an OxyGuard Commander Feeder control. This control system operated the Avro-tec feeders to distribute the feed. The controller determined when to activate the feeder to distribute the planned amount of feed according to the scheduled feeding regime based on a predetermined daily increase.

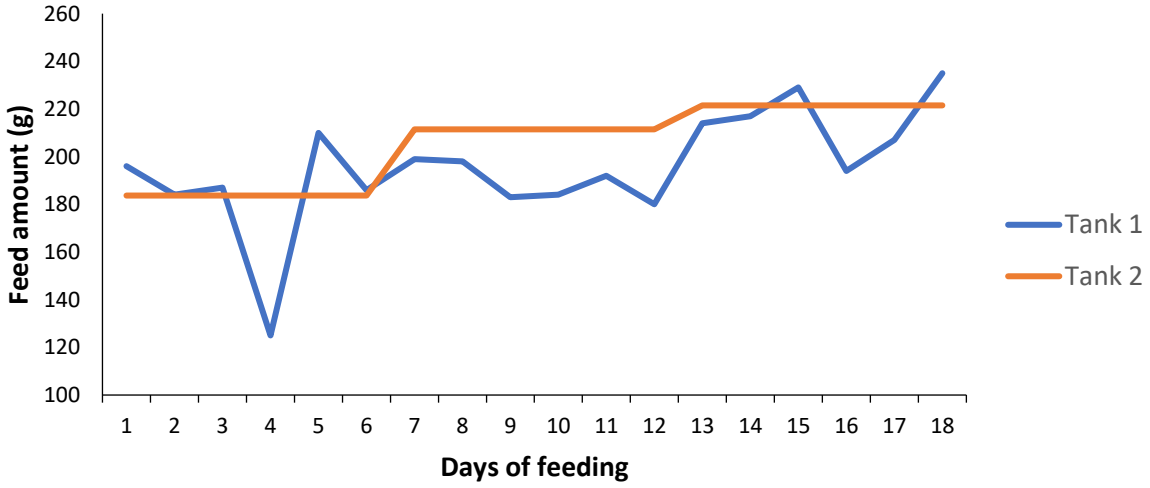

Figure 14. Feed amount provided per day during the demonstration trial.

During Phase 3, a smart feeding approach was introduced by establishing a connection between the feeder controller and the OxyGuard's Cobália Farm Management cloud. This connection enabled the analysis, adaptation, and communication of any changes in fish appetite. The measurement of appetite was derived from a computational analysis of the fish's behaviour recorded through the camera, as described in the previous section. The flow of data is illustrated in Figure 13.

The appetite measurement, also known as feeding score, was transmitted from iBOSS to the feeder controller and then processed by Cobália. The Cobália platform was responsible for calculating the appropriate feed amount based on behaviour observations and facilitating the exchange of information between iBOSS and the feeder controller.

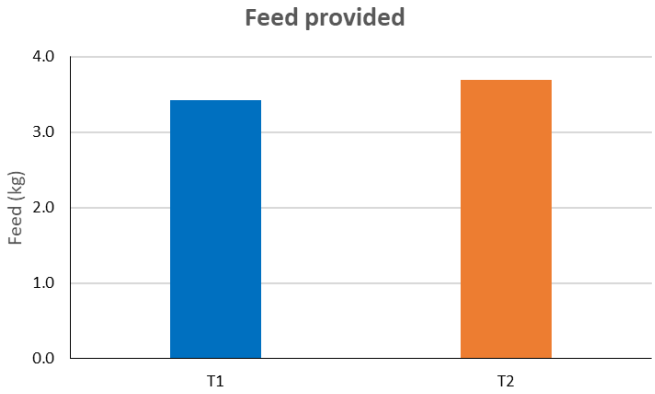

Figure 16. Total feed dispensed during the 3 weeks trial.

iBOSS successfully retrieved the feeder status and the camera monitoring data throughout the demonstration. Data were displayed in a dashboard and were used for the calibration of the feeding score. The feeding score was exposed on the API of the iBOSS, to be retrieved by the feeder, to adjust the amount of delivered food. This amount was also monitored by the iBOSS on an hourly basis.

During the demonstration, the movement monitoring and computation of the feeding score in real time after each feeding was calibrated on commercial regime, then monitored to make sure that the feeding score values are connected to the reality in the tank.

