Intelligent Fish feeding through Integration of ENabling technologies and Circular principles

Grant Agreement (GA) No: 818036

D16 *Demonstration Per*formance (KPIs) for land-based flow-through and pond systems

Version: 3.0

Date: 29-Jan-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: M53		Date:	21-July-2023
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Reviewer	Freya Robinson (ABT), Giovanni Marco Cussimano (ABT)				
WP	3	Deliverable Nr.	D16	Relative Nr.	D3.5
Comments					

Document change history

Version	Date	Author	Description
1	10.05.2023 – 20.07.2023	Balázs Kovacs (MATE), Julianna Kobolák (MATE), Réka Balogh (MATE), Milán Varju (GyE), Marton Orban (VT)	Inclusion of data from catfish and tilapia demonstrations
2	21-Jul-2023	Freya Robinson, Giovanni Marco Cusimano (ABT)	Final Review
3	29-Jan-2024	Balázs Kovacs (MATE), Julianna Kobolák (MATE), Freya Robinson (ABT)	Update for resubmission

Suggested reference to this deliverable: Deliverable number, Deliverable title (Year of publication), Intelligent Fish feeding through Integration of ENabling technologies and Circular principle (iFishIENCi) Horizon 2020 project under Grant Agreement (GA) No: 818036

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1 Introduction

Intensive fish production is increasingly important in aquaculture. However, sustainability is highly dependent on production costs and efficient use of resources. Feed, being one of the major costs in fish production in the intensive systems and representing 50-70% of the operational costs (Hasan 2007; Zlaugotne et al. 2022), is also one of the major constraints for industry growth due to limited sources of sustainable raw materials. Although fish meal and fish oil use have been dramatically reduced, they remain the main limiting factor for intensive fish production and the overall sustainability of aquaculture. Further reduction of fish meal in feed for high-value species (e.g., salmon, sea bass) and total replacement in diets of mass-produced lower value species (tilapia, carp, African catfish) is necessary to make the world aquaculture more sustainable (Rana et al. 2009). In addition, the growing worldwide demand for food also limits the amount of land-based agricultural production of raw materials for fish feed. To maintain the required growth in aquaculture, a large proportion of the feed ingredients should replace with cheaper and more sustainable alternatives from plant, algae, or insect-originated components (Idenyi et al. 2022). However, these changes in feed composition affect some reductions in production. The key challenge is to evolve fish lines (such as trout and catfish) that tolerate low levels of fish meal or fish oil and can utilise the alternative compounds more effectively, and to know the effect of alternative components in different production systems of different species (Le Boucher et al. 2012). This selection has been implemented in African catfish (*Clarias gariepinus*) during WP1 of the iFishIENCi project (see 1.2).

To investigate the result of the selection for fishmeal-reduced diets on African catfish the growth performance was analysed by comparing different genotypes (control and selected) In flow-through and Recirculating Aquaculture Systems (RAS) and the effect of alternative feed ingredients were tested in catfish and Nile tilapia (*Oreochromis niloticus*). The following experiments were carried out:

Experiment 1: Comparison of the catfish third offspring generation (F3) selected line and control line in flow-through system using the experimental feed and a control feed.

Experiment 2: Comparison of the catfish F3 generation selected line and control line in RAS using an experimental feed and a control feed.

Experiment 3: Comparison of the catfish F4 generation selected line and control line in flow-through system using the experimental feed and a control feed.

Experiment 4: Comparison of the catfish F4 generation selected line and control line in RAS using the experimental feed and a control feed.

Experiment 5 Comparison of the Hybrid-African catfish (produced by crossing the selected line with *Heterobranchus longifilis*) and Control Hybrid Catfish Hybrid-African catfish (produced by crossing the control line with *Heterobranchus longifilis*) in RAS using 5% algae containing feed and a control feed.

Experiment 6: Testing algae containing feed on African catfish in flow-through system.

Experiment 7: Testing alternative feed ingredients on Nile tilapia (*Oreochromis niloticus*) in a pond system.

1.1 Used nomenclature and labels

Generations: During the project four offspring generation were produced. The different generations are labelled as a follows:

Control groups: during the experiment we used three control groups. Labels:

- Control: An absolute control, which wasn't selected (control genotype) and fed with the control feed.
- Experimental I: first experimental feed control group, when the control genotype was fed with the experimental feed.
- Experimental II: second experimental feed control group, when the control genotype was fed with the experimental feed.

Selected groups: Three offspring groups were parallel selected. The groups were not mixed or crossed during the project. Labels:

- Positive selected 1 (PS1): Offspring group that was selected through four generations for higher growth rate using experimental feed.
- Positive selected 2 (PS2): Offspring group that was selected through four generations for higher growth rate using experimental feed.
- Positive selected 3 (PS3): Offspring group that was selected through four generations for higher growth rate using experimental feed.

1.2 New African catfish genetic line for efficient feed utilization

African catfish (*Clarias gariepinus*) and its hybrid with *Heterobranchus longifilis*, and other Clariidae catfish species are important cultured freshwater species with an exponentially growing mass of production worldwide. The total production is over 220,000 tons/year. They are produced in numerous African, South American, and European countries (including The Netherlands, Germany, France, Czech Republic, Poland, Romania, and Hungary). In temperate climates, African catfish can be produced in warm (geothermal) waters and Recirculating Aquaculture Systems (RAS). There is a rapidly increasing interest in Europe where Hungary is the largest producer of this species (its production volume is close to 3,000 tons per year) (FAO 2018). Despite the fact that African catfish is one of the most economical fish species and can be produced in intensive systems in Hungary, there are no selected breeding lines available. All stock originates from the Dutch line which was introduced to Europe more than 50 generations ago (more than 40 years ago) and maintained without selection and with uncontrolled gene flow. Selections carried out in other fish show that production efficiency can be significantly increased with selection and the use of alternative feeds.

Figure 1. Clarias gariepinus at GyE (Bajcshal) site in operation environment.

Clarias gariepinus (Burchell, 1822), or African sharp tooth catfish is a species of catfish of the family Clariidae, the airbreathing catfish ancient in African lakes and rivers. The main advantages of the species are its fast growth rate, ability to feed on a large variety of agriculture by-products (omnivores), and tolerance to adverse water quality conditions (Hogendoorn 1983).

Clarias gariepinus can be crossed with *Heterobranchus longifilis,* the so-call Vundu catfish, to get a hybrid. This cross has a higher growth rate as it has slow maturation, therefore does not spend energy on reproduction during flesh production. Furthermore, upon escape from farms is not a risk to the environment because these fish cannot survive in winter. The colour of the flesh of the hybrid is almost white, which is preferred among some customer groups (Nguenga et al. 2000).

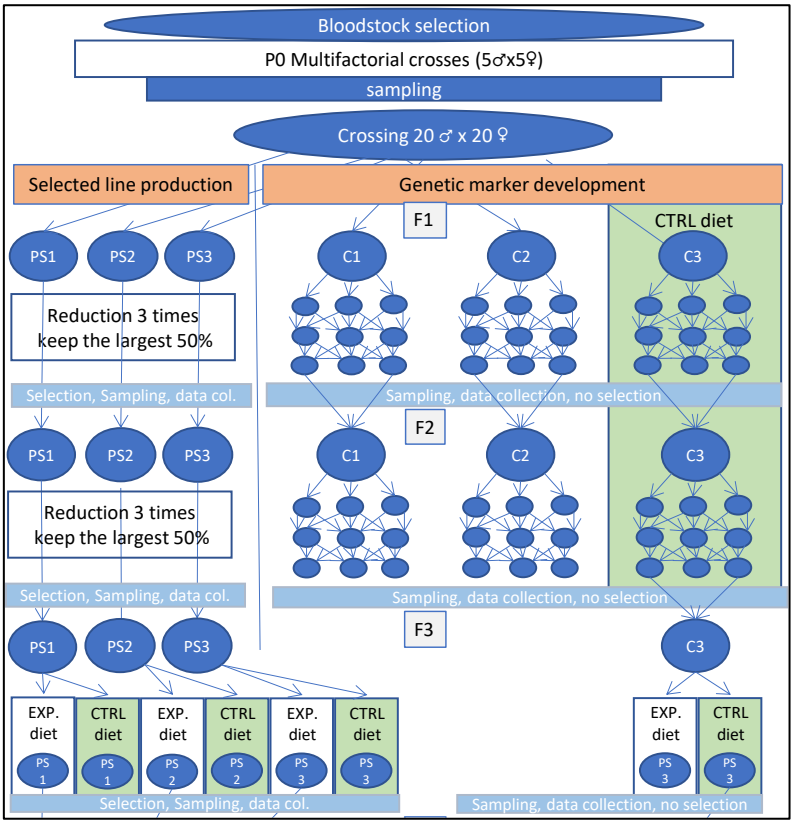

Figure 2. Schematic draw of the African catfish selection program from the F0 to F3 generation. Meaning of abbreviations EXP - Experimental; CTRL, control; F1, first offspring generation; F2, second offspring; F3third; offspring; P0, Parental generation; C1, control group 1; C2, control group 2; C3, control group 3; PS1 , Selected group 1; PS2 , Selected group 2; PS3, Selected group 3; the green background showing the groups were fed with control diet, the white background shows the groups were fed with experimental diet.

