NEXTGEN PROTEINS

Bioconversion of Underutilized Resources into Next Generation Proteins for Food and Feed

Project start: 01 October 2019 **Project duration: 48 months**

Deliverable No D4.4

Report on the effect of feeding alternative proteins on productive traits, physiological indicators, gut health and product quality and safety in salmon

Lead author/editor MATIS

Due Date of Submission: 28.03, 2023
Submission Date: 29.06, 2023

This Project has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement no. 862704.

www.nextgenproteins.eu

0 Document Information

Document Data

Work package related	Work package 4: Alternative protein application in feed products
Task related	Task 4.3: Dose response and field trials – Aquatic animals Task 4.4: Health, welfare and physiological indicators of animals in dose-response trials (subtask 4.4.1: Gut health, welfare and physiological indicators in poultry)
Type	Report
Dissemination level	e.g. PU (public) or CO (confidential)
Keywords	salmon, microalgae meal, insect meal, single-cell protein, performance, quality, plasma, gut, metabolome, microbiota.

Contributors

Authors	**Organisation's name**	**E-Mail**
Laura Martinez Rubio	Mowi Feed AS	Laura.rubio@mowi.com
Denise Bermudez	University of Stirling	dab00204@students.stir.ac.uk
Andrew Davie	Aquascot Ltd	Andrew.davie@aquascot.com
Emily Purvis	Aquascot Ltd	Emily.purvis@aquascot.com
Melissa Tillotson	Waitrose	melissa.tillotson@waitrose.co.uk
Georges Lamborelle	Matis	georges@matis.is
Sven-Ole Meiske	Matis	sven-ole@matis.is
Birgir Örn Smárason	Matis	birgir@matis.is
Serena Busti	Unibo	Serena-busti2@unibo.it

Document history

Document version #	**Date**	**Notes/Change**	**Status**
V1	7.3.2023	Matis	draft
V2	19.3.2023	Mowi/Aquascot/Waitrose	draft
V3	24.4.2023	Unibo	draft
V5	5.5.2023	Admin team review	Review
V6	29.6.2023	Final version reviewed and approved by Matis PST	Final

Table of content

1 Executive summary

This deliverable summarizes the main results concerning the effects of the dietary substitution of conventional ingredients with alternative protein sources, namely microalgae meal from VAXA, insect meal from Mutatec and single-cell proteins meal from Arbiom, "SylPro", on productive traits, physiological indicators, gut health and product quality in Atlantic salmon. For the dose-response trial, the effect of feeding alternative protein sources was assessed through a multi-disciplinary approach that included the evaluation of growth performance, plasma biochemistry and gut microbiota. It was intended to suggest what the best performing inclusion rate would be for use in the semi commercial scale trial, and to investigate plasma parameters and gut microbiota composition which are presented in this report. No statistically significant differences were found in initial body weight, final body weight and SGR. For FCR slight but significant effect was shown for Mutatec (FCR, $R^2 = 0.54$). This shows that a substitution with insect meal, SCPs meal from Arbiom and Processum, and Vaxa algae meal up to 21% does not affect salmon performance negatively in a dose-response trial on a pilot scale.

The semi commercial scale trial demonstrated that fish performance, as measured through a suite of routine production metrics, was comparable between the control diets and the test diets where all three alternative protein sources were included at 10% fixed inclusion. Broadly, flesh composition analysis was comparable across treatment groups and a consumer acceptance testing of the salmon samples showed that there was a perceived difference in the fillet samples with the pale ness of the algal protein fed salmon being a principle contributing factor to the overall reduction in purchase intent observed in the algal protein fed salmon compared to the control and SCP fed salmon. Ultimately this body of work has demonstrated that all three alternative protein sources can be used to substitute plant-based proteins in salmon feed formulations with no measurable impact on fish growth performance.

2 Introduction

The growth of the world population is increasing the demand for protein on a global scale, either for feed or food applications. Atlantic salmon farming is a rapidly growing industry, and with it comes the need for sustainable and cost-effective feed sources. Currently, most feed for farmed Atlantic salmon is based on different conventional raw materials such as soy, fishmeal and fish oil. The environmental impact of growing and importing soy and relying on wild fish stocks for feed has been a concern. Therefore, exploring alternative proteins for use in aquafeeds, to expand the list of available high-quality protein ingredients, is essential for the sustainability of the Atlantic salmon sector.

Recent research has investigated the potential of alternative protein sources, such as insect meal, single-cell proteins, and microalgae, as viable replacements for fishmeal in Atlantic salmon feeds (Borreal et al., 2019; Tacon et al., 2015). These alternative protein sources can potentially be more sustainable and have a lower environmental impact than many conventional sources, as they can be produced using fewer resources and do not rely on or compete with current food production. As research in this area continues to develop, it is expected that the use of alternative proteins in Atlantic salmon feeds will become more widespread and potentially lead to a more sustainable and efficient industry.

3 Dose-response trials

3.1 Aim

The aim of these dose-response trials for Atlantic salmon, carried out following a regression design, was to determine the optimal inclusion of the alternative proteins. Diets were formulated to contain increasing levels of alternative proteins by reducing the inclusion of conventional protein ingredients.

3.2 Materials and Methods

Feed formulation and approximate nutrients of feeds

Taking into account the nutritional needs of salmon, feeds containing the alternative proteins Mutatec *Hermetia illucens* larvae meal, VAXA microalgae, Arbiom SCPs, and Processum SCPs (*Paecilomyces variotii)* have been formulated. In the first dose-response trial, the diets tested were: a control diet based on 21% high-quality fish meal, and 9 (3 diets for each novel ingredient) experimental with increasing dietary level of torula meal (Arbiom), black soldier fly (Mutatec), and algae meal (Vaxa) in order to totally replace the fish meal dietary level.
In the second dose-response trial, the diets tested were 4: a control diet based on 21% high-quality fish meal, and 3 experimental diets containing SCPs from Processum. The diets were produced by extrusion technology at the Feed Technology Centre of Nofima, Bergen, Norway.
The proximate analysis was conducted on all diets in order to ensure a similar nutrient profile in all the treatments. The results of the diets' proximate analysis are shown in Table 3.2.1, while the ingredients composition of the experimental diets is shown in Table 3.2.2

Table 3.2.1: Proximate composition of diets.

Experimental diets	DM	CA	CP	CL
Control	89.4	8.1	51.8	23.9
Arbiom 7%	92.0	9.5	51.0	27.6
Arbiom 14%	92.8	14.0	52.7	26.8
Arbiom 21%	90.4	14.3	50.2	25.8
Mutatec 7%	92.3	9.4	53.4	26.1
Mutatec 14%	90.8	7.8	52.3	24.2
Mutatec 21%	92.3	9.7	52.6	28.1
Vaxa 7%	91.8	9.4	53.0	24.7
Vaxa 14%	91.9	9.3	50.6	24.6
Vaxa 21%	92.3	11.9	52.9	24.9
Processum 7%	91.9	9.4	53.8	24.7
Processum 14%	92.4	14.2	53.6	25.6
Processum 21%	91.6	10.3	53.9	27.9

DM= dry matter; CA= crude ash; CP=crude protein; CL= crude lipid

Table 3.2.2: Ingredients (%) composition of diets for dose-response trials on salmon containing different inclusion levels of Mutatec *Hermetia illucens* larvae meal, VAXA algae, Arbiom SCPs, and Processum SCPs (*Paecilomyces variotii*).

	Experimental diets												
Ingredients (%)	CTRL	7 Arbiom	14 Arbiom	21 Arbiom	7 Mutatec	14 Mutatec	21 Mutatec	7 Vaxa	14 Vaxa	21 Vaxa	7 Processum	14 Processum	21 Processum
ARBIOM torula yeast	-	7	14	21	-	-	-	-	-	-	-	-	-
Mutatec Black soldier fly	-	-	-	-	7	14	21	-	-	-	-	-	-
VAXA Algae meal	-	-	-	-	-	-	-	7	14	21	-	-	-
PROCESSUM SCPs	-	-	-	-	-	-	-	-	-	-	7	14	21
Fish meal	21	14	7	-	14	7	-	14	7	-	14	7	-
Fish oil	12.86	13.50	14.15	14.79	13.50	14.15	14.79	13.50	14.15	14.79	13.50	14.15	14.79
Soy protein concentrate	22.57	16.88	11.77	6.67	16.87	11.77	6.99	19.47	16.50	15.31	20.74	14.43	8.03
Wheat gluten meal	8.80	10.40	13.01	15.61	10.36	12.94	15.49	8.71	8.85	9.16	11.12	13.72	18.29
Pea protein concentrate	7.90	11.51	13.25	15.00	11.51	13.25	15.00	9.87	11.39	12.51	9.06	13.06	15.00
Wheat	5.88	5.45	5.01	4.56	6.03	6.17	6.00	6.44	6.99	6.02	3.31	2.38	1.32
Horse beans	5	5	5	5	5	5	5	5	5	5	5	5	5
Corn gluten meal	5	5	5	5	5	5	5	5	5	5	5	5	5
Rapeseed oil	5.54	5.15	4.84	4.53	4.49	3.53	2.57	4.75	3.98	3.23	4.93	4.12	3.36
Lecithin	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Synthetic amino acids	0.35	0.66	1.07	1.47	0.65	1.05	1.42	0.73	1.14	1.45	0.61	0.96	1.45
Astaxanthin	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Vitamin premixes	0.69	0.74	0.78	0.83	0.74	0.78	0.83	0.74	0.78	0.83	0.74	0.78	0.83
Mineral premixes	1.70	1.77	1.86	1.94	1.92	2.15	2.38	1.95	2.20	2.44	1.81	1.95	2.11
Water	1.15	1.39	1.70	2.02	1.36	1.66	1.97	1.29	1.44	1.71	1.61	1.88	2.26

BSF = Black soldier fly; SCP = Single Cell Protein; MAP = Mono Ammonium Phosphate; SCP = Soy Protein Concentrate; PPC = Pea Protein Concentrate

Fish and feeding

The feeding experiment consisted of 2 trials which were run for 56 days each in 12 °C artificial seawater (33 ppt) using Atlantic salmon (*Salmo salar*). The experiments were conducted at MARS (Matís Aquaculture Research Station) in 36 identical 200 l circular PE tanks with a slightly sloped bottom (**Figure 3.2.1**). The tanks were incorporated into a recirculation system. Water was cleaned biologically (moving bed filter), chemically (ozonisation), and mechanically (UV radiation, particle removal by filters, protein skimming). The water in the system was replaced with tap water at a rate of 0 – 5 % daily, depending on chemical water analyses and water loss in the system. The water quality parameters are shown in **Table 3.2.3** and **Table 3.2.4**, and the parameters never exceeded the suitable level for Atlantic salmon.

Fish were obtained from a local salmon farm and the trials started after an acclimation time of 2 weeks to ensure that the salmon had the desired start weight and got used to the experimental tanks. During this acclimation time, the fish were fed with commercial feed (Laxá ECO-LF 2.5 mm)

The fish were fed 3 times a day with an automatic feeding system and the feed amount was given in excess to ensure that the salmon were overfed during the whole trial period. Uneaten feed was collected once a day and dried to calculate the feed intake and FCR (Feed Conversion Ratio).

In the first dose-response trial, 18 fish were randomly allocated to each tank (30 tanks in total were used). The initial weight of 18 fish was on average 81g at the beginning of the trial, and approximately 170 g at the end of the trial.

In the 2nd dose-response trial, 20 fish were randomly distributed to each tank. The fish's initial body weight was on average 83 g and, at the end of the trial, the animals reached an average weight of 212 g.

During the trials temperature and oxygen were measured automatically and salinity, ammonia, nitrite and nitrate manually.

Table 3.2.3: Water quality parameter and measured value throughout trial.

	Measuring frequency	**Daily Average**	
Temperature	Every 10 minutes	12.81 ± 0.94	°C
Oxygen	Every 10 minutes	9.65 ± 0.58	mg l^{-1}
Salinity	Once a day	27.89 ± 1.02	ppt
pH	Once a day	7.03 ± 0.20	
Ammonia	Once a day	0.53 ± 0.12	mg l^{-1}
Nitrite	Once a day	0.59 ± 0.12	mg l^{-1}
Nitrate	Once a day	145.42 ± 46.08	mg l^{-1}

Table 3.2.4: Water quality parameter and measured value throughout trial 2.

	Measuring frequency	Daily Average	
Temperature	Every 10 minutes	12.49 ± 0.77	°C
Oxygen	Every 10 minutes	9.91 ± 0.52	mg l^{-1}
Salinity	Once a day	33.27 ± 1.53	ppt
pH	Once a day	6.78 ± 0.33	
Ammonia	Once a day	0.70 ± 0.11	mg l^{-1}
Nitrite	Once a day	0.32 ± 0.10	mg l^{-1}
Nitrate	Once a day	142.84 ± 56.88	mg l^{-1}

Figure 3.2.1: RAS used during trials.

Dose-response trials sampling

At the beginning, and at the end of the trials, all fish in each tank were individually weighted, for growth performance calculation (final body weight, specific growth rate, feed conversion rate).
At the end of trial 1, 3 fish per tank were sampled to collect blood and faeces for plasma biochemistry and gut microbiome analysis. The blood samples were centrifuged at 5000 rpm for 3 minutes to separate the blood plasma from the other components in the blood. The gut microbiome samples were taken from the hindgut and stored in dry ice right after sampling. The blood plasma samples and faeces samples were stored in 1.5 ml Eppendorf cups at -80°C until they were shipped on dry ice for analysis to the University of Bologna.
Also, at the end of trial 2, blood and faeces were collected. Blood samples were analysed at the University of Bologna, while gut microbiota analysis was conducted at Matís (Iceland).
The blood samples were centrifuged at 5000 rpm for 3 minutes to separate the blood plasma from the other components in the blood. The gut microbiome samples were taken from the hindgut and stored in dry ice right after sampling. The blood plasma samples were stored in 1.5 ml Eppendorf cups at -80°C until they were shipped on dry ice for analysis to the University of Bologna.