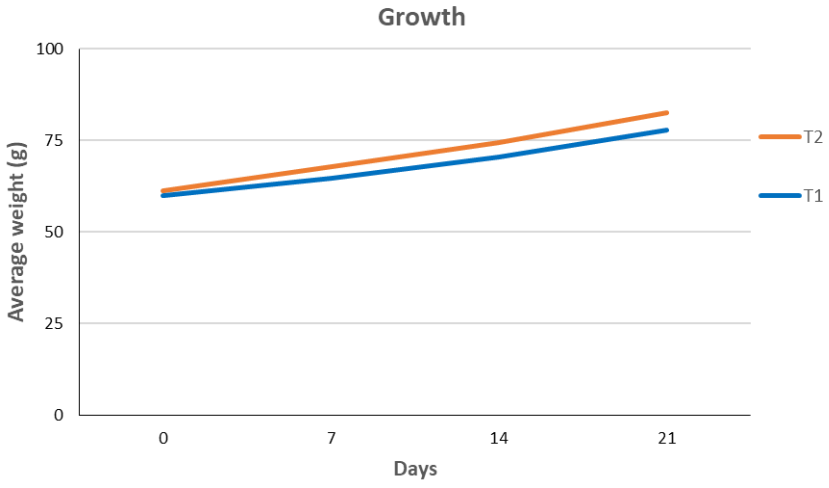

Figure 17. Overall fish growth during the 3 weeks experiment.

The results of the main trial, shown in Figure 16, compared both feeding strategies in term of the amount of feed provided per feeding event. In tank 2, where feeding was based on 1.5% of body weight, the feed quantity remained fixed at each feeding event throughout the days until a new fish

sampling occurred, at which point the ratio was recalculated. On the other hand, in tank 1, where feeding was based on the scoring system and fish behaviour, there were multiple fluctuation in the amount of feed provided, reflecting the variations in hunger exhibited by the fish. Figure 17 illustrates the graph displaying the amount of feed per day, and the trend is similar to the previous graph. The total amount of feed provided during the entire trial was lower in tank 1 compared to tank 2, as depicted in Figure 16.

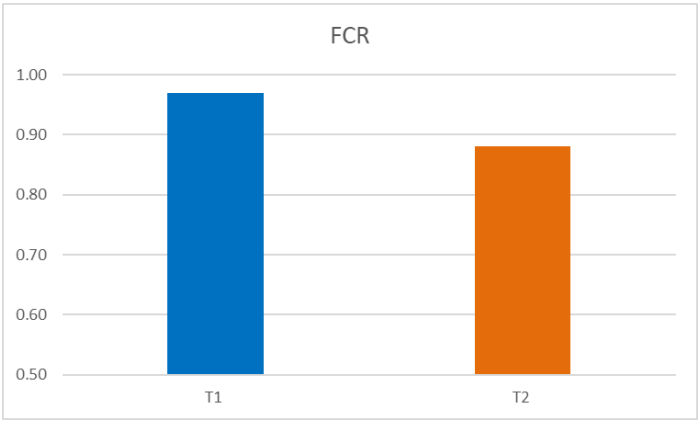

Figure 18. FCR during the 3 weeks demonstration. T1 fish fed based on behaviour; T2 fish fed based on percentage of body weight.

In terms of growth performance, as shown in Figure 17 and Figure 19, the fish fed based on the percentage of body weight demonstrated a higher growth and a lower FCR (82.7 g and 0.88) compared to the fish fed using the scoring system (77.8 and 0.97).

The predictions of the fish growth generated by the FishMet simulations are presented on the Figure 19. There is a good agreement (R^2>0.95) between the predicted fish body mass and the actual body mass observed in the trial.

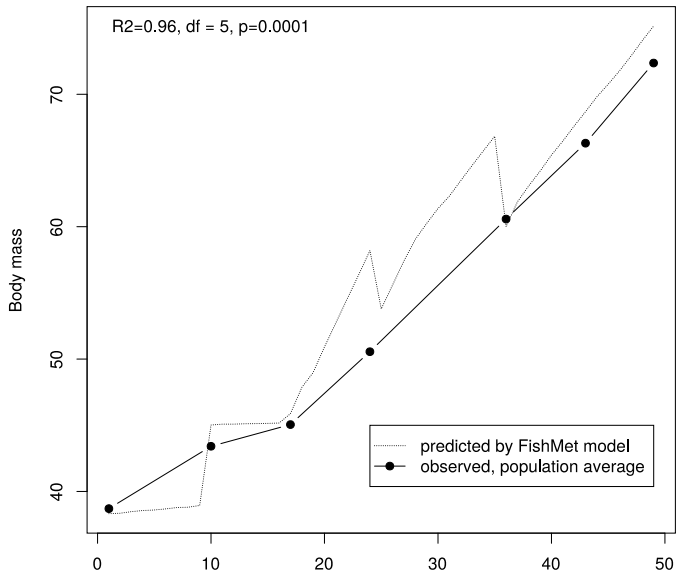

Figure 19. Prediction of growth generated by the FishMet model.

Additionally, the feed conversion ratio (FCR) predicted by the FishMet model during the latest month (the four latest trials, N=26 daily simulations) is 0.91±0.04, which is comparable with the observed data (0.92±0.06).