The use of alternative protein sources is increasingly important for economic and sustainability reasons. There are many new feed products available on the market for catfish species, including materials from waste streams of other industries. It is also known from previous studies that individual fish can utilise different feeds with various efficiency, probably related to their genetic background (Zlaugotne 2022). Previously, fish feeds were developed mainly with high fish meal content, and fish lines were adapted to this feed, but there are numerous new products on the market, that are using processed animal proteins (PAPs) or alternative protein sources in the feed. The aim of the selection is to develop an African catfish line which can efficiently utilize the lower fish meal-containing feeds or alternative protein sources such as algae or yeast proteins. Thereby allowing a more economical and sustainable production. The African catfish were selected through three generations during WP1 of the iFishIENCi project.

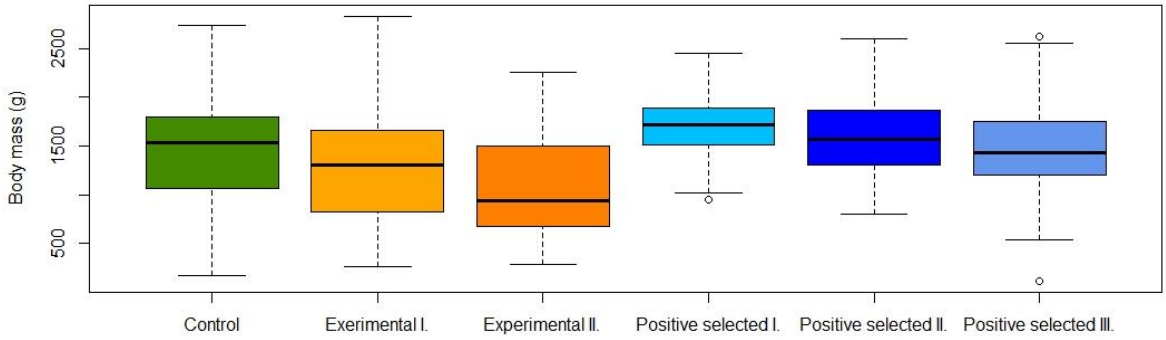

Figure 3. Average body mass of the different groups of F1 generation. The body mass distribution of the selected groups shows the body size of the fish chosen to for the broodstock of the F1 generation. Green-Control genotype fed with control diet. Orange – Control genotype fed with the experimental diet, and Blue-selected genotype groups fed with the experimental diet (number of the individuals=1,846).

The selection was carried out on a semi-industrial scale, in a flow-through system using 2 m^3 tanks, at the site of partner GyE (Figure 7 and Figure 8). The fish were fed with a control diet (conventional, high fish-meal content) and an experimental feed (low fish-meal content) in non-selected control groups. The experimental feed was used in three parallel selected experimental groups (positive selected groups I.-III.). The fish were reared according to the production technology. Size selection was performed in experimental groups (approximately 8% of the fish were selected for the next generation of breeders in every generation).

Control African catfish, bred with standard methods, were used as a starting point for the selection protocol. The original stock was not selected previously for any trait. Twenty male and female individuals were selected from a broodstock of approx. 1,000 individuals. The F1, F2 and F3 offspring were generated by 4 multifactorial crosses of 40 individuals (5 male and 5 female individuals from each parental group). Equalized egg (50g/female; approx. 3,500 eggs) and sperm (1ml/male) amounts were used, and sperm of the breeders were also cryopreserved for further purposes. The body mass of individuals was measured in every generation. Offspring were fed with a commercial (control) and an experimental feed. A special pilot feed with low fish-meal content was selected and used as experimental feed, which was developed earlier by Aller Aqua for African catfish. Fish were divided into six groups: three of them were used to produce the selected line for better utilization of the experimental feed and the other three were used as non-selected groups for genetic analyses (two of them were fed with experimental feed and one of them with the control feed); (Figure 3 and Figure 4). The body mass of 1,846, 1,783 and 3,083 individuals from the F1, F2 and F3 generations respectively, was measured when reaching the market size (approx. 1.2-2.5kg) and the largest individuals were selected two times during the rearing to produce the selected line. The non-selected groups were bred randomly.

In the F1 generation, the unselected fish utilised the experimental feed worse than the control feed. The average body mass in the F1 generation was 1,461.60 ± 490.34g for the control genotype group fed with the control feed and 1,180.26 ± 550.987g for the groups fed with the experimental diet, which is significantly lower, showing a negative effect of the lower fish-meal content diet, which is in line with the results found in other species (Figure 4). The body size of the fish chosen for the broodstock of the F1 generation were similar to the control group fed with control feed.

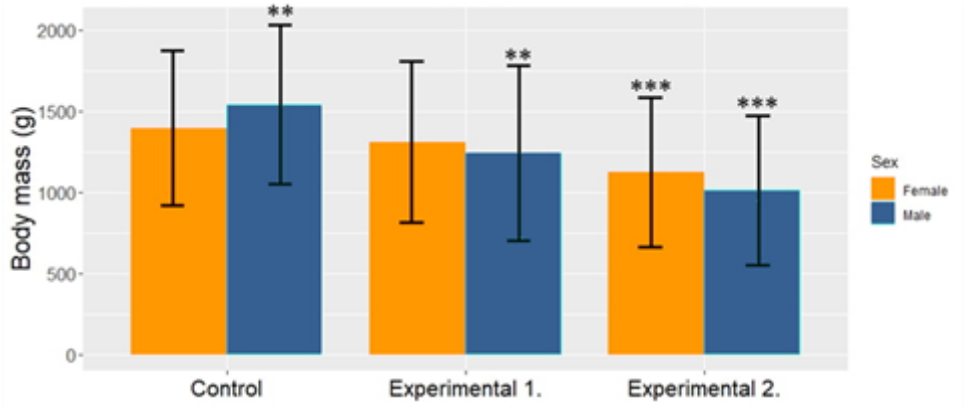

Figure 4. Average body mass of the male and female fish in the control groups of F1 generation. (number of the individuals=2252); Control – Control genotype fed with the experimental diet, and Experimental 1. and 2.-selected genotype groups fed with the experimental diet.

In addition, small differences were detected in the sex ratios of the F1 generation, the association between sex and feed was non-significant, suggesting that low-fish meal feed does not influence the sex of the fish. Additionally, a significant interaction was found between feed and sex, suggesting that utilization of different feeds might have been affected by sex (Figure 4).

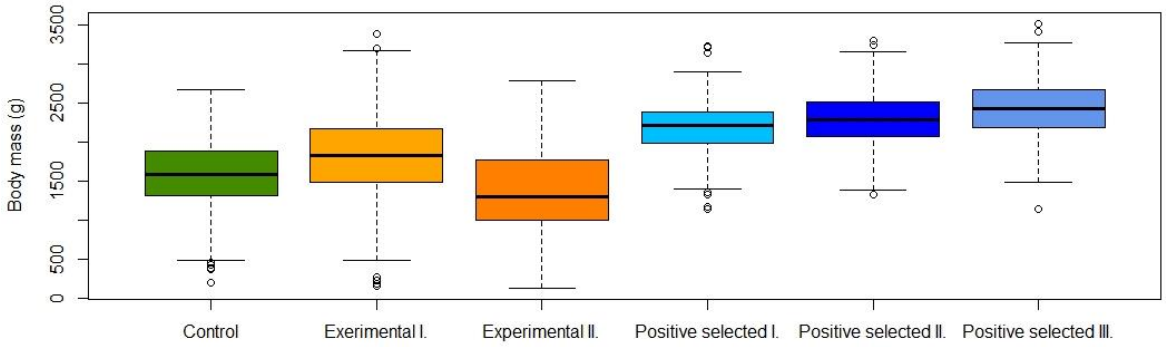

Figure 5. Average body mass of the different groups of F2 generation. Green - Control genotype fed with control diet. The body mass distribution of the selected groups shows the body size of the fish chosen for the broodstock of the F1 generation. Control genotype fed with control diet. Orange – Control genotype fed with experimental diet. Blue -selected genotype groups fed with the experimental diet (number of the individuals=2,783).

In the F2 generation, one experimental group had significantly lower (1,389.63 ± 507.22g) and the other one had significantly higher (1,827.48 ± 506.00g) body mass compared to the control group (1,597.05 ± 399.49g), which might be explained by habituation (Figure 5**Error! Reference source not found.**). Interaction between feed and sex could not be detected in this case (Figure 6). This result does

not support the F1 generation results. The different feeds do not seem to have been affected by sex. The body size of the fish chosen for the broodstock of the F1 generation were significantly higher than the control groups independently from the feed used.