Metabolic parameters in plasma

Glucose (GLU), urea, creatinine (CREA), uric acid, total bilirubin (Tot bill), cholesterol (CHOL), high-density lipoprotein (HDL), triglycerides (TRIG), total protein (TP), albumin (ALB), aspartate

aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), calcium (Ca^{+2}), phosphorus (P), potassium (K^{+}), sodium (Na^{+}), iron (Fe), chloride (Cl), magnesium (Mg), and ALB/globulins (A/G) were measured in the plasma using samples of 500 µL on an automated analyzer (AU 480; Olympus/Beckman Coulter, Brea, CA, United State) according to the manufacturer's instructions (Parma et al., 2023). The A/G, Na/K ratio, and Ca x P were calculated.

Gut bacterial community DNA extraction and sequencing in dose-response trial 1

Total microbial DNA was extracted, quantified with NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE), and stored at -20 °C until further processing. To perform the 16S rRNA gene analysis, the V3–V4 hypervariable regions were amplified using the 2 KAPA HiFi HotStart ReadyMix (KAPA Biosystems) using 341F and 785R primers with overhang Illumina sequencing adapters, as previously described (Pelusio et al., 2021). Briefly, the thermal cycle consisted of an initial denaturation at 95 °C for 3 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 C for the 30s and extension at 72°C for 30 s, and a final extension step at 72 °C for 5 min. As recommended in the Illumina protocol "16S Metagenomic Sequencing Library Preparation" for the MiSeq system, PCR reactions were cleaned up by using Agencourt AMPure XP magnetic beads. A limited-cycle PCR was performed to obtain the indexed library using Nextera technology, followed by a second AMPure XP magnetic beads clean-up step. Sequencing was performed on the Illumina MiSeq platform using a 2 x 250 bp paired-end protocol according to the manufacturer's instructions (Illumina, San Diego, CA). At the end of the sequencing process, raw sequences were processed combining PANDAseq and QIIME2 pipelines (Bolyen et al., 2019). High-quality reads, obtained after a filtering step for length (min/max = 350/550 bp) and quality with default parameters, were cleaned using DADA2 (Callahan et al., 2016) and clustered into amplicon sequence variants (ASVs) using VSEARCH algorithm (Rognes et al., 2016). Taxonomy was assigned using RDP classifier against SILVA database (Quast et al., 2013). Three different metrics were used to evaluate internal ecosystem diversity (alpha-diversity) – Faith's Phylogenetic Diversity (faith_pd), Shannon_entropy index, and number of observed ASVs (observed features). Unweighted UniFrac distances were computed to estimate inter-sample ecosystem diversity (beta-diversity) and used as input for Principal Coordinates Analysis (PCoA).

Statistical analysis

All data are presented in tables as mean ± standard deviation (SD), and in graphs. Growth and plasma biochemistry analysis results were analysed by one-way variance analysis (ANOVA). Statistical analyses were performed using GraphPad Prism 6.0 for Windows (Graph Pad Software, San Diego, CA, USA). Data were considered significant at $p \leq 0.05$.

Microbiota analysis and respective plots were produced using R software (https://www.r-project.org/) with "vegan" (http://www.cran.r-project.org/package-vegan/), "Made4" (Culhane et al., 2005) and "stats" packages (https://stat.ethz.ch/R-manual/R-devel/library/stats/html/00Index.html). Data separation was tested by a permutation test with pseudo-F ratios (function "Adonis" in "vegan" package). When required, Wilcoxon and Kruskal–Wallis test were used to assess significant differences in alpha diversity and taxon relative abundance between groups. p-values were corrected for multiple testing with the Benjamini–

Hochberg method, with a false discovery rate (FDR) ≤ 0.05 considered statistically significant (function p.adjust in the "stats" package).

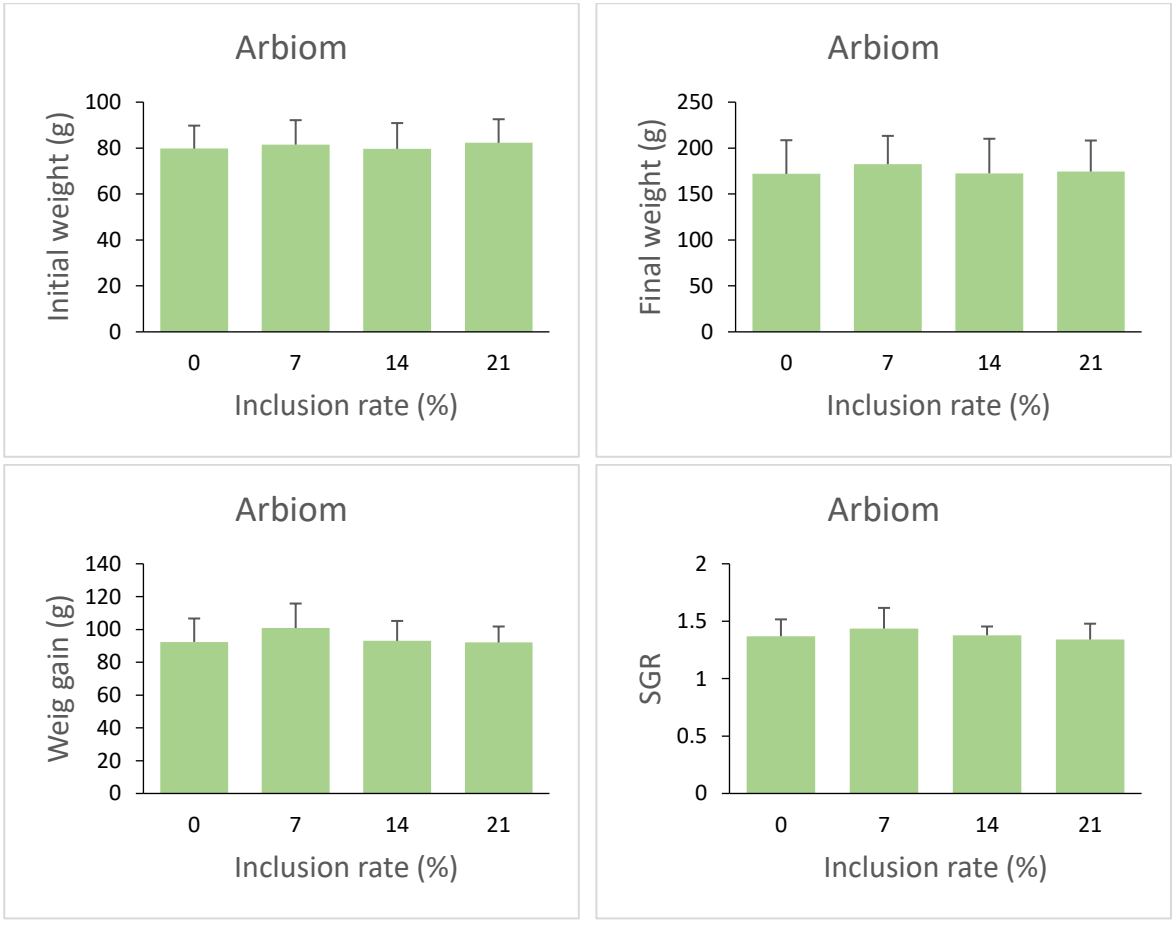
Calculations

The calculations for the determination of performance parameters were the following: Weight gain: Final body weight (FBW)- Initial body weight (IBW); Specific growth rate (SGR) (% day-1) = 100 * (ln FBW- ln IBW) / days; Feed conversion ratio (FCR) = feed intake/weight gain. Condition factor (CF) = (body weight (g)/total length (cm3)) x 100.

3.3 Results

Results of the first dose-response trial
Growth performance

The growth performance of the first dose-response trial on salmon is shown in Figure 3.3.1, Figure 3.3.2, Figure 3.3.3. No statistically significant differences were found in initial body weight, final body weight and SGR. For FCR slight but significant effect was shown for Mutatec (FCR, R^2 = 0.54). This shows that a substitution with insect meal, SCPs meal from Arbiom and Vaxa algae meal up to 21% does not affect salmon performance negatively (**Table 3.3.1**, **Table 3.3.2**, **Table 3.3.3**).



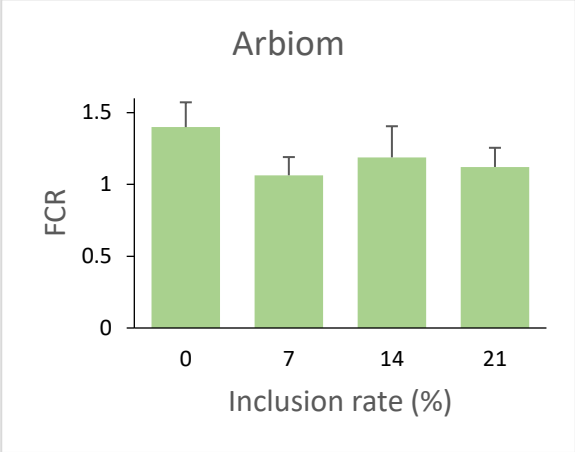


Figure 3.3.1: Visualisation of the parameters initial weight, final weight, weight gain, SGR and FCR of the experimental feeds with Arbiom single cell protein meal inclusions.

Table 3.3.1: Statistical parameters for initial weight, final weight, weight gain, SGR and FCR for all treatments of experimental feed with Arbiom single cell protein meal inclusion.

Initial	Value	Std.Error	DF	t-value	p-value
(Intercept)	79.94815	1.599344	204	49.9881	0
InclusionRate	0.08254	0.122126	10	0.67585	0.5145

Final	Value	Std.Error	DF	t-value	p-value
(Intercept)	175.9157	6.226568	190	28.25243	0
InclusionRate	-0.04561	0.473474	10	-0.09632	0.9252

Weight gain	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	95.867	5.895	16.262	1.60E-08
Inclusion	-0.1253	0.4501	-0.278	0.786

SGR	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.402258	0.062814	22.324	7.31E-10
Inclusion	-0.00205	0.004797	-0.427	0.679

FCR	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.402258	0.062814	22.324	7.31E-10
Inclusion	-0.00205	0.004797	-0.427	0.679

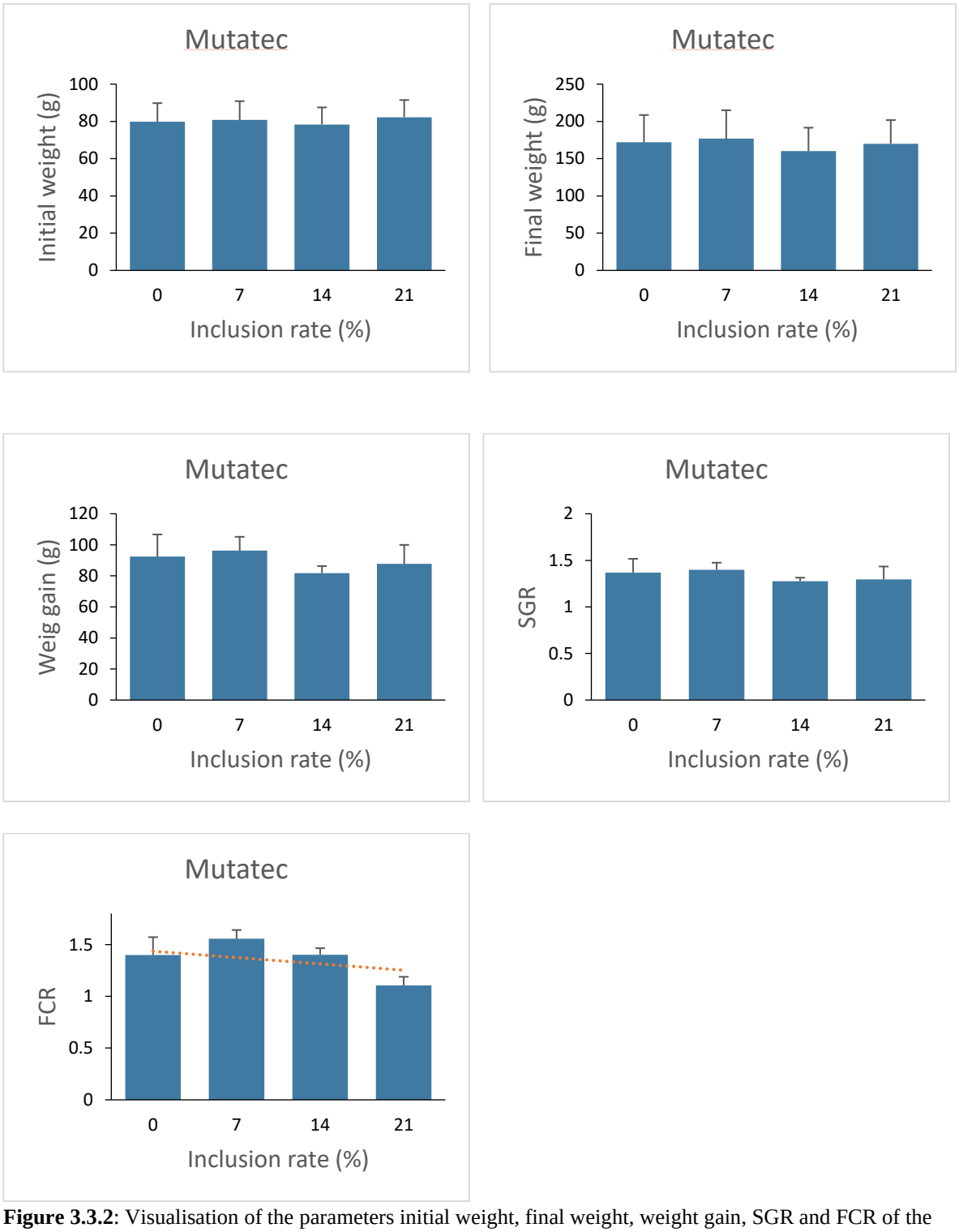


Figure 3.3.2: Visualisation of the parameters initial weight, final weight, weight gain, SGR and FCR of the experimental feeds with Mutatec insect meal inclusions.

Table 3.3.2: Statistical parameters for initial weight, final weight, weight gain, SGR and FCR for all treatments of experimental feed with Mutatec insect meal inclusion.