4.4 Conclusions, Recommendation for Application of the Results in Industry

The demonstration highlighted key considerations for AI development in closed land-based aquaculture (RAS), providing insights into potential applications despite the lack of robustness of the collected data. In phase 1 and 2, real-time fish detection and tracking, along with activity assessment under various feeding regimes, did not require specific replication due to ample data collected to calibrate the algorithm. However, phase 3 faced challenges with real time data analysis, hindering proper sample sizing and replication, resulting in weak statistical significance.

Future tests on the same species and studies on other commercially relevant species are necessary to assess the potential of scaling up in the aquaculture industry. The real-time monitoring of the fish could represent a useful tool especially in a pilot scale RAS where fish are confined in a closed system and the early detection of any abnormal behaviour is crucial, it is possible to further enhance the methodology by taking into account the complete behaviour dynamics of the fish.

The indications of feed reduction of fish fed based on behavioural observation compared to fish fed based on percentage of body weight can lead to a potential cost-reduction through the optimization of feeding, reduction of waste and manpower involved in the daily husbandry as consequence of the implementation of AI in RAS, however, based on the lower growth and higher FCR recorded, further evaluation is essential.

Concerning the iBOSS technology, it is the set of web services interconnected between each other through interoperable APIs. The use of web services makes it possible to connect them over the internet meaning that they don’t need to run locally in a server but can be distributed over the web. The security components used for encryption, authentication and authorization are meant to secure all data exchanges. This approach has several advantages:

- The services are available from any place where internet is available.
- The maintenance efforts are hidden and delegated to a third party to whom a subscription is paid.
- The use of interoperable interfaces makes the system open, allowing newcomers to join and propose new services (such as data processing or visualisation).

However, there may exist some fears of such situations:

- The first one is on the security level as organisations tend to think that a system isolated from the internet is safer. While this may be true, it is more and more complex to keep such systems isolated as at some point data is to be sent (i.e., alerts) and all security aspects have to be handled accordingly.
- Paying to access 'as a service' may provide less control than running locally (on-premises). However, cost of data storage, maintenance, backups, etc. should not be underestimated and each situation is analysed separately.

In addition, implementing distributed web services requires consideration of the bandwidth requirements. Acquiring video locally then sending it remotely for analysis rather than processing it locally through edge-processing may not be the best suited option in terms of cost and environmental impact.

Finally, one important aspect is data sovereignty. Data collected from the farms, whatever they are should remain the property of the farmer which should have access to it in raw and exploitable format, without any delays (e.g., for sensory data) whenever it is required.

The results indicate that the FishMet model can reliably predict the fish growth over a moderate time window (7 weeks), without recalibration and any parameter readjustment. This opens the possibility to use FishMet as a practical tool for "what-if" scenario modelling, such as predicting fish growth in response to planned changes of the feed scheduling, temperature alterations etc. Further work needs to be done to calibrate the model parameters to specific environments and to test the quality of the FishMet simulation-based predictions outside of the standard conditions, e.g., with unusually high or low temperatures, low oxygen etc. Additionally, further tests in a range of fish production environments will be required to make sure the quality of the simulations extends to a wider range of potential commercial conditions.

Nonetheless, the model-based predictive tool provided by the FishMet would be a source of revenue to the industry. It is much faster and cheaper to run computational simulations of possible planned changes in the production system than to conduct live experimental trials or rely on intuition. The FishMet predictions can also be used to determine the consequences and find the best mitigation measures in case of unplanned disruptions. There is a potential to integrate the FishMet predictive decision support tool into a web-based services like iBOSS using a *software as a service* model. One potential risk is scalability because the simulation model is computation intensive and involves high CPU load. But this may be mitigated by cloud-side dynamic virtual machine scaling.

4.5 Dissemination of the Demonstration

- Local event: SmartRAS Demonstration Event was held at the AquaBioTech Group facilities on 24th May 2023. Participants observed how the technologies of iFishIENCi were utilized in a research RAS facility for husbandry of Atlantic salmon including: iBOSS, FishMET and SmartRAS. The short webinar was followed by a visit to the facilities and an interactive tour of the smart monitoring and control systems. Approximately 10 industry representatives attended the event. A follow-up article was published on the iFishIENCi website - https://ifishienci.eu/smartras-demonstration-event/.
- As a lead up to the local SmartRAS Demonstration Event an article was published in the local media advertising the event and highlighting the demonstration activities ongoing at the AquaBioTech Group facilities. This article went live on 12th May 2023.
- The results were presented as part of the iFishIENCi final event under the iBOSS Technical Workshop on the 21st of June 2023. All partners involved in the demonstration presented the different technical aspects required to implement SmartRAS.
- A poster will be submitted (after the life of the project) to Aquaculture Europe 2023, hosted in Vienna, Austria in September. The poster will detail the scientific and industrially relevant results from the demonstration and highlight the future research direction and applications of SmartRAS and the other technologies demonstrated.