The selection had a remarkable effect on the growth rate. The calculated average gain was over 10% in the F2. To measure average gain and analyse the feed specificity of the result we evaluated the selection results in a demonstration experiment within WP3 and F4.

1.3 New feeds (catfish and tilapia)

The production cost is highly influenced by the cost of feeds and their ingredients, particularly for species requiring a high protein content. Some of these ingredients can be replaced with alternatives to reduce expenses. Numerous experiments have been conducted to substitute traditional fish meal or soya protein with plant or insect proteins. However, in many instances, this replacement has led to lower growth rates and compromised overall health of fish (Fontaínhas et al., 1999; Gómez-Requeni et al., 2004; Pongmaneerat et al., 1993), thereby limiting its practicality. Nonetheless, recent research indicates that feed containing alternative protein sources can be utilized effectively.

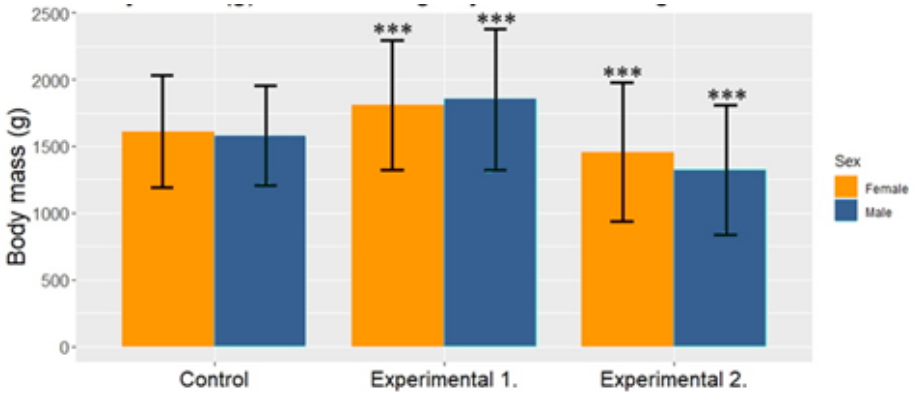

Figure 6. Average body mass of the male and female fish in the control groups of F2 generation. (number of the individuals=2,783); Control – Control genotype fed with the experimental diet, and Experimental 1. and 2.-selected genotype groups fed with the experimental diet

As part of the project, various alternative ingredients, such as algae, yeast and insect meals, were produced and tested. The iFishIENCi partners utilized the newly selected line of African catfish and Nile tilapia (*Oreochromis niloticus*) in both flow-through and pond systems. In these demonstration experiments, feeds based on a novel microalga (*Microchloropsis gaditana*) and insect meals were tested.

2 Feeding Optimisation Through Breeding

2.1 Key Performance Indicators

The key performance indicators (KPIs) for these demonstrations were as follows:

- Growth performance: The selected African catfish line can grow on a low fishmeal content diet with at least the same or better efficiency compared to the non-selected line fed a control feed.
- Identification of genetic markers for further selection of the African catfish.
- Catfish grow using algae meal as a novel protein source with no negative effect on growth performance and body quality traits.
- Using algae meal in feed does not have a negative effect on the fish flesh colour and taste.
- Tilapia grow using novel feed compounds with no negative effect on growth performance and body quality traits.
- Using insect protein and algae meal does not have negative effects on the immune status of tilapia.
- iBOSS water monitoring system is installed and operates in Laos.
- Organization and implementation of the closing workshop and tasting test at the end of the project.

2.2 Catfish Demonstrations

2.2.1 Experiment 1: Comparison of F3 generation selected line and control line in flow-through system using experimental feed

One control group and two of the three parallel selected lines were compared in a performance test at the GYE site using the same flow-through system as was used during the selection. It is a half-industrial scale flow-through system with fifteen 2m^3 capacity tanks. The tanks are supplied with thermal and freshwater wells (Figure 7 and Figure 8) The water exchange rate and the temperature (25°C) are monitored and regulated manually in each tank. The system requires manual feeding.

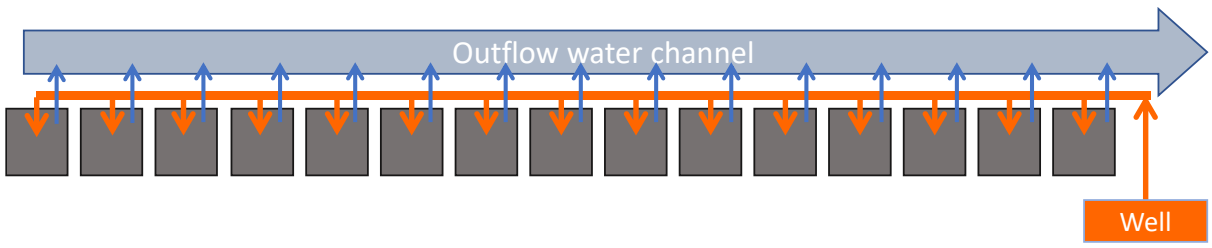

Figure 7. Schematic drawing of the flow-through system at the GYE site. The arrows show the direction of the water flow.

A low fishmeal content diet (Table 1) developed by Aller Aqua, was tested as a pilot feed for African catfish the breeding programme needed at least 3 generations and the newly designed algae-based feed was not available from the beginning of the project. Later it was tested with the selected line.

Table 1. Nutrient composition of the control and experimental diets used for the African catfish selection and pilot scale trials for growth performance (F1-F4 generation and hybrid catfish experiment).

Declared nutrient composition	Control diet 3mm (Aller Bona Float)	Control diet 4.5mm (Aller Bona Float)	Experimental diet 3mm (Aller Claria Float)	Experimental diet 4.5mm (Aller Claria Float)
Crude protein (%)	42	42	45	42
Crude lipid (%)	12	12	12	12
NFE (%)	28.2	28.2	26.4	29.5
Ash (%)	6.8	6.8	6	5.6
Fibre (%)	3.0	3.0	2.6	2,9
Phosphorus (%)	1	1	1	1
Gross energy (MJ)	20	20	20.3	20.2
Digestible energy (MJ)	15.8	15.8	17	16.6

Figure 8. Flow-through system at the GYE site.

However, one of the groups was not used in this experiment because the propagation of one of the selected lines had to be repeated and the age of the fish was different compared to the others. All the experimental groups were divided into two subgroups one was fed with the experimental diet and one with the control diet. All the subgroups contained 500 individuals (the average weight was 126g ± 10g). The length of the experiment was 14 weeks. The average harvesting weight was 1,520g ± 350g)

2.2.2 Results of demonstration experiment 1

The selected lines growth rates were higher in the case of both feeds and the difference were significant (Figure 9**Error! Reference source not found.**). The direct selection gain for the body mass w as 11% in the F3 generation fed with the experimental diet and 14% fed with the control diet, suggesting a relevant commercial potential of artificial selection in this species. However, no feed-

specific selection gain was detected in the flow-through system, which might be explained by the high selection response experienced in the groups fed with the control diet. The calculated heritability was 0.33 ± 0.08, which is relatively high and suggests that the difference is due to genetics.

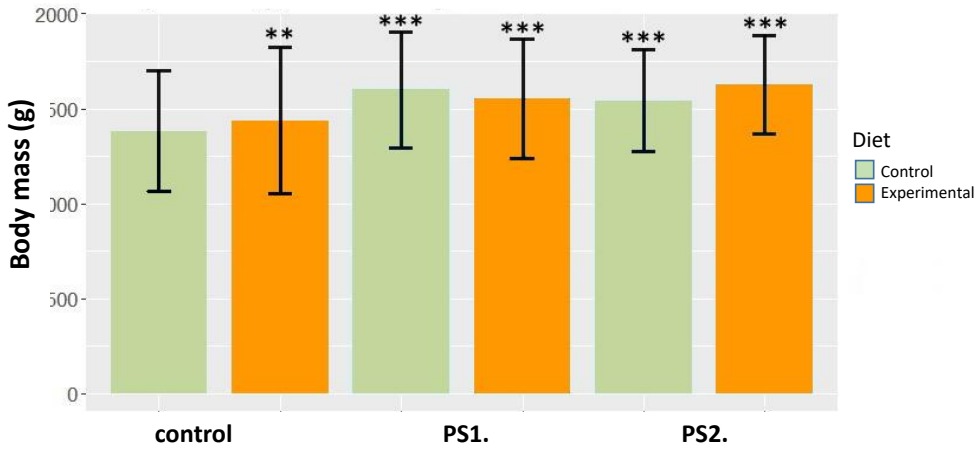

Figure 9. Average body mass of the F3 generation selected line and control line in flow-through system using the experimental feed and a control feed (number of the individuals=2,983).