Initial	Value	Std.Error	DF	t-value	p-value
(Intercept)	79.53704	1.261244	204	63.06239	0
InclusionRate	0.07011	0.096309	10	0.72793	0.4834

Final	Value	Std.Error	DF	t-value	p-value
(Intercept)	173.2307	5.785961	191	29.93983	0
InclusionRate	-0.32691	0.437695	10	-0.74688	0.4723

Weight gain	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	93.3827	4.1312	22.6	6.47E-10
Inclusion	-0.4419	0.3274	-1.35	0.207

SGR	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.378942	0.040574	33.986	1.15E-11
Inclusion	-0.00506	0.003215	-1.573	0.147

FCR	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.496995	0.055624	26.913	1.16E-10
Inclusion	-0.01502	0.004408	-3.407	0.00669

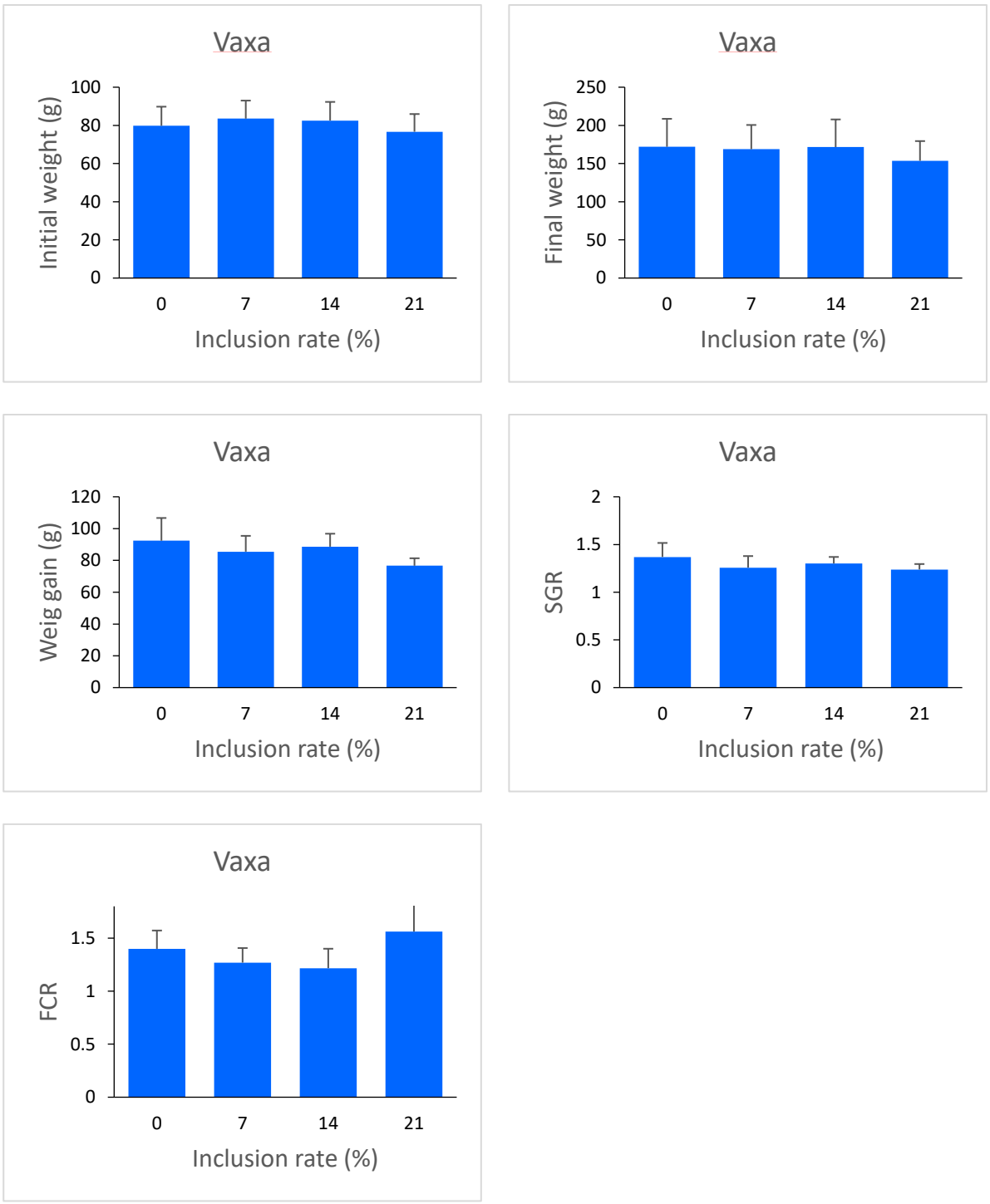


Figure 3.3.3: Visualisation of the parameters initial weight, final weight, weight gain, SGR and FCR of the experimental feeds with Vaxa algae meal inclusions.

Table 3.3.3: Statistical parameters for initial weight, final weight, weight gain, SGR and FCR for all treatments of experimental feed with Vaxa microalgae inclusion.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	82.17037	1.627199	204	50.49805	0
InclusionRate	-0.14762	0.12425	410	-1.18805	0.2623

	Value	Std.Error	DF	t-value	p-value
(Intercept)	174.45762	5.224585	186	33.39167	0
InclusionRate	-0.75895	0.39862	310	-1.90394	0.0861

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	89.8027	3.9627	22.662	6.31E-10
Inclusion	-0.4595	0.314	-1.463	0.174

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.31745	0.042071	31.315	2.59E-11
Inclusion	-0.002996	0.003334	-0.899	0.39

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.30422	0.114933	11.348	4.93E-07
Inclusion	0.006585	0.009108	0.723	0.486

Plasma biochemistry parameters

The plasma parameters investigated are shown in the tables below. In **Table 3.3.4** Plasma parameters of the control diet were compared to the ones of diets containing algae meal from Vaxa. In **Table 3.3.5** control diet was compared to diets containing insect meal from Mutatec, and in the last table (**Table 3.3.6**) control diet was compared to diets containing SCPs meal from Arbiom.
The diet VAX21 showed higher triglyceride values (P=0.0572) with respect to the control diet. Also, VAX21 showed higher Fe values (P=0.0016) with respect to Control and VAX7 diets. No significant differences were detected between the control diet and the diets containing insect meal. Higher urea values (P=0.0479) were found in Diet ARB21 with respect to ARB7, and higher triglyceride values (P=0.0181) were detected in ARB14 with respect to the control diet.

Table 3.3.4: Plasma biochemistry values for Atlantic salmon fed the Control diet and experimental diets containing microalgae meal from Vaxa.

Parameters	*Experimental diets*				
	CTRL	VAX7	VAX14	VAX 21	*P- value*
GLU (mg dL^{-1})	82.8 ± 5.26	79.8 ± 8.04	76.9 ± 15.19	79.4 ± 13.54	*0.7445*
Lactate (mg dL^{-1})	21.4 ± 9.23	18.3 ± 7.05	27.5 ± 7.97	19.6 ± 6.06	*0.0946*
Urea (mg dL^{-1})	5.19 ± 1.33	5.14 ± 1.68	4.69 ± 1.55	6.00 ± 0.80	*0.2618*
CREA (mg dL-1)	0.18 ± 0.03	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.04	*0.4018*
Uric acid (mg dL^{-1})	0.19 ± 0.09	0.19 ± 0.06	0.14 ± 0.07	0.16 ± 0.06	*0.3757*
Tot Bil (mg dL^{-1})	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	*0.4055*
CHOL (mg dL^{-1})	279.7 ± 56.0	261.3 ± 33.6	265.6 ± 61.0	235.6 ± 80.6	*0.4825*
HDL (mg dL^{-1})	126.1 ± 23.6	122.4 ± 14.5	118.13 ± 25.2	104.0 ± 30.4	*0.246*
TRIG (mg dL^{-1})	88.2 ± 21.6^{a}	108.8 ± 18.9^{ab}	99.6 ± 25.1^{ab}	120.0 ± 29.9^{b}	*0.0572*
TP (g dL^{-1})	4.81 ± 0.66	4.48 ± 0.79	4.68 ± 0.89	4.40 ± 1.08	*0.7424*
ALB (g dL-1)	1.51 ± 0.12	1.4 ± 0.32	1.35 ± 0.18	1.28 ± 0.32	*0.6863*
AST (U L^{-1})	535.56 ± 224.73	596.67 ± 427.23	471.38 ± 134.90	559.67 ± 300.18	*0.849*
ALT (U L^{-1})	11.89 ± 8.43	14.89 ± 24.14	13.33 ± 17.38	10.67 ± 11.63	*0.9537*
ALP (U L^{-1})	156.44 ± 43.60	148.56 ± 12.45	154.44 ± 43.59	168.11± 48.86	*0.7671*
CK (U L^{-1})	13671.6 ± 14385.6	13186.3 ± 11326.6	10227.5 ± 6976.9	15041.8 ± 17413.6	*0.9017*
LDH (U L^{-1})	3140.9 ± 2028.5	5920.9 ± 11649.8	2322.4 ± 966.4	4410.4 ± 8008.5	*0.7519*
Ca^{+2} (mg dL^{-1})	11.1 ± 0.46	10.9 ± 0.87	11.1 ± 1.00	10.8 ± 1.45	*0.8527*
P (mg dL^{-1})	13.6 ± 3.25	14.3 ± 5.25	12.9 ± 1.37	13.2 ± 3.57	*0.8727*
K^{+} (mEq L^{-1})	1.68 ± 0.81	2.34 ± 2.09	2.34 ± 1.70	2.68 ± 2.04	*0.6681*
Na^{+} (mEq L^{-1})	164 ± 2.96	163.1 ± 3.44	164.8 ± 3.03	162.8 ± 3.96	*0.592*
Fe (µg dL^{-1})	40.7 ± 20.5^{a}	43.9 ± 10.2^{ab}	64.3 ± 16.9^{bc}	72.6 ± 23.2^{c}	*0.0016*
Cl (mEq L^{-1})	129.6 ± 4.02	130.0 ± 3.34	132.3 ± 5.15	130.9 ± 4.18	*0.5759*
Mg (mg dL^{-1})	2.81 ± 0.47	2.82 ± 0.57	2.64 ± 0.44	2.73 ± 0.64	*0.8912*
A/G	0.44 ± 0.03	0.45 ± 0.04	0.41 ± 0.05	0.43 ± 0.02	*0.3117*

Data are given as the mean (n=3) ± SD. Different superscript letters indicate significant differences among treatments (One-way Anova $p \leq .05$). VAX, microalgae meal; GLU, Glucose; Tot Bil, total bilirubin; CHOL, cholesterol; HDL, high density lipoprotein; TRIG, triglycerides; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine amino transferase; ALP, alkaline phosphatase; CK, creatine kinase; LDH, lactate dehydrogenase, Ca^{+2}, calcium; P, inorganic phosphorus; K+, potassium; Na+, sodium; Fe, iron; Cl, chloride; Mg, magnesium; A/G, albumin/globulins.

Table 3.3.5: Plasma biochemistry values for Atlantic salmon fed the Control diet and experimental diets containing insect meal from Mutatec.

Parameters	*Experimental diets*				
	CTRL	MUT7	MUT14	MUT21	*P- value*
GLU (mg dL-1)	82.8 ± 5.26	75.2 ± 8.30	79.2 ± 12.92	77.2 ± 6.00	*0.3236*
Lactate (mg dL^{-1})	21.4 ± 9.23	19.4 ± 6.09	19.3 ± 8.64	28.1 ± 7.30	*0.0817*
Urea (mg dL^{-1})	5.19 ± 1.33	5.30 ± 1.33	4.67 ± 2.16	4.60 ± 1.73	*0.7485*
CREA (mg dL-1)	0.18 ± 0.03	0.16 ± 0.02	0.15 ± 0.01	0.17 ± 0.06	*0.2486*
Uric acid (mg dL^{-1})	0.19 ± 0.09	0.19 ± 0.05	0.19 ± 0.05	0.19 ± 0.04	*0.9654*
Tot Bil (mg dL^{-1})	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	*0.5404*
CHOL (mg dL-1)	279.7 ± 56.0	277.6 ± 54.0	252.9 ± 41.7	283.2 ± 50.7	*0.5801*
HDL (mg dL^{-1})	126.1 ± 23.6	125.2 ± 17.9	117.1 ± 25.1	124.3 ± 25.1	*0.8282*
TRIG (mg dL^{-1})	88.2 ± 21.6	115.8 ± 25.9	105.9 ± 30.5	120.6 ± 31.0	*0.0834*
TP (g dL^{-1})	4.81 ± 0.66	4.47 ± 0.49	4.29 ± 0.59	4.42 ± 0.43	*0.2462*
ALB (g dL^{-1})	1.51 ± 0.12	1.29 ± 0.11	1.31 ± 0.16	1.33 ± 0.17	*0.3567*
AST (U L^{-1})	535.56 ± 224.73	472.56 ± 103.00	452.78 ± 100.14	612.78 ± 484.96	*0.6125*
ALT (U L^{-1})	11.89 ± 8.43	6.44 ± 2.79	9.2 ± 4.29	14.44 ± 19.16	*0.4279*
ALP (U L^{-1})	156.44 ± 43.60	178.33 ± 63.04	180.8 ± 69.45	154.67 ± 38.13	*0.6309*
CK (U L^{-1})	13671.6 ± 14385.6	9598.8 ± 4192.1	10140.2 ± 4785.7	18845.4 ± 25351.5	*0.5402*
LDH (U L^{-1})	3140.9 ± 2028.5	2690.7 ± 1702.9	3062.9 ± 1691.1	6455.8 ± 12233.1	*0.5612*
Ca^{+2} (mg dL^{-1})	11.1 ± 0.46	10.9 ± 0.90	11.0 ± 1.09	10.9 ± 0.40	*0.8702*
P (mg dL^{-1})	13.6 ± 3.25	12.8 ± 1.70	13.4 ± 2.46	14.8 ± 3.44	*0.5019*
K^{+} (mEq L^{-1})	1.68 ± 0.81	2.71 ± 1.49	2.13 ± 1.46	2.00 ± 1.41	*0.4256*
Na^{+} (mEq L^{-1})	164 ± 2.96	163.6 ± 3.09	162.9 ± 2.47	165.2 ± 2.54	*0.3357*
Fe (µg dL^{-1})	40.7 ± 20.5	37.0 ± 13.2	34.9 ± 15.2	41.2 ± 8.5	*0.7778*
Cl (mEq L^{-1})	129.6 ± 4.02	132.5 ± 5.69	132.1 ± 6.44	132.4 ± 2.85	*0.5887*
Mg (mg dL^{-1})	2.81 ± 0.47	2.71 ± 0.36	2.72 ± 0.27	2.92 ± 0.45	*0.6406*
A/G	0.44 ± 0.03	0.43 ± 0.02	0.45 ± 0.03	0.44 ± 0.04	*0.4896*

Data are given as the mean (n=3) ± SD. Different superscript letters indicate significant differences among treatments (One-way Anova $p \leq .05$). MUT, mutatec meal; GLU, Glucose; Tot Bil, total bilirubin; CHOL, cholesterol; HDL, high density lipoprotein; TRIG, triglycerides; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine amino transferase; ALP, alkaline phosphatase; CK, creatine kinase; LDH, lactate dehydrogenase, Ca^{+2}, calcium; P, inorganic phosphorus; K+, potassium; Na+, sodium; Fe, iron; Cl, chloride; Mg, magnesium; A/G, albumin/globulins.