5 Other Demonstration Events

5.1 Microalgae Demo – NORCE

In September 2022 NORCE hosted a demonstration of their microalgae pilot facility in Mongstad, Norway. The target groups were high school students and relevant industry members, more than 50 students attended and 40 people from industry. The focus points of the visit were the iFishIENCi specific topics (microalgae as novel feed ingredient, and microalgae in a Waste2value approach).

Figure 20. 750L tubular photobioreactors at the algae pilot in Mongstad during production of Microchloropsis gaditana.

The microalgae pilot facility is owned by the University of Bergen and operated by NORCE. The infrastructure consists of various photobioreactors (three 40L Green Wall Panel photobioreactors, one 250L GemTube photobioreactor and 4,750L GemTube photobioreactors), as well as equipment for harvesting and other peripheral equipment for water treatment, pumping, etc.), seen in Figure 20. The pilot plant is used for optimisation and demonstration of microalgae production processes (TRL 5-6), scaled up from the lab facilities in Bergen, as well as for producing large amounts of microalgal biomass that is subsequently tested in applications ranging from food, to feed to materials. This testing is performed by partners, such as in iFishIENCi in the demonstration by Aller Aqua Research of the microalgae as feed ingredient in rainbow trout, and by MATE-GYE as feed ingredient for the African catfish.

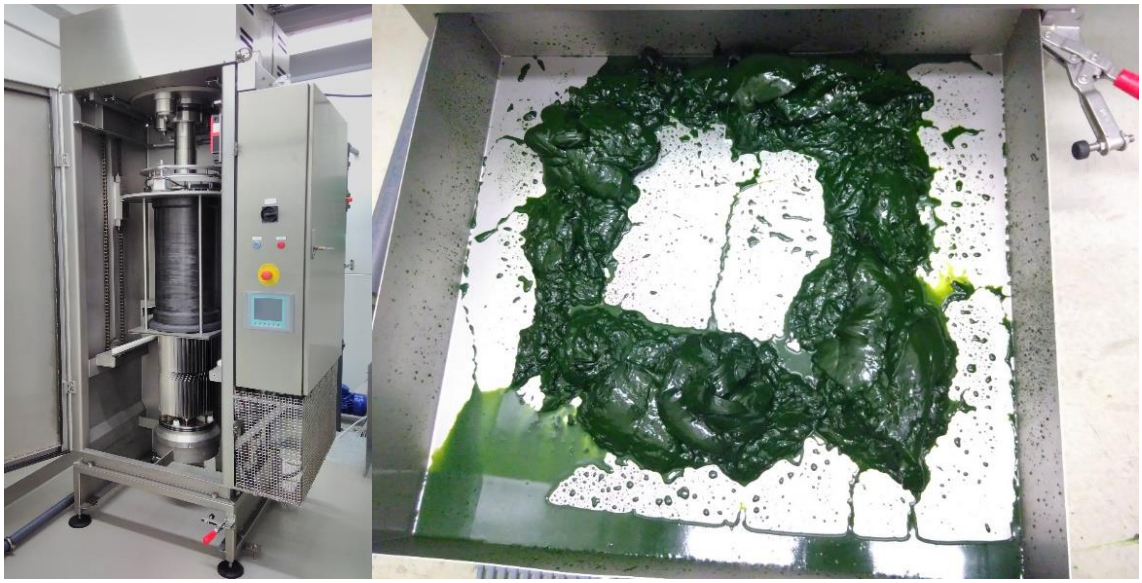

Figure 22. The Evodos 50 centrifuge for dewatering (left) and dewatered biomass (M. gaditana) (right).

The demonstration offered the participants the opportunity to view the microalgae pilot, learn about the operation and sample some of the products, algal biomass fresh or dried, fish feed with microalgae and commercially available products. Moreover, more general aspects of microalgae biotechnology and the role of microalgae in the circular bioeconomy were discussed, such as the use of wastewater from aquaculture as nutrient source for microalgae production, and there was plenty of time for questions. There were close to 100 visitors, from high school students to national industry. The demonstration event was hosted in combination with Science Week ("Forskningsdagene"), which focused on "Ocean" and allowed NORCE to use the programmes communication channels to advertise the event which led to greater number of participants and a wider awareness range. The demonstration was successful, NORCE have already had interest from several industrial partners for possible future collaboration, some of which have already entered into new projects and proposals.

Figure 21. Visitors during the demonstration event (left) in the greenhouse with the reactors and (right) around the water and harvesting tanks of the pilot facility.