2.2.3 Experiment 2: Comparison of F3 generation of the selected line and control line in RAS using experimental feed

The F3 generation selected lines were compared in RAS (Figure 10) at the MATE site. The system has a 3m^3 thermoregulated, quadratic tank, separated into three equal parts with internal walls for separated rearing of groups (Figure 10). The tank was connected to a bead filter during the F1-F3 selection and F3 experiment, which was later changed to a drum filter (during the F4 experiments) and a water reservoir tank (with a 1m^3 capacity). The system was equipped with a WTW sensor for dissolved oxygen, temperature and pH monitoring and there were separate probes for refreshing water flow, and water recycling flow. All these probes were connected to a tailor-made Programmable Logic Controller (PLC) -based monitoring/alarm system. The temperature of the circulated water, the circulation rate, and the water change rate were regulated and there were aerators (atomizer) for each section of the tank. Light was provided by a LED system, where photoperiod and light intensity is adjustable. The temperature, the flow rate and the oxygen level were monitored continuously. The feeding was performed manually, three times a day during the early rearing and once a day for

juveniles and adults. The water temperature was 23-24°C. The photoperiod was 16 hours of daylight and 8 hours of dark in every experiment.

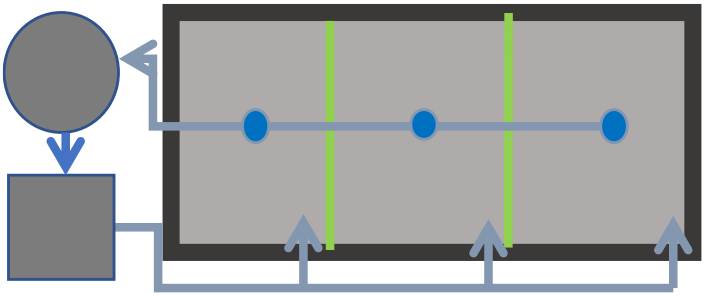

Figure 10. Systematic drawing of the RAS system at the MATE site. The arrows show the direction of the water flow. The grey circle is the bead/drum filter. The grey square is a water reservoir.

A performance test was carried out at the MATE facilities. In this experiment, to avoid the different environmental effects (tank differences, minor human effects, etc.), the control and the selected groups were reared mixed in the same tank. To be able to identify the individuals belonging to each group, they were labelled with PIT-tag (Passive Integrated Transponder). Similar to the experiments in flow-through systems, one control group and two of the three parallel selected lines were compared in a performance test. All experimental groups were divided into three subgroups and distributed equally in the three tanks of the RAS. One tank was fed with the control diet and two with the experimental diet. All the subgroups contained 89 individuals, the average weight was 206g ±62g. The length of the experiment was 16 weeks. The weight of the fish was monitored every second week. The average harvesting weight was 1,950g ± 346g.

2.2.4 Results of demonstration experiment 2

The two selected lines showed a higher growth rate with both diets (Figure 11). The average selection gain was 32% fed with the experimental diet, and 12% fed with the control diet in the F3 generation, respectively. In this system, a feed specific selection gain of 21% was also present in F3 generations. Heritability estimates for the body mass 0.58 ± 0.15 in the RAS systems and feed conversion ratios for the different feeds were almost equal in RAS.

2.2.5 Experiment 3: Comparison of F4 generation of the selected line and control line in flow-through system using the experimental feed

To confirm the result of Experiment 1, it was repeated with the F4 generation with the same design. Using the same flow-through system (Figure 7 and Figure 8) and feed (Table 1). In this case the propagation of the used line was carried out at the same time and all three selected groups were used in the experiment. All experimental groups were divided into two subgroups one was fed with the experimental diet and one with the control diet. All subgroups contained 1,000 individuals (the average weight was 212 ± 58g). The length of the experiment was 6 weeks. The average harvesting weight was 555 ± 80g).

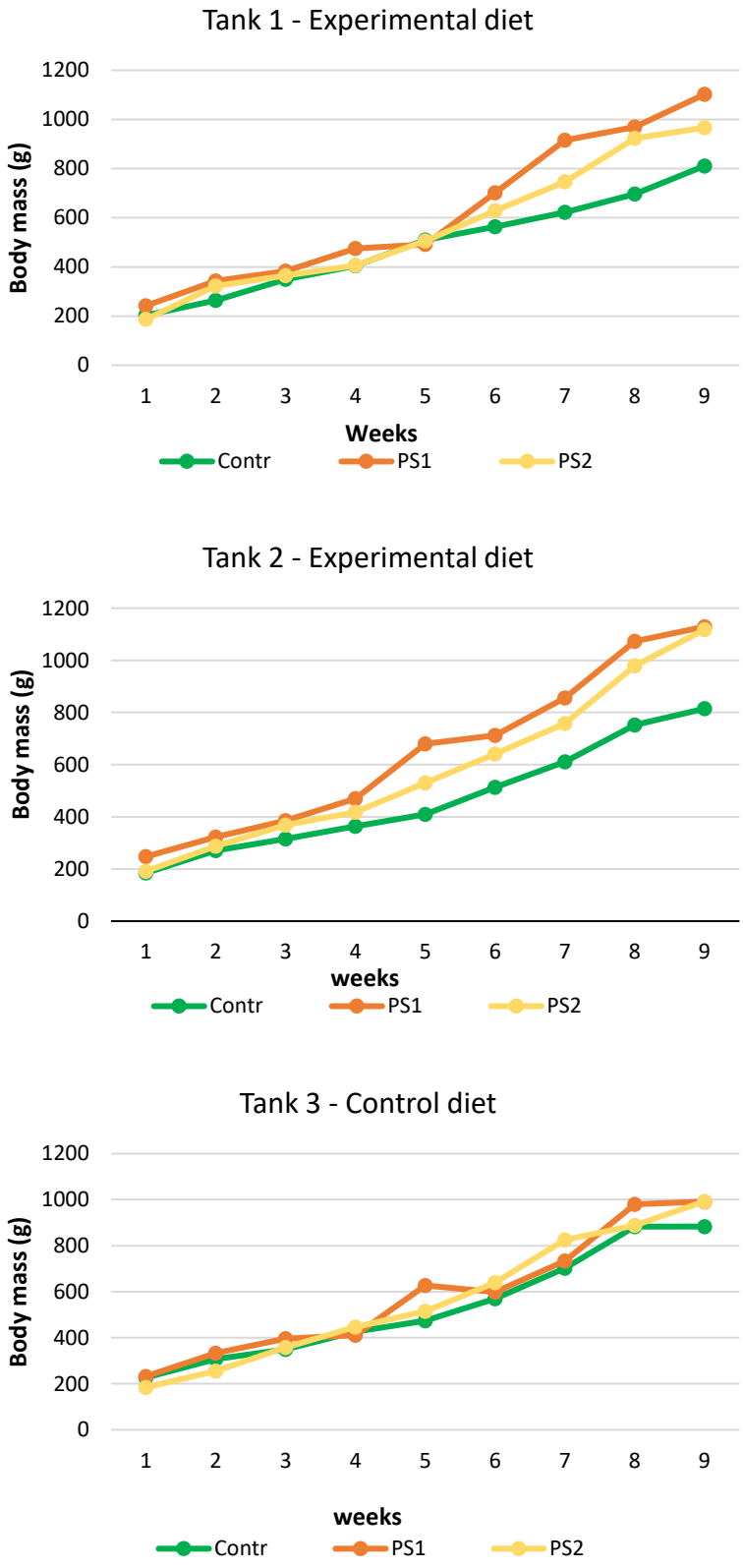

Figure 11. Average individual body mass of the F3 generation selected line and control line in RAS using the experimental feed and a control feed (number of individuals=263). Panel 1: experimental diet groups 1, Panel 2: experimental diet group 2. Panel 3: control diet groups. green: control genotype, orange: group one - selected for low fish meal diet. yellow: group two- selected for low fish meal diet. Cont, Control; PS1, xxx, PS2, xxxx;

2.2.6 Results of demonstration experiment 3

The selected lines growth rates were higher in the case of both feeds and the differences were significant like in generation F3 (Figure 12). The direct selection gain for the body mass was higher than in the F3 generation. The gain was 23% in the groups fed with the experimental diet and 26% fed with the control diet, suggesting a relevant commercial potential of artificial selection in this species. However, the difference between the gains with different feeds was not significant.