Table 3.3.6: Plasma biochemistry values for Atlantic salmon fed the Control diet and experimental diets containing SCPs meal from Arbiom.

Parameters	*Experimental diets*				
	CTRL	ARB7	ARB14	ARB21	P- value
GLU (mg dL^{-1})	82.8 ± 5.26	76.7 ± 5.83	82.4 ± 5.83	88.1 ± 14.90	0.0944
Lactate (mg dL^{-1})	21.4 ± 9.23	23.4 ± 9.26	20.7 ± 6.73	23.9 ± 10.42	0.8589
Urea (mg dL^{-1})	5.19 ± 1.33^{ab}	4.03 ± 1.43^{a}	4.51 ± 1.09^{ab}	5.89 ± 1.74^{b}	0.0479
CREA (mg dL^{-1})	0.18 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.16 ± 0.03	0.3097
Uric acid (mg dL^{-1})	0.19 ± 0.09	0.20 ± 0.03	0.17 ± 0.03	0.21 ± 0.04	0.5667
Tot Bil (mg dL^{-1})	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.7797
CHOL (mg dL^{-1})	279.7 ± 56.0	261.0 ± 48.7	305.0 ± 29.4	256.2 ± 39.2	0.1042
HDL (mg dL^{-1})	126.1 ± 23.6	124.6 ± 20.9	137.0 ± 8.2	125.0 ± 20.7	0.478
TRIG (mg dL^{-1})	88.2 ± 21.6^{a}	101.7 ± 25.5^{ab}	119.4 ± 15.7^{b}	99.0 ± 13.9^{ab}	0.0181
TP (g dL^{-1})	4.81 ± 0.66	4.34 ± 0.73	4.65 ± 0.53	4.49 ± 0.82	0.5169
ALB (g dL^{-1})	1.51 ± 0.12	1.32 ± 0.25	1.41 ± 0.28	1.27 ± 0.15	0.3652
AST (U L^{-1})	535.56 ± 224.73	570.33 ± 336.64	562.56 ± 151.72	561.11 ± 368.90	0.9944
ALT (U L^{-1})	11.89 ± 8.43	9.78 ± 10.57	10.78 ± 5.54	7.11 ± 7.30	0.6434
ALP (U L-1)	156.44 ± 43.60	150.11± 43.00	180.78 ± 38.66	175.11 ± 33.25	0.3191
CK (U L-1)	13671.6 ± 14385.6	21890.7 ± 27118.7	18289.9 ± 12166.4	16886.4 ± 23852.9	0.8596
LDH (U L-1)	3140.9 ± 2028.5	2002.9 ± 1616.7	2854 ± 1901.6	2015.9 ± 1304.8	0.3995
Ca+2 (mg dL^{-1})	11.1 ± 0.46	10.7 ± 0.70	11.1 ± 0.83	10.9 ± 1.07	0.5641
P (mg $dL^{-1)}$	13.6 ± 3.25	12.2 ± 2.10	13.8 ± 2.16	14.0 ± 3.77	0.5419
K+ (mEq L^{-1})	1.68 ± 0.81	1.83 ± 1.22	2.43 ± 2.13	3.09 ± 1.69	0.2169
Na+ (mEq L^{-1})	164 ± 2.96	163.3 ± 3.04	164.0 ± 4.95	163.3 ± 3.54	0.9611
Fe (µg dL^{-1})	40.7 ± 20.5	44.3 ± 14.5	40.4 ± 9.3	34.6 ± 8.0	0.5278
Cl (mEq L^{-1})	129.6 ± 4.02	132.3 ± 2.56	132.4 ± 5.39	133.1 ± 4.89	0.3694
Mg (mg dL^{-1})	2.81 ± 0.47	2.52 ± 0.43	2.93 ± 0.41	2.59 ± 0.55	0.2332
A/G	0.44 ± 0.03	0.43 ± 0.03	0.44 ± 0.04	0.42 ± 0.05	0.7278

Data are given as the mean (n=3) ± SD. Different superscript letters indicate significant differences among treatments (One-way Anova $p \leq .05$). ARB, arbiom meal; GLU, Glucose; Tot Bil, total bilirubin; CHOL, cholesterol; HDL, high density lipoprotein; TRIG, triglycerides; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine amino transferase; ALP, alkaline phosphatase; CK, creatine kinase; LDH, lactate dehydrogenase, Ca+2, calcium; P, inorganic phosphorus; K+, potassium; Na+, sodium; Fe, iron; Cl, chloride; Mg, magnesium; A/G, albumin/globulins.

Gut microbiota analysis

To highlight the gut microbiota (GM) composition of salmon in the different dietary groups, the overall composition at various phylogenetic levels was investigated at the family level (Figure 3.3.4). In diets containing SCPs meal from Arbiom, the microbial family composition was dominated by Streptococcaceae at 50%, followed by Lactobacillaceae at 34.2%, and Bacillaceae at 7.37%. In diets containing insect meal from Mutatec, the dominant microbial family was Paenibacillaceae with 38% of abundance, followed by Streptococcaceae at 20.1%, and Lactobacillaceae at 17.6%. The microbial family abundance of diets containing algae meal from Vaxa was dominated by Lactobacillaceae at 38.5%, followed by Streptococcaceae at 29.7%, and Actinobacteria at 4.36%. Finally, the control diet microbial family composition was dominated by Lactobacillaceae at 49.9%, Streptococcaceae at 34.7%, and Bacillaceae at 2.38%.

The internal ecosystem diversity for each dietary group (alpha-diversity), and the GM variations between samples (beta-diversity) were assessed respectively by the calculation of three different metrics: PD_whole_tree, obvserved_feature (number of ASV) and Shannon index (**Figure 3.3.5**), and by the principal coordinates Analysis (PCoA) based on unweighted and weighted Unifrac distances (**Figure 3.3.6**).

Concerning internal ecosystems diversity, in all the 3 metrics calculated a significantly higher diversity was displayed by diets containing Vaxa with respect to the control diet ($P<0.05$). According to the Shannon index, diets containing Arbiom SCPs meals were significantly lower compared to the control group ($P<0.05$). No significant differences were detected between diets containing Mutatec insect meal and the control diet in all the 3 metrics calculated $P>0.05$).

Results of beta diversity showed that dietary insect meal levels significantly affected GM in terms of overall GM composition. In fact, diets containing Mutatec insect meal displayed a clear and significant separation from the other diets ($P<0.05$).

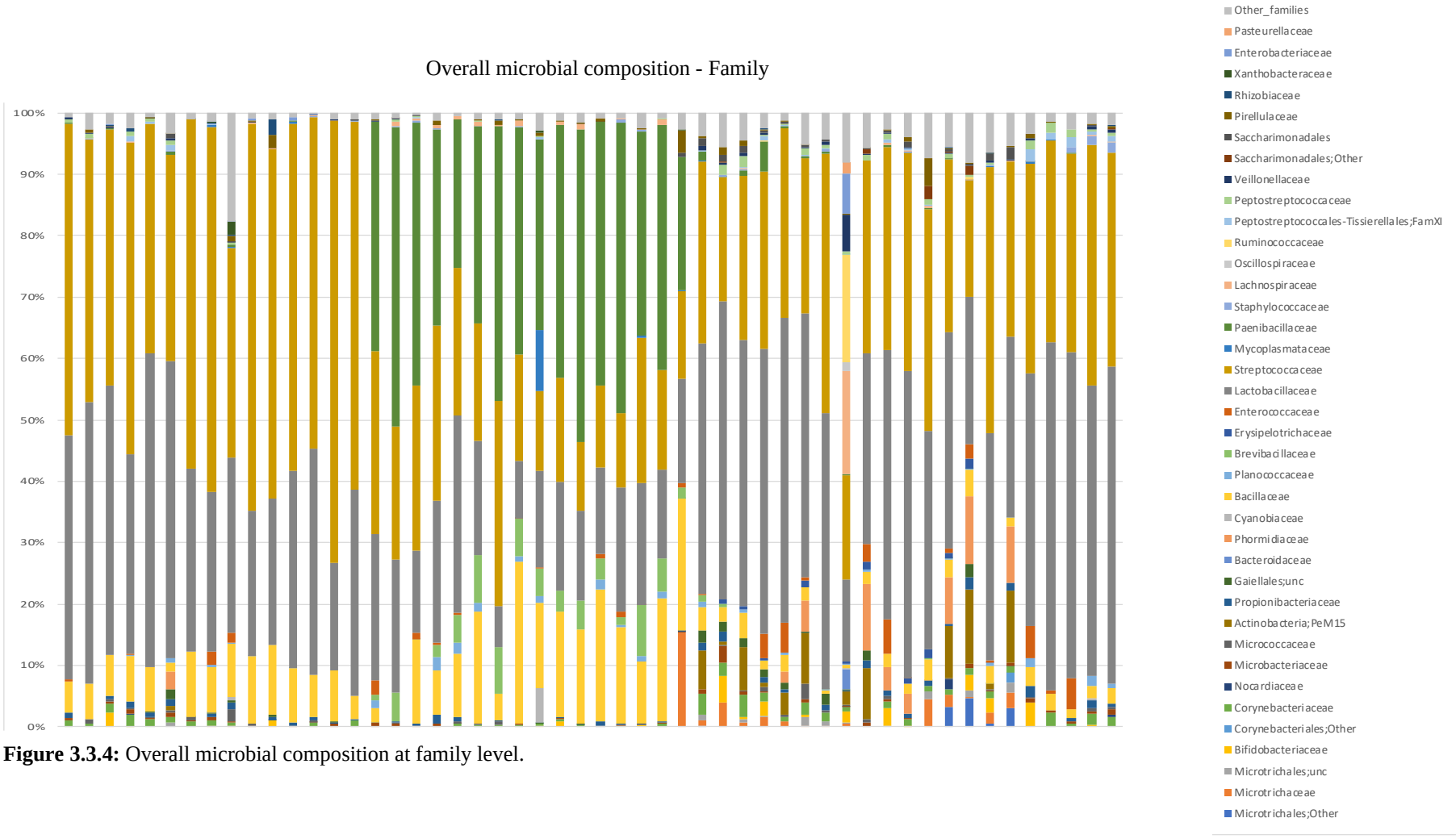
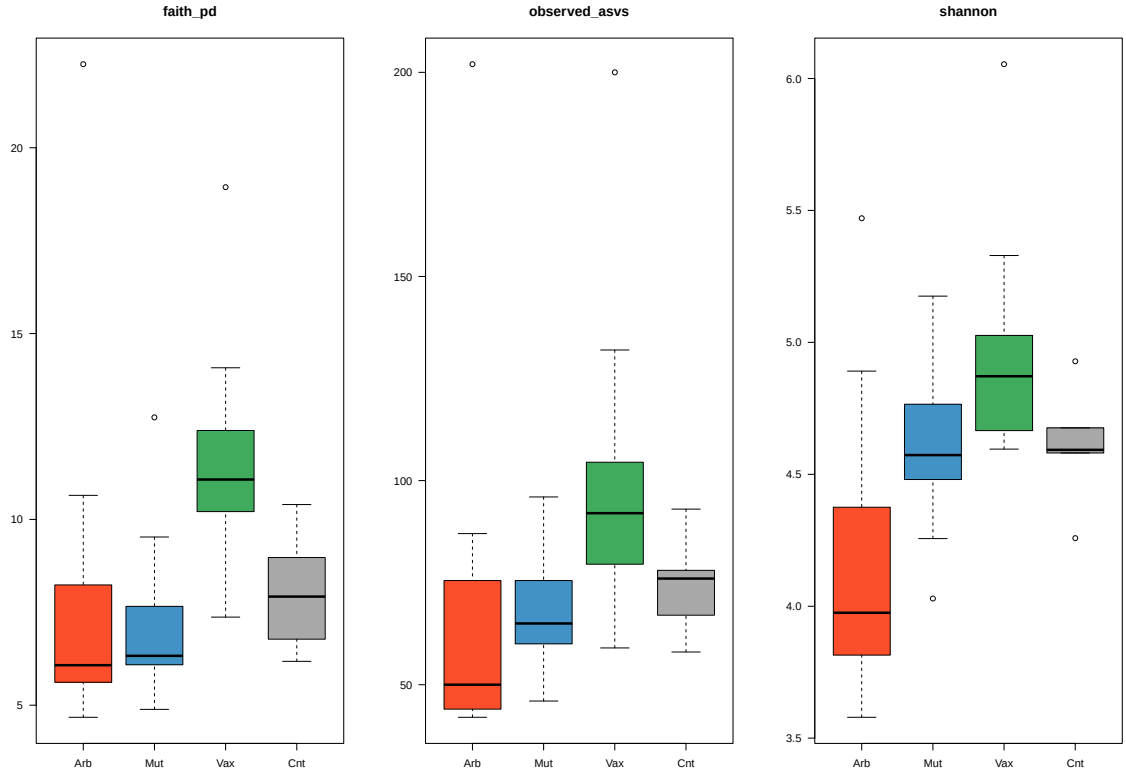


Figure 3.3.4: Overall microbial composition at family level.