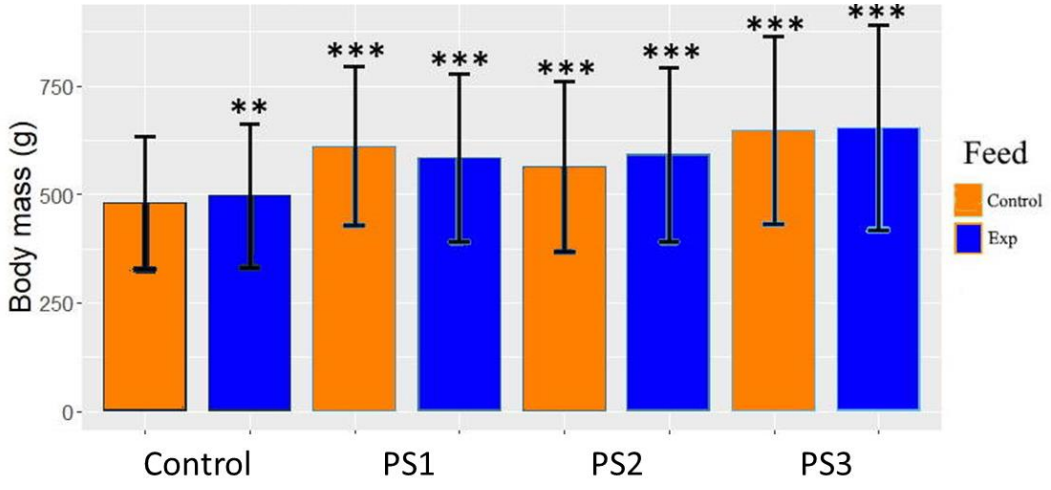

Figure 12. Average individual weight of the F4 generation selected lines and control line in flow-through system using the experimental feed (Exp.) and a control feed (number of individuals = 2,552).

2.2.7 Experiment 4: Comparison of F4 generation of the selected line and the control line in RAS using the experimental feed

Similar to the flow-through experiments, the RAS experiments were repeated with the F4 generation to confirm the results. In experiment 4, performed in the same RAS (Figure 7) at the MATE site, the same design and feed was used as in experiment 2. Similarly, all three selected groups were used in the experiment. All experimental groups were divided into two subgroups one was fed with the experimental diet and one with the control diet (Table 1) according to the industrial production protocols. The number of individuals was 220, the average weight was 555 ± 80g at stocking. The length of the experiment was 6 weeks. The average harvesting weight was 990 ± 256g)

2.2.8 Results of demonstration experiment 4

The three selected lines showed a higher growth rate with both the experimental and control diets (Figure 13). The average selection gain was 33% fed with the experimental diet, and 12% fed with the control diet in the F4 generation. While a feed specific selection gain of 21% was also present in F4 generations. Heritability estimates for the body mass 0.47 ± 0.14 in the RAS systems and feed conversion ratios for the different feeds were almost equal in RAS.

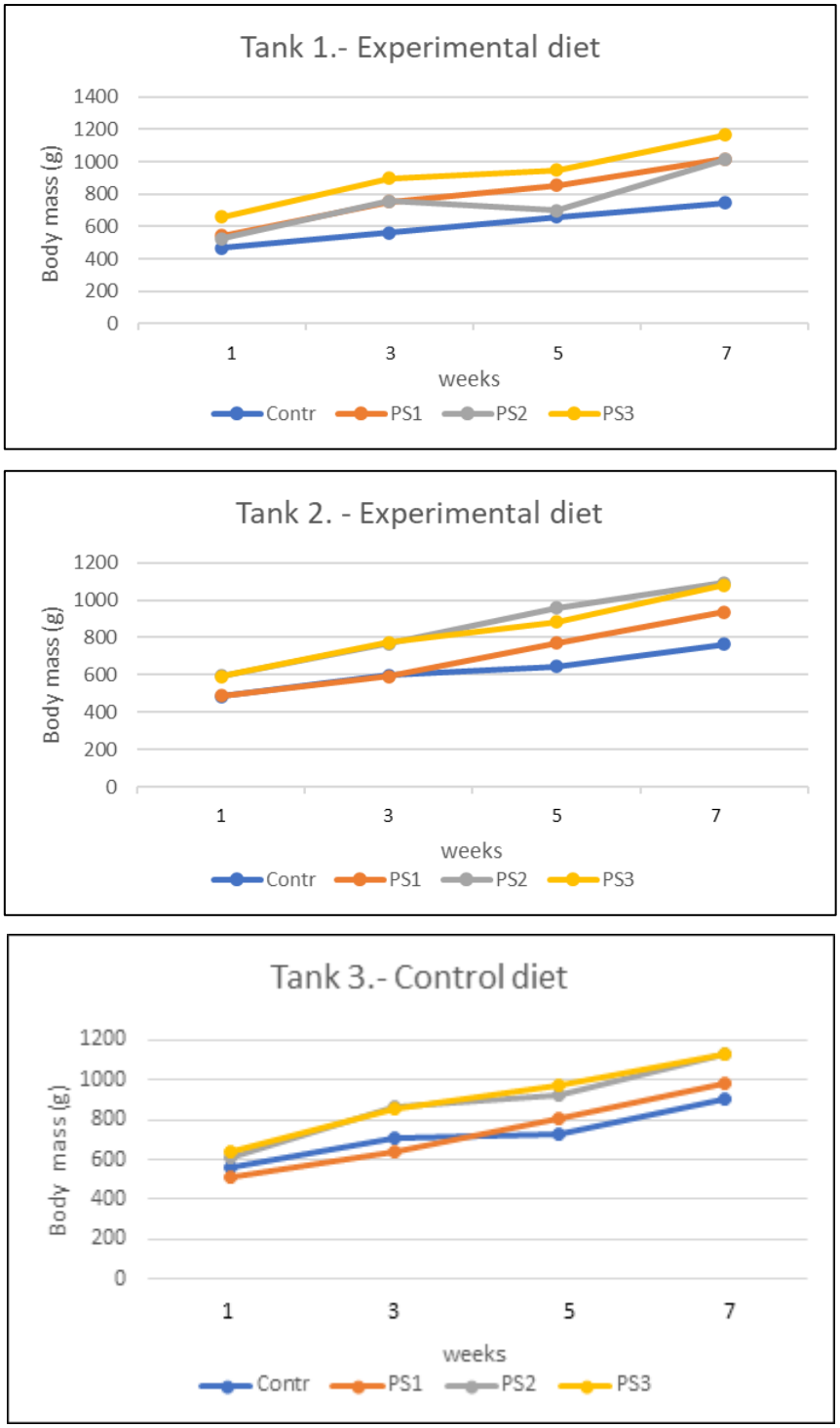

Figure 13. Average body moss of the F4 generation selected line and control line in RAS using the experimental feed and a control feed (number of individuals=263). Panel 1: experimental diet groups 1, Panel 2: experimental diet group 2. Panel 3: control diet groups. blue: control genotype, orange: group one (PS1) selected for low fish meal diet. Grey: group two (PS2) selected for low fish meal diet. Yellow: group three (PS3) selected for low fish meal diet.

2.2.8.1 Genetic Analysis of the feed utilisation

Samples for genetic analyses were collected from all generations. A new generation ddRAD (Double digest restriction-site associated DNA) sequencing was performed to produce genome-wide data for the identification of genetic markers (Single Nucleotide Polymorphism) for better feed utilisation. In

total, 192 (78 F2 offspring with a high growth rate and 78 with a small growth rate and 36 F0 parents) individuals were used for genomic library preparation. The restriction was performed by MspI and PstI enzymes, the insert size was 150-300bp and sequencing was done on an Illumina NextSeq platform with 2x150bp paired-end sequencing. A minimum of 1M sequence reads were generated per individual. The bioinformatical data evaluation was made by Stacks software.

2.2.8.2 Results of the genetic analyses

During the analyses, 22,971 single nucleotide polymorphism (SNP) markers were identified. Among them, 4,075 showed significant differences between high and small growth rates on the level of P <= 0.05, and 1,921 on the level of P <= 0.01. The distribution of the F_{ST} values (Fixation index) showed high differences (Table 2). Among them, only 21 marks have F_{ST} values over 0.1. These markers can most likely be used effectively in further selection work. The analyses of this are under process.

Table 2. Distribution of the FST values of the SNP markets that showed significant differences.

significance level	number of the markers	FST					
		Minimum	1st Quarilis	Median	Mean	3rd Quartilish	Maximum
P <= 0.05	4,075	0.01294	0.02096	0.02714	0.03132	0.0371	0.20559
P <= 0.01	1,921	0.02171	0.03017	0.03789	0.0416	0.0476	0.20559

2.2.9 Experiment 5: Comparison of two genetic lines of hybrid catfish on feed utilisation and growth performance in RAS

The trial was conducted at the ABT facilities using hybrid catfish to demonstrate the effect of selected African catfish on hybrid catfish production. The experimental hybrids were produced by crossing of selected *Clarias gariepinus* ♀ with non-selected *Heterobranchus longifilis* ♂ (hybrid catfish) and as a control non-selected *Clarias gariepinus* ♀ with non-selected *Heterobranchus longifilis* ♂ (control hybrid catfish). The performance of the two groups was compared in a recirculating aquaculture system, using 12x500L 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 fish were fed with the experimental diet is shown in Table 1. The length of the experiment was eight weeks.