Faith_PD

diet 1	diet 2	p-value
Arb	Mut	0.5
Arb	Vax	0.00007
Arb	Cnt	0.2
Mut	Vax	0.000004
Mut	Cnt	0.1
Vax	Cnt	0.003

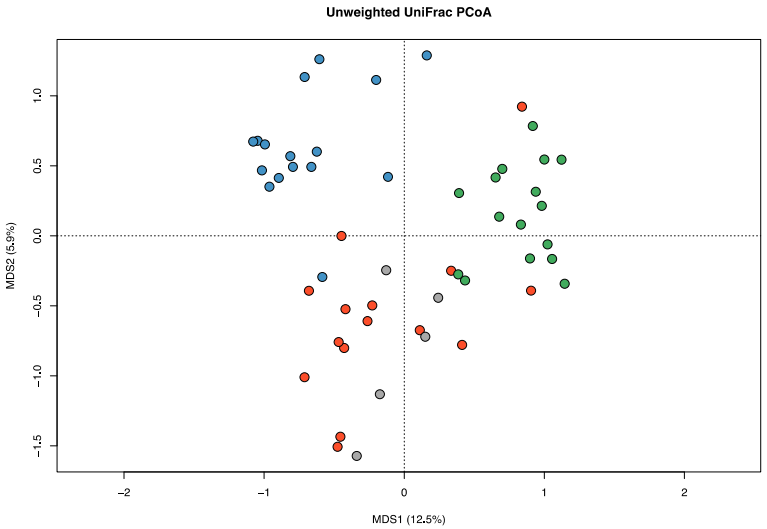
Observed ASVs

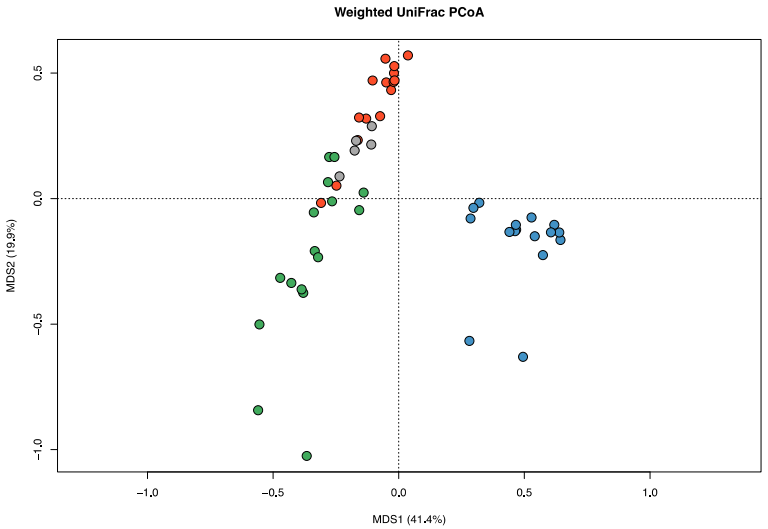
diet 1	diet 2	p-value
Arb	Mut	0.05
Arb	Vax	0.0007
Arb	Cnt	0.1
Mut	Vax	0.0006
Mut	Cnt	0.5
Vax	Cnt	0.05

Shannon

diet 1	diet 2	p-value
Arb	Mut	0.003
Arb	Vax	0.00007
Arb	Cnt	0.04
Mut	Vax	0.002
Mut	Cnt	0.8
Vax	Cnt	0.03

Figure 3.3.5: Alpha diversity analysis of overall diets.





Unweighted
Adonis p-value = 0.001
Arb vs Mut = 0.006
Arb vs Vax = 0.006
Arb vs Cnt = 1
Mut vs Vax = 0.006
Mut vs Cnt = 0.006
Vax vs Cnt = 0.006

Weighted
Adonis p-value = 0.001
Arb vs Mut = 0.006
Arb vs Vax = 0.006
Arb vs Cnt = 0.006
Mut vs Vax = 0.006
Mut vs Cnt = 0.006
Vax vs Cnt = 0.09

Figure 3.3.6: Beta diversity analysis of overall diets.

3.4 Results of the second dose-response trial

Growth performance

The growth performance of the second dose-response trial on salmon has showed no significant differences between the treatments (**Figure 3.4.1** and **Table 3.4.1**). This gives a similar indication then in trial 1 that the experimental diets with an inclusion rate of up to 21 % with Processum meal doesn't affect performance parameters of Atlantic salmon.

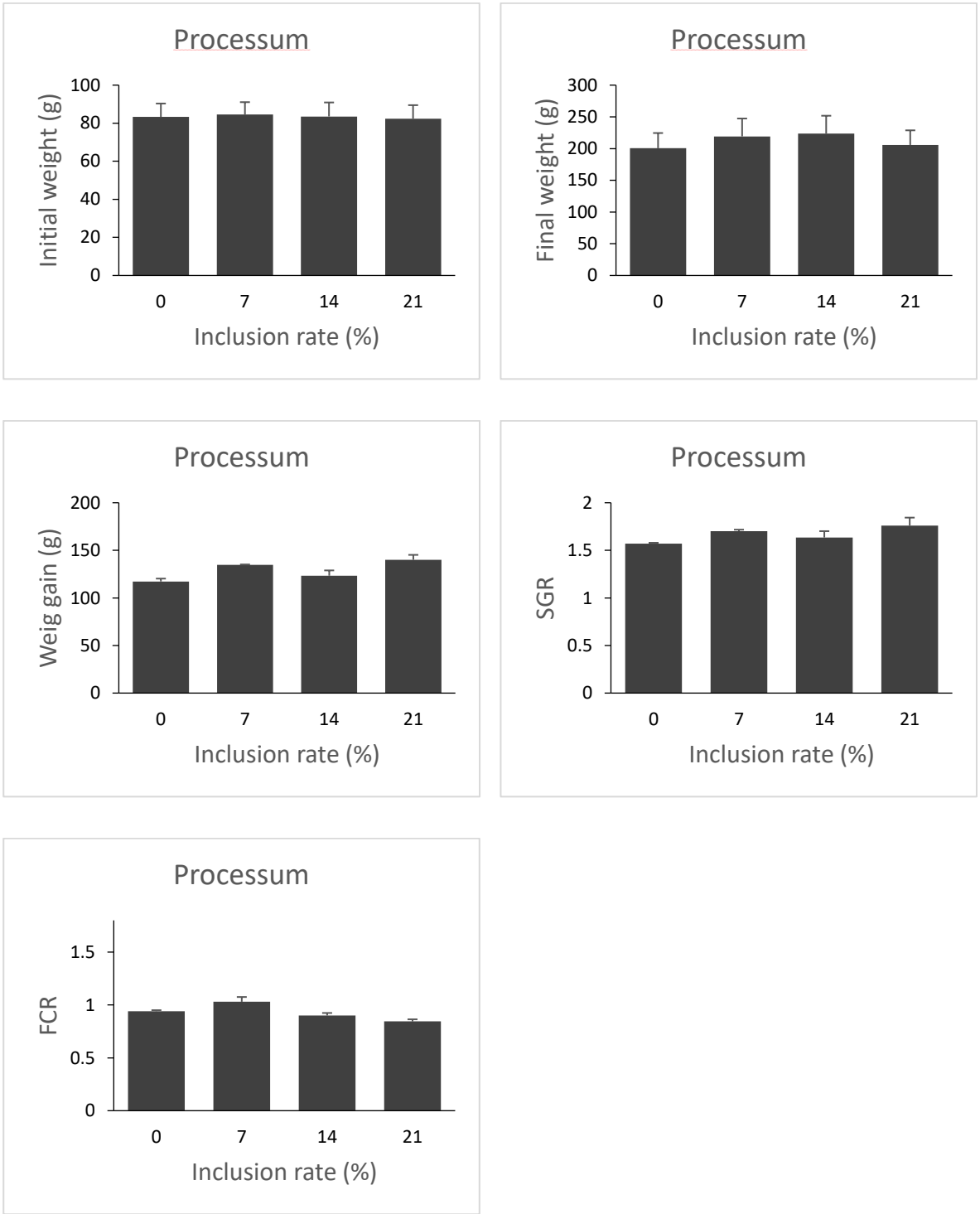


Figure 3.4.1: Visualisation of the parameters initial weight, final weight, weight gain, SGR and FCR of the experimental feeds with Processum meal inclusions.

Table 3.4.1: Statistical parameters for initial weight, final weight, weight gain, SGR and FCR for all treatments of experimental feed with Processum meal inclusion.

Initial	Value	Std.Error	DF	t-value	p-value
(Intercept)	83.90189	1.12602	227	74.51188	0
InclusionRate	-0.05096	0.08592	710	-0.59307	0.5663

Final	Value	Std.Error	DF	t-value	p-value
(Intercept)	209.1288	5.127683	226	40.78426	0
InclusionRate	0.2884	0.39118	410	0.73724	0.4779

Weight Gain	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	125.3534	4.8906	25.632	1.88E-10
Inclusion	0.3348	0.3734	0.897	0.391

SGR	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.628673	0.041866	38.902	3.01E-12
Inclusion	0.003608	0.003197	1.128	0.285

FCR	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.974772	0.033203	29.358	4.91E-11
Inclusion	-0.004388	0.002535	-1.731	0.114

Plasma biochemistry analysis

The plasma parameters investigated are shown in Table 3.4.2. In this analysis, plasma parameters of the control diet were compared to the ones of diets containing SCPs from Processum. No significant differences (P>0.05) were found among treatments.

Table 3.4.2: Plasma biochemistry values for Atlantic salmon fed the control diet and the experimental diets containing SCPs from Processum.

Parameters	*Experimental diets*				
	CTRL	Processum7	Processum14	Prtocessum 21	*P- value*
GLU (mg dL^{-1})	89.4 ± 6.50	89.8 ± 4.84	88.11 ± 5.11	92.78 ± 10.2	*0.5502*
Lactate (mg dL^{-1})	22.4 ± 6.52	29.9 ± 9.85	20.0 ± 5.76	26.4 ± 12.2	*0.1128*
Urea (mg dL^{-1})	5.36 ± 1.76	5.70 ± 1.35	3.68 ± 1.52	5.02 ± 1.67	*0.0555*
CREA (mg dL^{-1})	0.18 ± 0.02	0.19 ± 0.02	0.17 ± 0.01	0.19 ± 0.04	*0.08113*
Uric acid (mg dL^{-1})	0.24 ± 0.06	0.29 ± 0.10	0.25 ± 0.05	0.29 ± 0.09	*0.428*
CHOL (mg dL^{-1})	312.2 ± 29.2	346.7 ± 31.0	330.0 ± 57.7	305.3 ± 27.3	*0.1195*
HDL (mg dL^{-1})	153.4 ± 13.4	167.2 ± 17.7	159.6 ± 20.3	151.7 ± 9.9	*0.1725*
TRIG (mg dL^{-1})	107.6 ± 16.3	141.4 ± 23.8	121.3 ± 44.2	1109 ± 21.5	*0.0708*
TP (g dL^{-1})	4.03 ± 4.32	4.29. ± 026	4.06 ± 0.44	4.01 ± 0.34	*0.3048*
ALB (g dL^{-1})	1.49 ± 0.10	1.51 ± 0.08	1.48 ± 0.20	1.47 ± 0.15	*0.9437*
ALT (U L^{-1})	32.78 ± 11.96	36.9 ± 18.15	23.0 ± 3.12	36.89 ± 32.20	*0.3316*
ALP (U L^{-1})	325.7 ± 77.2	321.1 ± 46.6	297.8 ± 51.9	317.2 ± 75.2	*0.8044*
LDH (U L^{-1})	2736.3 ± 2314.9	2206.1 ± 1371.5	1443.3 ± 1044.6	4119.2 ± 8188.2	*0.6149*
Ca^{+2} (mg dL^{-1})	10.89 ± 0.59	10.96 ± 0.35	10.72 ± 0.57	10.86 ± 0.84	*0.8739*
P (mg dL^{-1})	14.46 ± 1.61	15.78 ± 2.24	12.91 ± 1.81	16.62 ± 6.98	*0.212*
K^{+} (mEq L^{-1})	3.57 ± 1.48	2.64 ± 2.09	3.61 ± 1.39	2.83 ± 1.39	*0.4751*
Na^{+} (mEq L^{-1})	157.8 ± 2.33	174.3 ± 6.86	167.2 ± 4.15	172.0 ± 8.17	*0.0414*
Fe (µg dL^{-1})	57.9 ± 12.2	64.4 ± 24.0	64.0 ± 19.6	56.2 ± 23.2	*0.7645*
Cl (mEq L^{-1})	143.2 ± 2.87	144.3 ± 6.30	144.2 ± 2.65	145.5 ± 8.14	*0.8522*
Mg (mg dL^{-1})	3.01 ± 0.49	3.08 ± 0.64	2.64 ± 0.45	3.20 ± 0.73	*0.2249*
A/G	0.59 ± 0.03	0.54 ± 0.05	0.58 ± 0.04	0.58 ± 0.05	*0.1155*

Data are given as the mean (n=3) ± SD. Different superscript letters indicate significant differences among treatments (One-way Anova $p \leq .05$). GLU, Glucose; Tot Bil, total bilirubin; CHOL, cholesterol; HDL, high density lipoprotein; TRIG, triglycerides; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine amino transferase; ALP, alkaline phosphatase; CK, creatine kinase; LDH, lactate dehydrogenase, Ca^{+2}, calcium; P, inorganic phosphorus; K+, potassium; Na+, sodium; Fe, iron; Cl, chloride; Mg, magnesium; A/G, albumin/globulins.

Gut microbiota analysis

To highlight the gut microbiota (GM) composition of salmon in the different dietary groups, the overall composition at various phylogenetic levels was investigated at the family level (**Figure 3.4.2**). The figure depicts the taxonomic distribution at the Family level in each of the samples, which showed a domain of *Planococcaceae* (34%) and *Streptococcaceae* (29%). However, the abundance of some taxa varied between study groups, such as *Bacilli* (8%) in diets containing 7%

Processum, *Lachnospiraceae* (12%) in diets containing 14% Processum, and *Pectobacteriaceae* (35%) in samples containing 21% Processum.

The internal ecosystem diversity for each dietary group (alpha-diversity), and the GM variations between samples (beta-diversity) were assessed respectively by the calculation of Shannon index (Figure 3.4.3 and Table 3.4.3), and by the principal coordinates Analysis (PCoA) based on unweighted and weighted Unifrac distances (Figure 3.4.4 and Table 3.4.4).

Concerning internal ecosystems diversity and according to the Shannon index, there were no statistically significant differences between the study groups.

Results of beta diversity showed that there is a difference between 14% Processum and 21% Processum (pv alue < 0.039) and between 7% Processum and 14% Processum (p value < 0.039).

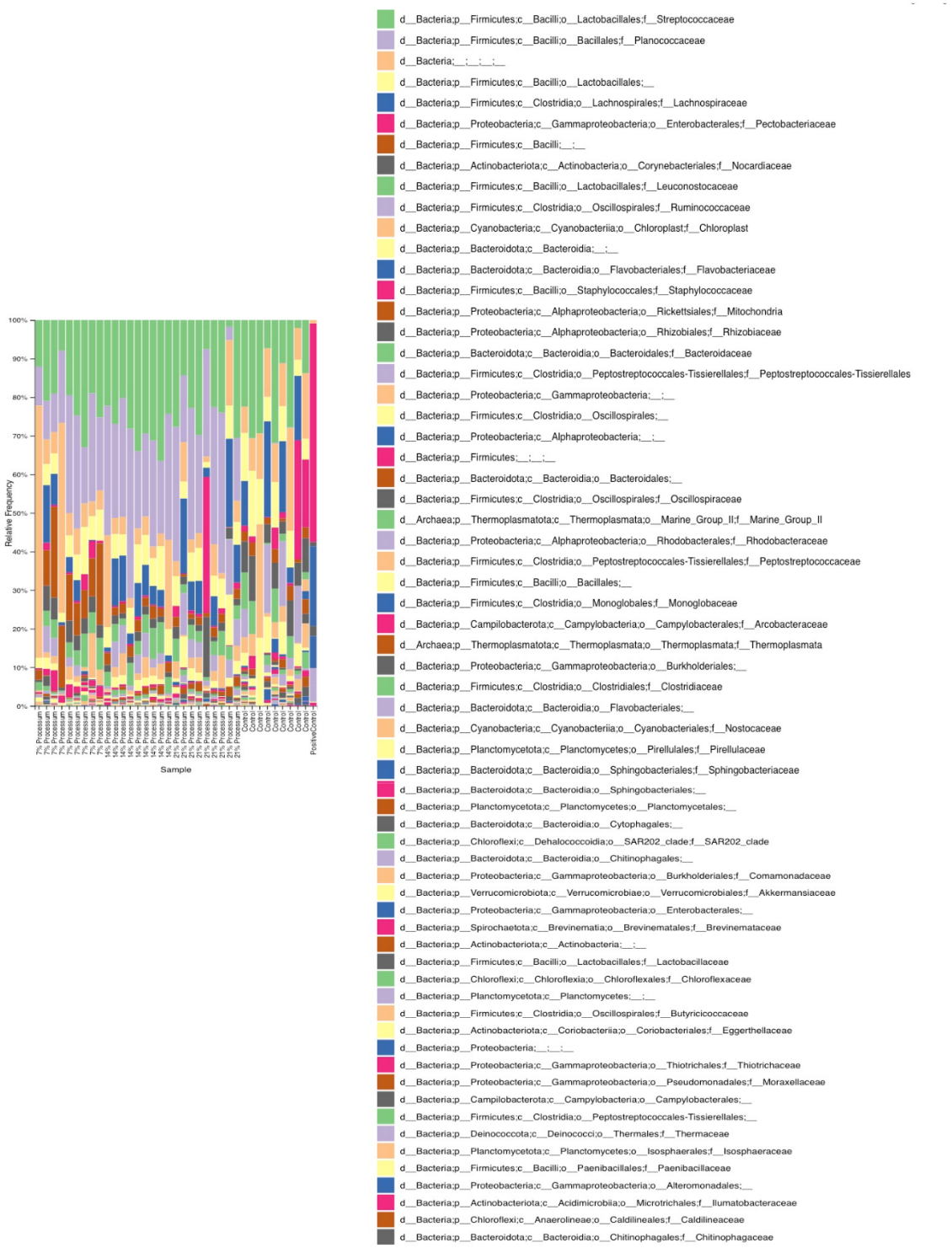


Figure 3.4.2: Overall microbial composition – Family.

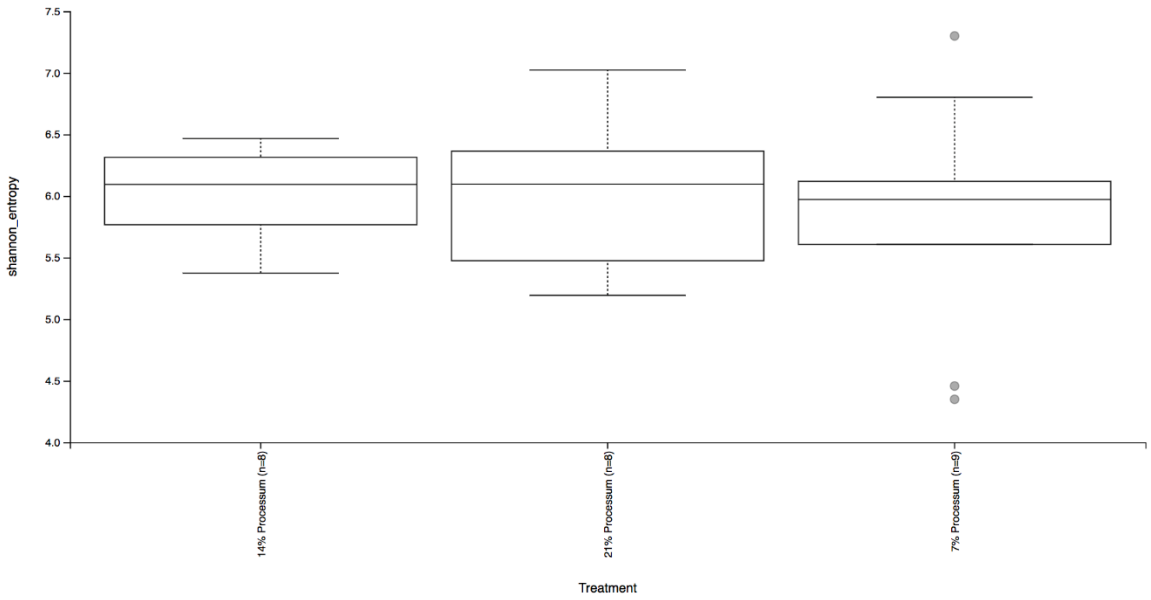


Figure 3.4.3: Alpha Diversity Boxplots.

Table 3.4.3: Sample Shannon entropy.

Treatment	shannon_entropy
categorical	numeric
7% Processum	7.298843719
s7% Processum	6.118518353
7% Processum	6.800844433
14% Processum	5.373471972
14% Processum	5.444544951
7% Processum	5.705074508
7% Processum	5.606895075
7% Processum	4.457245998
21% Processum	5.523798809
21% Processum	5.326734517
21% Processum	5.193400899
14% Processum	6.013763222
14% Processum	6.46608953
14% Processum	6.44020401
7% Processum	5.971449343
7% Processum	4.349362769
7% Processum	5.991657099
21% Processum	6.62487363
21% Processum	6.158425987
14% Processum	6.173352394
14% Processum	6.271290619
14% Processum	5.873858683
21% Processum	6.276828897
21% Processum	7.02200313
21% Processum	6.031991815

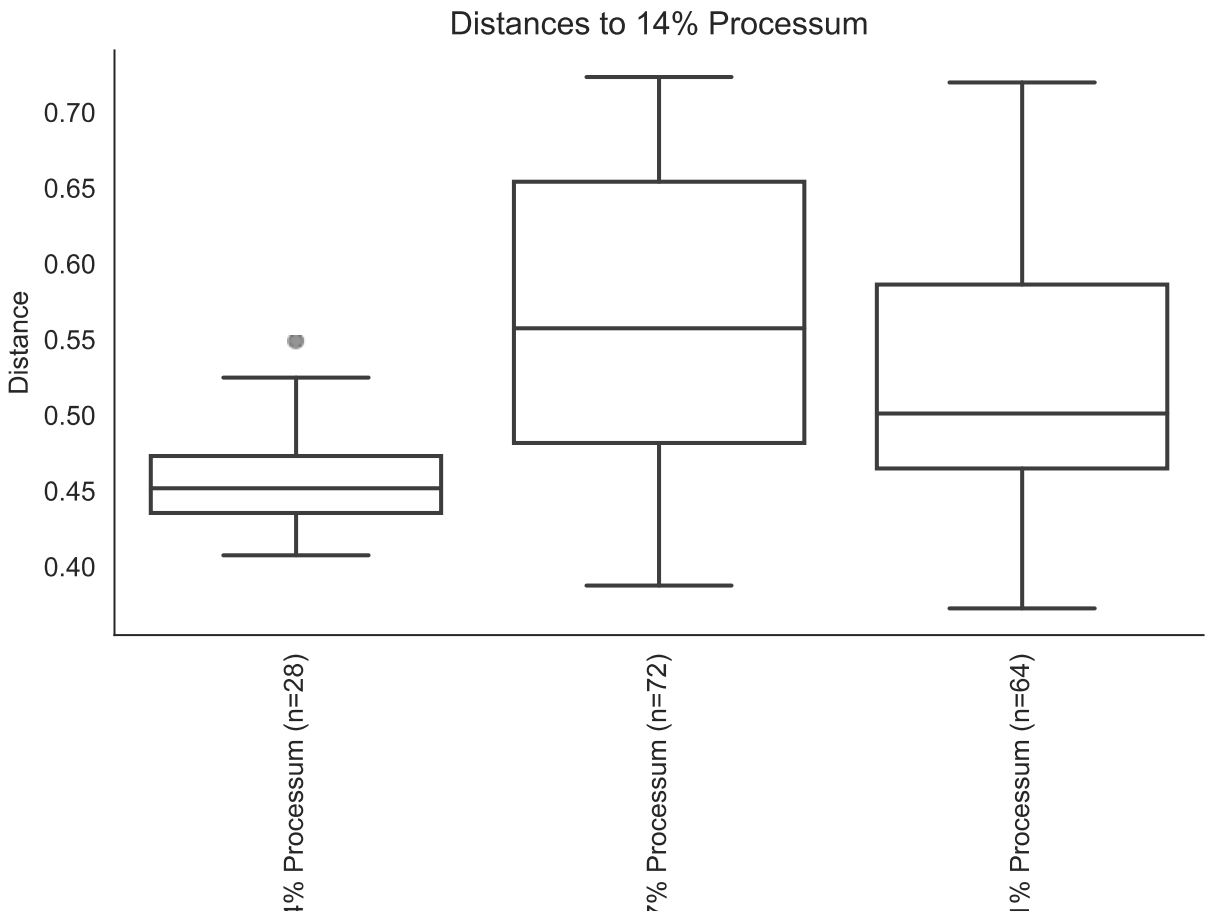


Figure 3.4.4: Group significance plots.

Table 3.4.4: Pairwise permanova results.

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
14% Processum	21% Processum	16	999	1.56472054	0.026	0.039
7% Processum	14% Processum	17	999	1.982770364	0.018	0.039
7% Processum	21% Processum	17	999	1.678552401	0.075	0.075

4 Salmon field trial

4.1 Aim

The main objective in this trial was to establish the utility of the three emerging alternative proteins as feed ingredients for salmon in seawater and to assess the subsequent impact on flesh composition and ultimately consumer acceptance.

4.2 Materials and Methods

Diets

Four diets were formulated following commercial standards that were covering the nutritional requirements of grower size salmon. The four diets were isolipidic, isoproteic and isoenergetic. The levels of fish meal in this trial, were fixed to a standard commercial level of 10%. The emerging raw materials were included in the formulations with a fix inclusion rate of 10% as well. Without forcing the replacement, those raw materials ended up replacing mainly the Soya Protein concentrate from the control diet. The protein content and other attributes of the emerging raw materials selected, were closer to the nutritional content of SPC than to other plant ingredients commonly used in salmon feeds, hence the selected replacement by the formulation software.

Table 4.2.1: Summary of the formulations of the 4 diets of the field trial.

	NextGen CTRL	NextGen SCP	NextGen IP	NextGen AP
Fish Meal	10	10	10	10
Fish oil	16.26	16.16	16.27	16.02
Soya Protein concentrate	18.25	8.07	7.32	5.71
Wheat Gluten	10.71	11.41	12.15	12.69
Pea Protein concentrate	10	10	10	10
Rapeseed Oil	15.34	14.81	14.69	14.43
Lecithin	1	1	1	1
Wheat	5.20	5.85	4.94	7.15
Beans	8	8	8	8
ARBIOM single cell protein	0	10	0	0
Mutatec Black soldier fly	0	0	10	0
VAXA Algae meal	0	0	0	10
Synthetic aminoacids	0.299	0.267	0.408	0.432
Yeast derivatives	0.416	0.416	0.416	0.415
Vitamin premixes	0.793	0.794	0.793	0.792
Mineral premixes	1.625	1.224	1.525	1.561
Astaxanthin	0.065	0.065	0.065	0.065
Technical ingredients	0.329	0.323	0.323	0.318
Water	1.69	1.59	2.09	1.42
Yttrium (Digesibility marker)	0.01	0.01	0.01	0.01

Fish and sampling

Following a quadruplicate design for each one of the experimental diets, fish of approximately 2kg size were stocked in 16 pens at Mowi's Feed Trials Unit (Ardnish, Scotland) for 14 weeks. Daily feed intake was monitored in each pen by the collection of waste feed, to calculate the feed conversion ratio (FCR) for each diet group. Feed ration was adjusted to always offer an excess of feed so, fish had the opportunity to reach satiation in each meal.
Performance parameters including daily feed intake, growth, FCR & survival were collected during the trial.

At the end of the trial, samples were collected for the following assessments:

- Digestibility of the main nutrients in the feeds
- Simple morphometrics including condition factor and carcass yield
- Flesh quality including colour (SalmoFan) and nutrient values (NIR and wet chemistry)
- Histopathology of key tissues within the gut (Pyloric caeca, Mid-intestine & Distal intestine) and liver (UNIBO)-
- Consumer acceptance study and cooked nutritional analysis

Analyses

The feeds, flesh and faeces were sent to the Institute of Aquaculture, University of Stirling where flesh samples were homogenised using a food processor (Blixer® 4 V.V.; Robot-Coupe, Vincennes, France) before storing at -20 oC prior to analysis. Feed pellets were ground into a fine powder using a Knifetecttm 1095 (Foss Analytical AB, Högnäs, Sweden) and stored -20 °C, prior to proximate composition according to standardised methods (AOAC,2000). Collected faeces were freeze dried before ground into a fine powder, using a mortar and pestle, before being stored in a desiccator until analysis. Ethical approval for the study was granted by the Animal Welfare and Ethical Review Body (AWERB) at the University of Stirling (AWERB/7860/New Non ASPA).