2.2.10 Results of demonstration experiment 5

At the end of the experiment, the biomass was measured, and feed conversion ratio (FCR), specific growth rate (SGR), specific feeding rate (SFR), and survival rate were calculated. The comparison of growth performance and survival between the selected and control lines did not show a significant

difference (Table 3). The final mean weight and SGR were slightly higher in the control line than in the selected line, while the SFR and survival rate were higher in the selected line than in the control line. Details of this study can be found in the public Deliverable 3.4 - Demonstration Performance (KPIs) for Recirculating Aquaculture Systems. as Catfish Experiment 2.

Table 3. 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

2.2.11 Experiment 6: Comparison of F4 generation selected line and control line in flow-through system using 5% algae containing feed

The three selected and one non-selected African catfish lines were used to test *M. gaditana* in a flow-through system at the GYE site (Figure 7 and Figure 8). In the experiment eight 2m^3 tanks were used, two tanks per line, with 300 fish/tank deployed. One tank per line was fed with custom-made control and one with custom-made experimental feed (Table 4). The average weight of the fish was 550 ± 80g at the start and the average final weight was 1,181 ± 125g. The length of the experiment was 6 weeks. The fish were fed three times/day manually.

Both diets were formulated with low fish meal content (6%), the control diet contained 24% soybean meal and 8% extruded soybean meal, while the algae diet contained 5% microchloropsis meal which substituted the soybean meal (22%) and extruded soybean meal (5%) (Figure 14).

Following the experiment, nutrient component analyses, flesh colour analyses and organoleptic evaluation were performed to evaluate the effect of algae on fish flesh quality. At the end of the trial 50 left-side fillets per treatment were sampled and frozen at -20°C and sent to a food analytical lab for analysis and later sent for composition analyses.

Figure 14. Formulated control and 5% algae-containing feed.

Table 4. Nutrient composition of algae and control diets used for the African catfish pilot scale trial.

Nutrient composition of feed Parameter (% OM)	Control diet	Test diet Algae
Moisture	9.815	8.565
Crude protein	41.709	41,899
Crude fat	12.448	12.455
NfE	12.565	12.565
Crude fibre	3.166	2.817
Crude ash	2.857	2.993
P	0.620	0.652
Ca	0.520	0.517

2.2.12 Results of demonstration experiment 6

There was no significant difference in the daily consumption. All groups fed with algae-containing feed had higher growth rates (Figure 15). The average body weight was 5.3% higher than the control. In the case of the control feed, the average selection gain was 19.3%, while in the groups fed with the algae-containing feed the selection gain was 21.3%. Significant difference was in FCR of the algae diet and the control diet (1.13 ± 0.07; 0.98 ± 0.03; P: 0.005).

During the nutrient composition analyses of the African catfish fillets, 39 components were measured. Most of them were similar in the control and algae diet fed groups, only a few fatty acids (Palmitoleic acid, Behenic acid, Erucic acid), the total ash, vitamin A and the Beta-Carotene showed a significant

increase, while the level of Lauric acid was significantly lower in the flesh of the algae fed groups (Table 5).

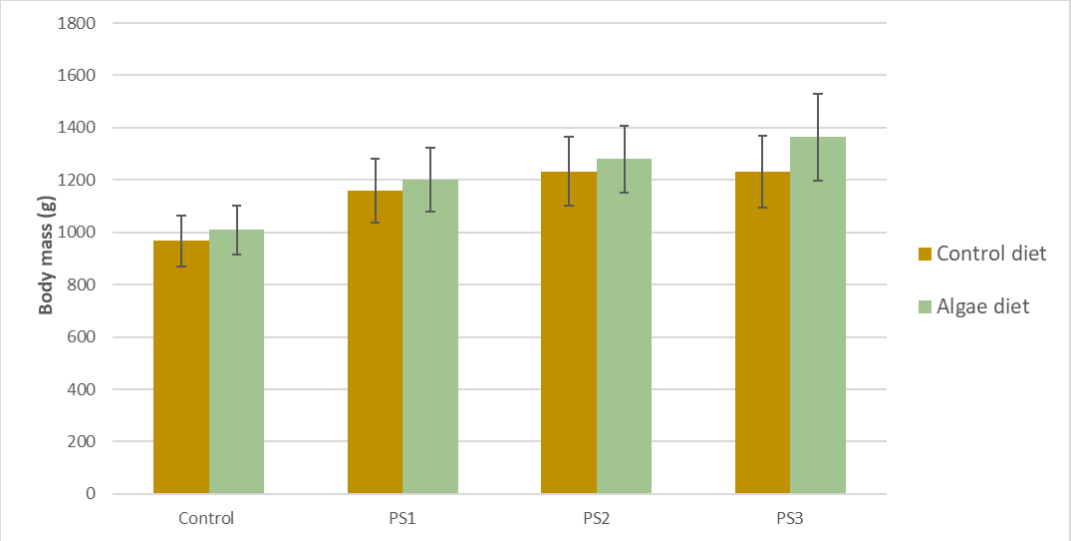

Figure 15. Average individual body weight of African catfish in flow-through system using microchloropsis (5%) and control diet, PS1 – group1, selected for low fish meal diet. PS2– group2, selected for low fish meal diet. PS3– group1, selected for low fish meal diet (number of the individuals =2,400).

Table 5. Result summary of African catfish fillets composition measurements.

	Control average	SD	Algae average	SD	differece (%)	T-test
Lauric acid (C 12:0) [a] (m/m %)	0.284	0.129	0.11	0.04637	-61.2676	0.0218409
Palmitoleic acid (C 16:1) [a] (m/m %)	3.066	0.238	3.566	0.2072	16.30789	0.0076025
Behenic acid (C 22:0) [a] (m/m %)	0.338	0.041	0.654	0.24141	93.49112	0.020365
Erucic acid (C 22:1) [a] (m/m %)	0.056	0.005	0.104	0.03975	85.71429	0.0281461
Total ash content [a] (m/m %)	1.066	0.019	1.112	0.03899	4.315197	0.0459761
Vitamin A [a] (all-trans-retinol) [a] (mg/100g)	0.011	0.003	0.0174	0.00445	58.18182	0.0264852
β-carotene [a] (mg/100g)	0	0	0.014	0.00894	0	0.0080791

The colour analyses of the fillets showed minimal but significant differences between algae and the control diet fed fish fillets (Figure 16). The difference is on the edge of visibility and caused by the yellow component of the colour palette. 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.

Figure 16. African catfish filets fed with algae (left) and control (right) diets.

The sensory evaluation results indicated that there were no significant differences in the rating of the two samples. However, the fish fed the algae feed showed a slight advantage over the fish fed the control feed. The group fed with algae displayed a more pronounced "salmon colour" appearance compared to the control group, which exhibited more green or white colouration. Furthermore, the algae fed group had a more neutral odour compared to the control group. The algae fed fish also emitted noticeable aromas of corn and nuts. In terms of taste, the algae group maintained a neutral flavour, but hints of corn and nutty flavours were detected alongside the chicken taste. While the mouthfeel did not undergo significant changes, there was a slight decrease in stickiness and a less firm texture observed in the algae fed group. Moreover, the buying intention was higher for the algae fed fish. The introduction of the new algae feed did not have any impact on the sensory acceptance of the fish fillets as assessed by the evaluators. Details of this study can be found in the public Deliverable 3.6.

2.3 Nile Tilapia Demonstration

2.3.1 Experiment 7: Testing alternative feed ingredients in Nile tilapia (*Oreochromis niloticus*) in pond system (Experiment 7)

The experiments were performed by VT in a pond system in Laos. The location and infrastructure for the implementation of the experiment were provided by Aquatic Development Co. (a joint Hungarian-Laos venture). For the feeding trials, a net cage system was used in a tropical freshwater lake. In the lake systems, the water exchange rate was quite fast since the lake is directly supplied by the Nam Houm reservoir. The levels of organic compounds and turbidity were low, the temperature is affected by the environmental conditions and the lighting period corresponds to natural light. The 3.5% algae flour (16 kg of algae (microchloropsis) meal was produced by NORCE.) and the 3.5% black soldier fly larvae-originated insect protein-supplemented products were produced during WP1. The “Bio Stimulant” premix was produced at Vitafort Zrt. in Hungary after which it was sent to Laos, where the

Nongteng feed mill used it for the third experimental feed. This feed contained a special premix created with the use of high biological value vitamins, antioxidants, and additives (e.g., yeast cell walls, ß-glucans, fermented wheat germ extract, protected vitamin C). The 100g monosex tilapia hybrids produced by the combination of GIFT (Genetically Improved Farmed Tilapia) and Big Nin strains, were purchased from there as well.

The cage system containing eight cages, two cages for each group. According to the growth potential of Nile tilapia, individuals of 100g were expected to reach 300g live weight each during a nine-week period. After the two-week-long habituation period, two cage systems were used for the task which were installed in the same lake. Each system consisted of 4 cages; the water supply of the lake was provided by the Nam Houm reservoir. The fish stock was divided into 4 groups, one group occupied 2 cages with 45m^3 volume each. According to the experimental design, 100-125 fish were accommodated in each unit (Figure 17).