Proximate composition

The proximate compositions of the diets, homogenized flesh and dried faecal samples were determined using standardised methods (AOAC 2000). The moisture content for feeds were performed by drying a known quantity of feed in an oven at 103 °C for 4 hours, whereas salmon flesh and faeces were left overnight at the same temperature. Crude protein content was measured by the Kjeldahl method using the Opsis LiquidLINE system (OPSIS AB, Furulund, Sweden). Briefly, samples (~0.20 g) were added to 5 ml of sulphuric acid and two copper catalyst tablets followed by digestion at 400 oC for 1 hour. Protein content was determined as nitrogen content (N x 6.25). Ash content for feeds and flesh were determined after incineration at 600 °C for 16 hours in a muffle furnace.

Lipid and fatty acid analysis

Total lipids (TL) from flesh and feeds were extracted according to Folch et al (1957). Briefly, TL was extracted from ~0.5 g sample kept on ice, then homogenized in either 20 or 36 ml of chloroform/methanol (C:M) (2:1 by vol.) for flesh and feeds respectively, using an UltraTurrax

tissue disruptor (Fisher Scientific, Loughborough, UK). The non-lipid impurities were isolated by washing with 0.88 % KCl and the upper layer was aspirated, with the remaining lower solvent layer dried under oxygen-free nitrogen to dryness. Lipid content was determined gravimetrically and overnight desiccation in vacuo.

Fatty acid methyl esters (FAME) were prepared by acid-catalyzed transesterification of total lipid to determine fatty acid composition by gas liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy), based on the American Oil Chemists Society (AOCS) official method for marine oils. Total lipid extracts, together with 17:0 free fatty acid internal standard, were incubated overnight at 50 °C using 2 ml a 1 % solution of 95 % sulphuric acid in methanol and 1 ml of toluene (Christie.1993). FAMES were then extracted and purified according to Tocher and Harvie (1988), before separated and quantified by gas liquid chromatography (GC) using on-column injection and a 30 m × 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K). Hydrogen was used as carrier gas with a thermal gradient from 50 oC to a final temperature of 230 oC. FAMES were compared to known standards and published data (Tocher and Harvie, 1988). Data were compiled using Chromocard for Windows (Version 1.19; Thermoquest Italian S.p.A., Milan, Italy).

Carotenoid pigment

Flesh astaxanthin levels were determined using a modified method of Barua et al.(1993). Approximately ~1 gram of flesh was homogenized in 10 ml of absolute 1:1 concentration of ethanol/ethyl acetate using an Ultra Turax tissue disrupter, before centrifuging at 1300 rpm for 5 minutes. The supernatant was then decanted to a clean glass tube. For the feeds, these were homogenised and centrifuged for a further 2 times, first adding 5 ml ethyl acetate, then adding 5ml of isohexane, with removal of the supernatant each time. The total supernatant was combined and place under a nitrogen stream and desiccated overnight in vacuo before resuspending in 2 ml isohexane prior to HPLC analysis. Samples were injected on a Thermo Scientific Ultimate 300 UHPLC system equipped with a 50 × 3 mm 1.7 µ Synchronis Silica Column (Thermo Scientific, Hemel Hempstead, UK) with exposure at a wavelength of 470 nm. An isocratic solvent system was used containing isohexane/acetone/isopropanol (82:16:2 v/v/v) at a flow rate of 0.5 mL min^{-1}. The carotenoid pigments were quantified using an external standard of astaxanthin.

Mineral and heavy metal analysis

Macro minerals: Na (sodium), Mg (magnesium), P (phosphorus), K (potassium), Ca (calcium); Microminerals: V(vanadium), Cr (chromium), Mn (manganese), Fe (iron), Co (cobalt), Cu (copper), Zn (zinc), Se (selenium); and Heavy Metals: As (arsenic), Hg (mercury), Cd (cadmium), Pb (lead), Ni (nickel) were determined for diets, flesh, and faces. Briefly, approximate 0.04-0.08 g of sample were added to Teflon tubes, before addition of 5 ml of 69 % nitric acid, followed by microwave digestion (MARS Xpress; CEM Microwave Technology Ltd., Buckingham, UK) for 30 minutes, with a cooling period of 20 min. In addition, 200 µl of gold standard solution for heavy metals and 200 µl methanol solution for selenium analysis respectively were added for enhancing sensitivity before the digested samples were diluted with Milli-Q water in a 10 ml volumetric flask. Finally, samples were analysed using iCAP-RQ Thermo Scientific inductively coupled plasma mass spectrometer (ICP-MS). The ICP-MS operated in kinetic energy discrimination (KED) mode using 100% helium as collision gas to rectify any meddling, and argon as plasma gas.

Digestibility analysis

Yttrium oxide concentration were done in feeds and freeze-dried faeces to calculate the Apparent Digestibility Coefficients (ADC). Briefly, yttrium was quantified by ICP-MS after the same microwave digestion previously mentioned in minerals and heavy metal analysis. The ADC of amino acids and selected minerals were calculated as:

$$ADC = 100 - 100 * (Yd * Yf) * (CXf * CXd)$$

where Y is yttrium concentration, d is diet, f is faeces and CX is nutrient concentration.

Amino Acids analysis (AAA)

Amino acids content for feeds, flesh and faeces were analysed using AccQTag ultra method and performed by High Performance Liquid Chromatography (HPLC). Briefly, samples (~ 250 mg) were hydrolysed with 10 mL of 6 N HCl in Teflon tubes and flushed with oxygen free nitrogen; following a microwave run digestion at 190 oC and 150 oC automatic program, and a subsequent cooling period. Hydrolysis is important to destroy fat and carbohydrate. Samples were diluted to volume (250 ml) with milliQ-water, then filtered into 150 polypropylene tubes: following a derivation (AccQ-Tag Ultra Derivation Kit C & U) of standards and samples. Preparation of mobile phases: Concisely, the AccQ-Tag Ultra Reagent Powder was prepared, by transferring 250 ml of eluent A into 500 ml reagent bottle, then transferred 225ml of Milli-Q water into a 500 ml reagent bottle and added 25ml of eluent B. Mobile phase C, moved 500 ml of ultra-pure water into 500ml reagent bottle. Mobile phase D, transferred 250 ml of eluent B into 500ml reagent bottle. Ultimately, sample mixtures of amino acids standard and blank were prepared by adding 10 µL of the gradient with 70 µL borate buffers and 20 µL derivation AccQ reagent. A 5 µL of the samples, standard and blank were injected for analysis by HPLC. Finally, AA were separated by HPLC analysis, part of the Waters UPLC Amino Acid Analysis (AAA). The UPLC conditions, chromatography integration and data processing were pre-determined and fixed, access is in H-Class System Guide. The concentration for every amino acid was calculated using the average peak areas compared with the standard and expressed as g/100 g of samples. Results from the UPLC were measured in area units.

Fillet processing

A total of 160 whole fish (40 from each group) were harvested at Mowi's Farms Trial Unit and transported to a processing facility for gutting. Following chilled storage to allow rigour to pass,the head-on-gutted salmon were processed through Aquascot's Primary processing facility (Fyrish Way, Alness) where they were filleted and portioned into single 130g servings. For each of the four groups, 35 fillets were portioned and the remaining 35 fillets were frozen down as contingency stock. For the consumer acceptance panel, 130 of the 130g portions from each diet were required and a further 44 portions were packaged for a separate internal Waitrose/Aquascot assessment. An additional 9 fillets per diet were kept for the cooked nutritional analysis. Portions for the consumer acceptance study were packaged individually and labelled on site before being shipped to the testing facility using a chilled overnight courier service.

Consumer Acceptance Study

A group of 80 consumers trained in organoleptic assessment, took part in a consumer acceptance study at Campden BRI testing centre in Leamington Spa, UK. The consumers had undergone a pre-selection process using the Campden BRI Online Panel Database and were all identified as being regular consumers of Waitrose/Aquascot chilled salmon. The study was conducted in accordance with Market Research Society (MRS) code of conduct and BS ISO 11136:2014.

The samples were presented monadically, and each feed group was assigned a 3-digit code to prevent identification. During the cooked analysis, palate cleansers of water and plain crackers were provided between tastings. The testing sessions took 45 minutes to complete with 6 groups of 18 consumers entering the testing area each time.

Samples were presented first for a raw assessment where consumers were asked to score each one on appearance and odour liking using a 9-point hedonic scale, the depth of colour using a 'Just About Right' or JAR scale, as well as providing comments on their overall likes and dislikes of the fish.

For the cooked assessment, samples were prepared according to the standard Waitrose cooking instructions of oven baking at 180ºC for 20-22 minutes. Participants were asked to score each portion on its appearance, odour, flavour, texture, aftertaste and overall liking using the 9-point hedonic scale, the strength of colour using the JAR scale and finally an overall purchase intent score using a 5-point scale. A comparison of overall liking of the raw product versus once It had been cooked was also made.

Cooked Nutritional Analysis

Nine fillets for each of the diets were sent to Eurofins lab for cooked fillet nutritional analysis. The samples were sent off as whole, cooked fillets with the skin removed. The protocol for cooking follows the standard Waitrose salmon cooking instructions of oven baking at 180ºC for 20-22 minutes. The samples were then chilled and packaged and transported to the laboratory (Eurofins, Grimsby). On arrival, the samples were macerated to produce a set of three samples each containing a mash of three fillets. Samples were tested using wet chemistry and gas chromatography.

Statistical analysis

Statistical analyses were performed either using using Minitab® v18.1 statistical software package (Minitab Inc., USA) by a one-way analysis of variance (ANOVA) using Tukey posthoc test. Data are presented as mean and SD of the mean and significance level of $P>0.05$ was applied to all statistical tests performed. Different superscript lettering was used to indicate significant differences between data in tables.

4.3 Results

Diet composition

The analysed values of proximate composition and fatty acids of the control and experimental diets are presented in Table 4.3.1.

The Essential Amino Acids (EAAs) and Non-Essential Amino Acids (NEAAs) composition were similar between feeds, covering the nutritional requirement for Atlantic Salmon (Table 4.3.2). Overall, the results showed that IP contained the highest amount of total amino acids content in the diets (23.24g/100g), followed by SCP (19.60 g/100g), AP (19.10 g/100g) and the control with the lowest composition (18.22g/100g).

The diet carotenoid pigments are shown in **Table 4.3.3**. The control, SCP and IP feeds had a similar astaxanthin level average of 56.07 mg.kg-1, whereas the AP feed exhibited a lower astaxanthin content of 45.20 mg.kg-1. In addition, the AP diet had a higher level of Beta Carotene 140.44 mg.kg-1 which helped result in a much higher total carotenoid value of 276.09 mg.kg-1, as compared to the control, SCP and IP feeds that ranged a total value of 65.21 mg.kg-1.

Table 4.3.1: Diet proximate composition (%) and fatty acid content (% of total fatty acids) of the experimental diets.

	CONTROL	SCP	IP	AP
Proximate composition (%)				
Lipid	32.65	33.18	32.70	33.25
Moisture	6.63	6.80	6.55	6.65
Protein	37.38	36.83	38.85	38.80
Ash	4.21	4.10	4.31	4.49
Fatty acid composition (% total lipid)				
14:0	3.17	3.16	3.17	3.11
16:0	9.14	9.22	9.27	9.75
18:0	2.21	2.19	2.19	2.16
20:0	0.44	0.44	0.43	0.44
22:0	0.61	0.58	0.60	0.56
Total saturated[1]	***15.93***	***15.95***	***16.01***	***16.39***
16:1n-7	2.47	2.49	2.53	2.56
18:1n-9	33.07	32.35	32.42	32.13
18:1n-7	2.26	2.21	2.20	2.24
20:1n-9	6.14	6.17	6.20	6.18
22:1n-11	8.97	9.02	9.01	9.02
Total monounsaturated[2]	***55.15***	***54.53***	***54.64***	***54.50***
18:2n-6	13.29	13.27	13.32	13.27
20:2n-6	0.15	0.15	0.15	0.16
20:4n-6	0.16	0.19	0.17	0.18
Total n-6 PUFA[3]	***13.59***	***13.76***	***13.81***	***14.17***
18:3n-3	4.34	4.37	4.19	4.08
18:4n-3	1.83	1.85	1.85	1.79
20:5n-3	3.60	3.66	3.70	3.49
22:5n-3	0.37	0.39	0.39	0.37
22:6n-3	3.90	4.07	4.03	3.81
Total n-3 PUFA[4]	***14.52***	***14.93***	***14.74***	***14.13***
1 includes 15:0, 24:0				
Total PUFA[5]	***28.93***	***29.52***	***29.35***	***29.11***

2 includes 16:1n-9, 17:1, 20:1n-11, 20:1n-7, 22:1n-9, 24:1n-9
3 includes 18:3n-6, 20:3n-6, 22:4n-6, 22:5n-6
4 20:3n-3, 20:4n-3, 21:5n-3
5 includes 16:2, 16:3 and 16:4

Table 4.3.2: Diet Table amino acid composition (g/100g) of the four experimental diets.

	CONTROL	SCP	IP	AP
EAA Composition (g/100g)				
Arginine	2.35	2.21	2.40	1.90
Histidine	0.76	0.72	0.68	0.64
Isoleucine	1.42	1.45	1.29	1.42
Leucine	2.74	2.71	2.74	2.94
Lysine	2.04	2.04	2.08	2.14
Methionine	0.90	1.00	1.06	1.11
Phenylalanine	1.81	1.76	1.49	1.55
Threonine	1.29	1.32	2.08	1.52
Valine	1.58	1.62	1.72	1.76
Σ EAA	**14.88**	**14.82**	**15.56**	**14.99**
NEAA Composition (g/100g)				
Alanine	1.51	1.64	2.69	2.77
Aspartic acid	3.18	3.01	3.03	3.05
Cysteine	0.39	0.39	0.45	0.65
Glutamic acid	7.87	7.96	6.87	7.28
Glycine	1.68	1.66	3.47	3.45
Proline	2.45	2.40	2.98	2.99
Serine	1.88	1.84	2.31	2.35
Taurine	0.04	0.04	0.06	0.06
Tyrosine	1.34	1.34	1.17	1.09
Σ NEAA	**20.33**	**20.28**	**23.03**	**23.69**
Σ TOTAL AA	**35.22**	**35.10**	**38.59**	**38.69**
PROTEIN %	**37.38**	**36.83**	**38.85**	**38.80**

Table 4.3.3: Diet Table carotenoid pigments (mg.kg-1).