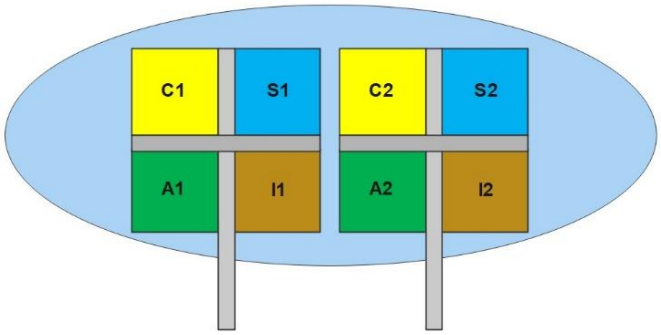

Figure 17. Distribution of feeding groups during the tilapia trial: A: tilapia groups fed with algae containing feed premix; I: tilapia groups fed with insect protein supplementation; S: tilapia groups fed commercially viable bioactive additives; C: control group.

On the second week of habituation, the iBOSS system was installed. The following parameters were recorded: salinity, O_2, pH, temperature, turbidity, and conductivity. The measurement of the fish and the general health checks were performed weekly. The length parameters of 30 fishes (15 fish/cage) and the weight of 30 animals (15 fish/cage) by every feeding group was recorded to provide data for the calculation of average weight, length, and gain of the population. The feed was offered twice a day for the animals, the daily ratio was determined as equal to 3.5% of the stock body weight during the first week of habituation. The ratio decreased to 2% of the stock weight for the 6th week, this was not changed until the end of the trial.

After habituation, before the feeding of the experimental feeds, and at the end of the trial, liver and head kidney samples were collected to evaluate the immune status of the fish stock. During the first sampling, 12 samples were collected (6 liver and 6 head kidneys in every group) to define the initial immune condition. At the end of the trial, all 4 groups were divided into a stressed and a non-stressed

category. While in the stressed category the fish were exposed to stress before sampling, in the case of the non-stressed group the animals were sedated immediately. The detailed structure of the stress experiment, which resulted in 96 samples, was the following:

Stressed category

- Group 1.: Algae 6 fish - 12 samples;
- Group 2.: Insect protein 6 fish - 12 samples;
- Group 3.: Bio Stimulant additive 6 fish - 12 samples;
- Group 4.: Control 6 fish - 12 samples;

Total: 48 samples

Non-stressed category

- Group 1.: Algae 6 fish - 12 samples;
- Group 2.: Insect protein 6 fish - 12 samples;
- Group 3.: Bio Stimulant additive 6 fish - 12 samples;
- Group 4.: Control 6 fish - 12 samples;

Total: 48 samples

Overall: 96 samples

The samples were taken to Hungary by the contribution of Hungarian experts and were tested in the laboratories of the Research Institute of Fisheries and Aquaculture of MATE. Vitafort organized an organoleptic test in cooperation with TTZ to evaluate the hedonic acceptance of the fish raised during the activity.

2.3.2 Results of demonstration experiment 7

During the trial, every feeding group reached the planned weight and length. There was no tendentious difference between the groups in the average weight and body length at the end of the trial (Table 6, Figure 18 and Figure 19).

Table 6. Result summary of the different feeding groups of tilapia.

Dates of measurements	Average weight (g)				Average length (mm)			
	Control	Algae	Insect	BioS	Control	Algae	Insect	BioS
Start size	100	100	100	100				
03.09.2021.	147.5	142.5	140	144	147.8	142.5	140.2	144.0
10.09.2021.	158	170.5	161.5	179.5	158.0	162.2	157.3	162.8
17.09.2021.	210	209.5	189.5	200.5	175.2	175.2	171.7	171.0
24.09.2021.	224.5	223	220.5	239	180.0	179.2	184.3	181.2
01.10.2021.	259	257.5	243.5	245	185.0	184.2	184.3	182.0
08.10.2021.	268	296	262	285.5	191.3	198.0	190.7	194.8
15.10.2021.	305.50	313	305.5	311.5	202.8	197.0	200.8	200.8
21.10.2021.	337.5	348	344	344.5	206.5	205.7	200.3	207.3

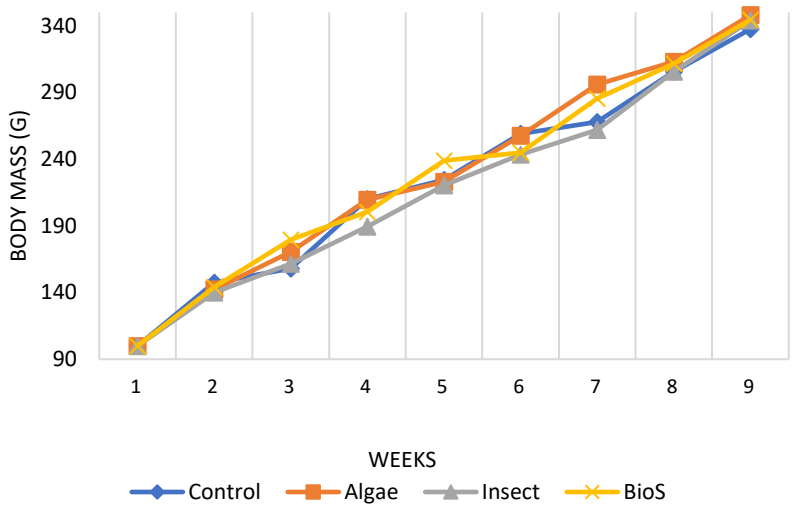

Figure 18. The average body length of the different feeding groups during the tilapia trial.

During the immunity status analysis, gene expression analysis was carried out. Based on the results the effect of feeding significantly ($p<0.05$) increased the expression of TNF-α and IFN-γ genes in the head kidney of groups fed with insect protein (I) and algae (A) supplementation compared to the control. In addition, IL-8 gene expression was significantly higher in group A than in the control as well. The applied environmental stress did not significantly affect the gene expression in either the liver or the head kidney within the groups, however, in the post-stress samples, there was a significant ($p<0.05$) increase in group A compared to the control in expression of SOD and GPx genes.

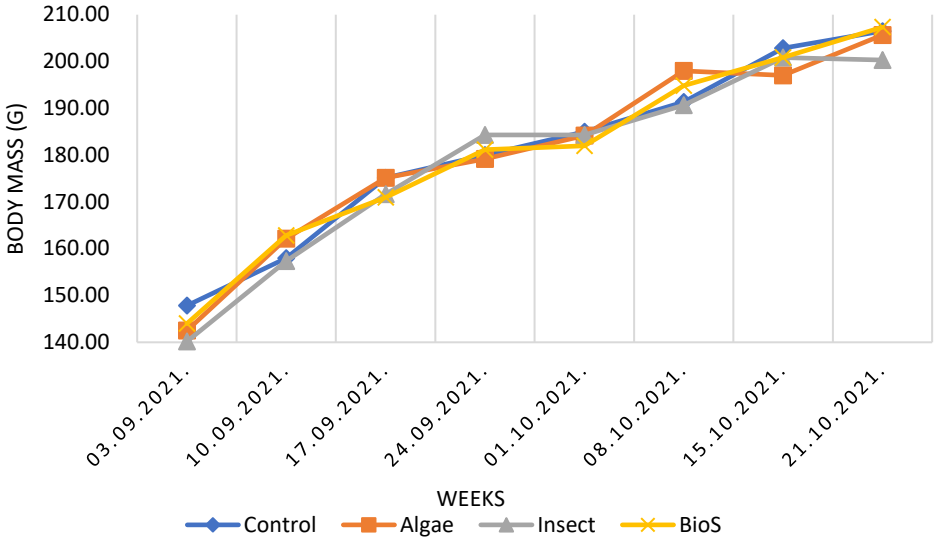

Figure 19. The average weight and body length measured during the tilapia trial.

Due to the Covid 19 regulations in place at the time of the experiment, it was possible to evaluate the flesh quality by sensory event only in small groups. The testers assessment shows that there were minor differences between the individual fish samples, and it can be concluded from this that the different feeds could have a minor influence on the sensory quality of the fish. Control and algae diets were evaluated slightly better than insect and bioactive supplemented diets. Details of this study can be found in the public deliverable 3.6.

Conclusions, recommendations for application of the results in the industry

The selection system developed and applied worked well on African catfish. The selected lines performed better than the control population in independent experiments of the F3 and F4 generations. They utilised both the experimental and control feed better. This was demonstrated in both flow-through systems and RAS experiments. However, during the flow-through rearing, when environmental factors exerted a stronger influence, there was no significant growth difference between the diets, however, both were utilised better by the selected lines. In contrast, in the RAS system, where the conditions were much more controlled, there was a significant 21% feed-specific selection again in the F3 and F4 generations. This fulfils the performance indicator goal that the selected African catfish line can grow with at least the same or better efficiency on low fishmeal feed than the non-selected line on normal feed.

A genetic comparison was performed of fast and slow-growing individuals derived from the F2 generation using ddRAD method based on new generation sequencing, resulting in the identification of 21 SNP putative genetic markers that are likely to be useful for growth selection of African catfish in the future. However, the KPI was only partially met, because further targeted studies are needed to confirm the usability of the marker.

Furthermore, no detectable difference was observed in the growth or feed utilization of the two hybrid catfish crosses, which is likely due to the induced heterosis effect, or the dominant genetic background derived from the paternal species (*Heterobranchus longifilis*). However, further investigations are needed to confirm this.

On the other hand, the selected lines not only performed better in the selection experiment but also showed more efficient utilization of both the algae-based and control feed in the experiment conducted with algae-based diet, demonstrating the overall growth advantage of the selected line over the initial control line. The efficacy of the applied method is demonstrated by obtaining consistent results in all three parallel selected groups.

The same experiment also shows that the inclusion of 5% algae meal as a replacement for soy protein positively influences the growth of African catfish, as both the control and selected lines showed greater growth on the algae-based diet compared to the control diet. No negative effect was observed during organoleptic testing. On the other hand, minor colour change was measured with a sensitive instrument, which is on the edge of visibility to the naked eye (in the yellow colour range), but this

does not affect the flesh quality. Among the analysed compositional parameters, only a few fatty acids and vitamin levels increased with algae supplementation, which can be considered a positive result. This fulfils the KPI goal, as the algae meal did not have a negative effect on the colour and taste of African catfish flesh.

In the case of experiments conducted in the pond system, neither the algae and insect meal nor the bioactive additive showed any significant effect on growth. The increased expression levels of certain genes (TNF-α and IFN-γ genes) indicate an elevated cellular immune response due to supplementation with algae and insect meal, and additional genes showed increased levels in response to stress in the algae diet. Based on these findings, both feed supplements, black soldier fly larvae meal at a 3.5% dosage and algae meal at the same dosage, improved the natural immune response of tilapia. Furthermore, the latter treatment also enhanced resistance to transportation stress through more effective activation of the antioxidant system. Furthermore, taste tests indicated only minimal, taster-specific differences among the fish fed different feeds. Overall, it can be said that none of the feed ingredients negatively affected the growth and flesh quality of tilapia, which meets the expected key performance indicators.

The created line of African catfish, although further improvements can be made through additional selection steps, can be introduced into production, significantly increasing the growth efficiency. The line's application in a closed RAS could be even more effective. The selection method used can be effective for other lines and species as well, such as in the case of *Heterobranchus longifilis,* which is used as the paternal partner in the hybrid catfish production. The method can be particularly effective for species that have not been subject to selection so far, which is true of the less commercially cultured fish species. Therefore, the significance of these species can also increase.

None of the tested feed supplements negatively affected the growth of either fish species and the algae supplementation had a specifically positive effect at a dosage of 3.5-5% for both species. Therefore, a more detailed examination of the applicability and cost-effectiveness of this supplementation on a larger scale is highly recommended.

Dissemination of the Demonstration

The results of these demonstrations were presented in a training event and in the Fisheries and Angler Specialists Meeting in Hungary. The training event was hosted at the GYE (Bajcshal Ltd.) site in Kisbajcs, Hungary. Its primary objective was to facilitate networking among African catfish producers, industry leaders, and professionals, including researchers. Additionally, the event aimed to showcase the innovative accomplishments of the iFishIENCi project, with a particular focus on the development of the novel genetic line for African catfish. Moreover, it sought to raise awareness about the significance of selective breeding and the selection process for improving feed utilization, especially concerning sustainable protein sources. The participants in the event represented seven distinct African catfish producers (the biggest among them), which collectively accounted for the majority of African catfish production in the country. Furthermore, attendees comprised of representatives from universities, the Agricultural Ministry, and the press. This gathering provided an exceptional opportunity for networking within the sector.

Five presentations were held during the event, two of these were presented by GYE representatives, while three were given by researchers from MATE. The presentations encompassed various aspects of the iFishIENCI project with special emphasis on genetic breeding and its outcomes, including recent studies on catfish species in general. According to the best of our knowledge, this event marked the first occasion where professionals actively engaged in African catfish production in the country had a dedicated platform, despite Hungary's status as the primary producer of this species within the European Union. Consequently, this event had long been anticipated and was in high demand.

https://eurofish.dk/news/hungary-eu-funded-research-project-to-boost-production-of-african-catfish/

The other demonstration of the iFishIENCi project results was during the two-day long Fisheries and Angler Specialists Meeting at Gödöllő, Hungary. More than 200 people were registered from all over the country. Most of them were from different aquaculture companies but governmental representatives, ministry decision-makers and scientific researchers were among them.

Many lectures of the meeting concentrated on the presentation and potential applications of iFishIENCi results in Hungarian aquaculture. This included a virtual farm visit (pre-recorded video tour) at the Bajcshal Ltd. and lectures from iFishIENCi partners (NORCE, HCMR, ABT, MATE-AKI, GYE (Bajcshal) and VT). The presented lectures included the examination of feed additives and potential raw materials in the iFishIENCi project; innovative developments in cage aquaculture of sea bream and sea bass in Greece; the technological developments in aquaculture digitization in Norwegian

salmon farming; opportunities of the circular economy in aquaculture based on the results of the iFishIENCi project; African catfish selection breeding program within the frame of iFishIENCi project; and results achieved by the iFishIENCi project at Bajcshal Ltd. All the lectures focused on the industry applications and support to use by local farmers.

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/

Besides the Hungarian programs, two dissemination event were held in Laos to promote the iFishIENCI project and share the experiences and results gained during the tilapia feeding trials.

After the closing of the feeding trial, Vitafort performed a small workshop with a taste test in cooperation with TTZ on the 11th of November 2021 to evaluate the hedonic acceptance of the fish raised during the activity. Due to the Covid-19 pandemic situation, the event was carried out with an attendance of 15 participants. To provide some insight into the background of the filets prepared for the tasting test, the professionals of Vitafort introduced the iFishIENCi project and the roles and tasks of Vitafort within the program in the frame of a small workshop. After the presentations, the participants tasted and ranked the steamed filets (algae, insect, bioactive, control) based on the questionnaire prepared by TTZ. The filled questionnaires were collected and sent to TTZ for further evaluation.

As Vitafort has an active role in the implementation of the Hungarian Tied Aid Loan programs in Laos through its subsidiary, Vitafort Agro Asia Co., a great opportunity emerged in the frame of the Laotian-Vietnamese-Hungarian Forum to share the results of the tilapia feeding experiments and introduce iFishIENCi project. The high-level meeting was held on the 14-15th of November 2022, with the attendance and contribution of the representatives of the Laotian Agricultural Ministry and the Vietnam National University of Agriculture.

References

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

Hasan MR, editor. Economics of aquaculture feeding practices in selected Asian countries. Rome: FAO Fisheries Technical Paper No. 505; 2007.

Hogendoorn, H. (1983). *The African catfish (Clarias lazera C. and V., 1840) : a new species for aquaculture*. [internal PhD, WU]. Landbouwhogeschool. https://edepot.wur.nl/200656

Idenyi, John N., Jonathan C. Eya, Amechi S. Nwankwegu, Emeka G. Nwoba, Aquaculture sustainability through alternative dietary ingredients: Microalgal value-added products, Engineering Microbiology, Volume 2, Issue 4, 2022,doi.org/10.1016/j.engmic.2022.100049.

Le Boucher R, Dupont-Nivet M, Vandeputte M, Kerneı¨s T, Goardon L, et al. (2012) Selection for Adaptation to Dietary Shifts: Towards Sustainable. Breeding of Carnivorous Fish. PLoS ONE 7(9): e44898. doi:10.1371/journal.pone.0044898

Nguenga, D., Teugels, G.G., & Ollevier, F. (2000). Fertilization, hatching, survival and growth rates in reciprocal crosses of two strains of an African catfish Heterobranchus longifilis Valenciennes 1840 under controlled hatchery conditions. Aquaculture Research, 31(7), 565-573. https://doi.org/10.1046/j.1365-2109.2000.00468.x

Rana, K.J.; Siriwardena, S.; Hasan, M.R. Impact of Rising Feed Ingredient Prices on Aquafeeds and Aquaculture Production; FAO Fisheries and Aquaculture Technical Paper; FAO: Rome, Italy, 2009; p. 541.

Zlaugotne B, Pubule J, Blumberga D. Advantages and disadvantages of using more sustainable ingredients in fish feed. Heliyon. 2022 Sep 6;8(9):e10527. doi: 10.1016/j.heliyon.2022.e10527. PMID: 36119893; PMCID: PMC9475308.