	CONTROL	SCP	IP	AP
Carotenoid pigments (mg.kg-1)				
Lutein	5.32	4.86	5.32	54.75
Canthaxanthin	-	-	-	-
Beta Carotene	1.66	1.79	1.76	140.44
Astaxanthin	58.53	54.45	55.22	45.20
Unknown Carotenoids	-	-	-	-
Diester/asteroidenone	-	-	-	-
Astacene/Adonirubin	2.38	1.96	2.38	35.70
Total Carotenoids	**67.89**	**63.06**	**64.68**	**276.09**

-not detected

Flesh quality parameters and performance data

Data for flesh lipid, moisture, protein, and ash analysis showed no significant differences (P> 0.05) between the different dietary groups (**Table 4.3.4**). Wet weight was unaffected by the diet with an average of 64.64 % moisture, 11.98 % crude lipid, 20.36 % crude protein, and 1.46 % of ash.

The growth performance and feed utilization for the Atlantic Salmon fed the four diets are shown in **Table 4.3.4**. There were no significant differences (P >0.05) between the final average body weights of salmon fed the control, and the other three treatments. Salmon fed the IP and control diet demonstrated the highest final mean weight 3.30 and 3.27 kg compared to AP 3.12 and SCP 3.08 kg. There was also no significant difference in the specific growth rate (SGR) of fish with SGR reducing from 0.38 in the control and IP, 0.34 in the AP and 0.32 SCP. Similarly, there we no significant between the control group and the other diets with respect to daily feed intake with levels ranging between 0.39 IP, 0.38 control, 0.36 AP and 0.32 SCP. Feed convention ratio (FCR) values also did not differ significantly (P>0.005) between the control diet (1.01) and the other treatments (SCP 1.03, IP 1.04, AL 1.01).

However, the differences in the colour of the flesh were significant between the group with the Algal meal added in the diet and the other groups. Both methods, NIR and wet chemistry were in agreement showing paler fillets in this dietary groups.

Table 4.3.4: Flesh proximate composition (%), Growth performance and feed utilisation of the four dietary groups of fish in this trial. Colour measured instrumentally by a Minolta chromamether and also using the SalmoFan scored. Astaxanthin and Total pigment measured in this table by scanning the NQC are with a Near-infrared spectroscopy (NIR).

	Control		SCP meal		Insect Meal		Algal meal		p-value
Performance	Avg	+/- s.d.	Avg	+/- s.d.	Avg	+/- s.d.	Avg	+/- s.d.	
Weight gain (kg)	1.17	0.19	0.97	0.15	1.20	0.16	1.02	0.03	N.S
Final weight	3.27	0.19	3.08	0.16	3.30	0.17	3.12	0.04	N.S.
SGR (%/day)	0.38	0.05	0.32	0.04	0.38	0.04	0.34	0.01	N.S
FCRb	1.00	0.12	1.00	0.11	1.02	0.11	1.14	0.18	N.S
Condition factor	1.49	0.08	1.48	0.03	1.48	0.09	1.49	0.08	N.S
Carcass yield	89.95	0.30	90.12	0.23	89.91	0.29	89.49	0.42	N.S
Proximate composition (% ww)									
Lipid	11.66	0.86	12.85	1.94	11.83	1.02	11.58	1.26	N.S
Moisture	65.38	1.62	63.58	2.53	64.58	1.40	65.02	1.10	N.S
Protein	20.67	0.83	20.23	0.57	20.41	0.18	20.14	0.53	N.S
Ash	1.47	0.18	1.46	0.15	1.38	0.08	1.54	0.11	N.S
Pigmentation & colour (NIR)									
Minolta a* dorsal	24.04	0.05	24.09	0.97	24.02	0.77	22.84	0.62	0.04
SalmoFan (Roche)	25.34	0.40	24.90	0.48	25.17	0.08	24.17	0.38	0.003
Free Astaxanthin (mg/kg)	4.12	0.19	4.06	0.23	3.89	0.17	3.41	0.04	<0.001
Total pigment (mg/kg)	4.53	0.21	4.47	0.25	4.28	0.18	3.75	0.04	<0.001

Total flesh carotenoid levels measured by wet chemistry are provided in **Table 4.3.5**. The total carotenoid and astaxanthin levels showed no significant differences (P> 0.05) between the control,

SCP, and IP dietary treatments. However, the total carotenoid levels as well as astaxanthin levels in the AP treatment were significantly (P<0.002), lower than all other treatment groups.

There are not so many references in the literature about the impact in fillet colour of adding Spirulina algal meal at a moderate inclusion in the salmon feeds, but this result was really clear pointing out that the other pigments present in this ingredient clearly interfere with the Astaxanthin deposition in the fillet.

Table 4.3.5: Flesh carotenoid pigments (mg/kg) of fish fed with the four experimental diets.

	CONTROL	SCP	IP	AP
Carotenoid pigments (mg.kg^{-1})				
Astaxanthin	3.24 ±0.18^{a}	3.08 ± 0.25^{a}	3.16 ± 0.28^{a}	2.47 ± 0.21^{b}
Canthaxanthin	0.10 ±0.02	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Lutein	0.02 ±0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Zeaxanthin	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Unknown Carotenoids	0.07 ±0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Diester/asteroidenone	0.02 ±0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Astacene/Adonirubin	0.47 ±0.09	0.49 ± 0.02	0.50 ± 0.04	0.48 ± 0.05
Total Carotenoids	**3.91 ±0.23^{a}**	**3.79 ± 0.21^{a}**	**3.88 ± 0.31^{a}**	**3.18 ± 0.27^{b}**

Results are means ± SD (n = 4). Values within a row with different superscript letters are significantly different as determined by ANOVA

The flesh fatty acid profile, as a proportion of the total lipid, of the salmon fillets at harvest were, as expected, similar between treatments, they mostly reflect their respective dietary treatment (Table 4.3.6). However, a slight, but significant (P<0.026) difference, was noted in the proportion of total saturates between the control (15.90 %) and AP dietary treatments (16.29 %), This appeared to bedriven by insignificant, but higher levels of 16:0 and 18:0 in the AP flesh (10.58 and 2.66 %, respectively) than control (10.28 and 2.63 %, respectively). In terms of flesh PUFA levels, no significant differences were observed between treatment. SCP had the highest EPA+DHA (0.91 g.100g-1), followed by IP (0.88 g.100g-1), control (0.87 g.100g-1) and then AP (0.86 g.100g-1), although these were not significant.

The mineral composition of the flesh analysed is shown in Table 4.3.7. Of the heavy metals, only arsenic presented a significant higher (P<0.020) level for the AP treatment (0.69 mg.kg-1) compared to the control, SCP and IP treatments (0.62, 0.54 and 0.39 mg.kg-1, respectively) . No further differences in the mineral and heavy metal composition between treatments were found, with all treatments exhibiting a similar profile.

The amino acid composition of the flesh is shown in Table 4.3.8. The total essential amino acids of the IP treatment had a significant (P<0.016) higher level than both the control and SCP groups, which appeared to be driven by a higher level in arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine although these were not significant. Total amino acids contents were also significantly (P<0.042) higher in the IP treatment (23.24 g/100g) compared to the control group (18.22 g/100g), with SCP (19.60 g/100g) and IP (19.10 g/100g) showing intermediary values. Glutamic acid was the highest contributor to the total amount of amino acids in all four diets followed by alanine and glycine. Among all essential amino acids, lysine was found to be of the highest content while histidine and methionine were the two lowest essential amino acids in the four diets.

Table 4.3.6: Flesh fatty acid content (% of total fatty acids) of the four experimental diets (Control, SCP, IP and AP) fed to Atlantic salmon (Salmo *salar* L.).

	CONTROL	SCP	IP	AP
Fatty acid (% total lipid)				
14:0	2.33 ± 0.10	2.38 ± 0.04	2.36 ± 0.10	2.38 ± 0.08
16:0	10.28 ± 0.15	10.31 ± 0.25	10.33 ± 0.06	10.58 ± 0.14
18:0	2.63 ± 0.06	2.62 ± 0.07	2.65 ± 0.05	2.66 ± 0.04
20:0	0.30 ± 0.01	0.30 ± 0.02	0.30 ± 0.01	0.29 ± 0.01
Total saturated[1]	***15.90 ± 0.12 [b]***	***15.99 ± 0.27 [ab]***	***16.03 ± 0.06 [ab]***	***16.29 ± 0.11 [a]***
16:1n-7	2.75 ± 0.02	2.82 ± 0.04	2.77 ± 0.03	2.78 ± 0.05
18:1n-9	36.18 ± 0.25	36.19 ± 0.34	36.11 ± 0.14	35.96 ± 0.19
18:1n-7	2.80 ± 0.03	2.79 ± 0.04	2.79 ± 0.03	2.77 ± 0.02
20:1n-9	4.44 ± 0.21	4.41 ± 0.16	4.42 ± 0.20	4.37 ± 0.09
22:1n-11	3.99 ± 0.33	3.96 ± 0.25	3.95 ± 0.25	3.95 ± 0.19
22:1n-9	0.60 ± 0.04	0.59 ± 0.03	0.58 ± 0.02	0.59 ± 0.02
24:1n-9	0.41 ± 0.01	0.39 ± 0.02	0.39 ± 0.02	0.42 ± 0.03
Total monounsaturated[2]	***52.38 ± 0.90***	***52.12 ± 0.31***	***51.97 ± 0.30***	***51.78 ± 0.33***
18:2n-6	12.67 ± 0.13	12.86 ± 0.21	12.81 ± 0.11	12.78 ± 0.05
20:2n-6	0.94 ± 0.04	0.95 ± 0.02	0.91 ± 0.03	0.91 ± 0.02
20:3n-6	0.26 ± 0.02	0.25 ± 0.01	0.25 ± 0.01	0.27 ± 0.18
20:4n-6	0.26 ± 0.03	0.26 ± 0.01	0.27 ± 0.02	0.28 ± 0.01
22:5n-6	0.07 ± 0.05	0.09 ± 0.01	0.07 ± 0.05	0.10 ± 0.00
Total n-6 PUFA[3]	***14.31 ± 0.17***	***14.52 ± 0.19***	***14.43 ± 0.01***	***14.52 ± 0.18***
18:3n-3	4.22 ± 0.06	4.31 ± 0.11	4.20 ± 0.06	4.17 ± 0.06
18:4n-3	0.88 ± 0.04	0.88 ± 0.01	0.88 ± 0.02	0.90 ± 0.03
20:4n-3	0.95 ± 0.05	0.94 ± 0.04	0.95 ± 0.03	0.91 ± 0.03
20:5n-3	3.51 ± 0.20	3.52 ± 0.13	3.65 ± 0.06	3.53 ± 0.08
22:5n-3	1.49 ± 0.08	1.51 ± 0.07	1.52 ± 0.04	1.51 ± 0.03
22:6n-3	4.97 ± 0.28	4.84 ± 0.14	5.02 ± 0.23	5.02 ± 0.17
Total n-3 PUFA[4]	***16.60 ± 0.62***	***16.56 ± 0.29***	***16.74 ± 0.24***	***16.60 ± 0.31***
Total PUFA[5]	***31.71 ± 0.80***	***31.90 ± 0.46***	***32.00 ± 0.29***	***31.93 ± 0.24***

Table 4.3.7: Flesh mineral composition (mg.kg-1) of the four experimental diets (Control, SCP, IP and AP) fed to Atlantic salmon (Salmo *salar* L.).

	CONTROL	SCP	IP	AP
Macrominerals (mg.kg⁻¹)				
Na (sodium)	302.93 ±20.61	319.72 ± 30.42	402.90 ± 122.70	340.99 ± 56.78
Mg (magnesium)	228.03 ±11.28	243.38 ± 22.29	274.77 ± 60.11	247.90 ± 42.55
P (phosphorus)	2051.67 ±142.28	2173.94 ± 47.91	2624.23 ± 618.82	2358.57 ± 383.20
K (potassium)	3315.99 ±251.79	3476.56 ± 100.97	4148.32 ± 930.65	3758.62 ± 593.03
Ca (calcium)	107.95 ±23.20	124.89 ± 22.81	147.72 ± 19.77	129.81 ± 25.47
Microminerals (mg.kg⁻¹)				
V (vanadium)	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cr (chromium)	0.14 ±0.07	0.13 ± 0.04	0.23 ± 0.09	0.11 ± 0.04
Mn (manganese)	0.14 ±0.02	0.15 ± 0.04	0.18 ± 0.09	0.15 ± 0.08
Fe (iron)	7.29 ±1.19	8.52 ± 1.31	11.22 ± 7.79	9.64 ± 5.15
Co (cobalt)	0.38 ±0.36	0.29 ± 0.22	0.40 ± 0.20	0.37 ± 0.33
Cu (copper)	1.08 ±0.42	0.70 ± 0.16	0.80 ± 0.30	0.80 ± 0.28
Zn (zinc)	39.24 ±10.49	43.55 ± 4.12	39.96 ± 4.78	35.22 ± 2.45
Se (selenium)	0.11 ±0.01	0.10 ± 0.03	0.14 ± 0.04	0.12 ± 0.04
Heavy Metals (mg.kg⁻¹)				
As (arsenic)	0.62 ±0.06^{b}	0.54 ± 0.06^{b}	0.39 ± 0.15^{b}	0.69 ± 0.17^{a}
Hg (mercury)	0.01 ±0.02	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Cd (cadmium)	0.21 ±0.03	0.26 ± 0.03	0.25 ± 0.06	0.21 ± 0.05
Pb (lead)	1.70 ±0.61	1.58 ± 0.28	1.80 ± 0.50	1.57 ± 0.56
Ni (nickel)	0.03 ±0.02	0.03 ± 0.03	0.13 ± 0.09	0.09 ± 0.10

Results are means ± SD. Values within a row with different superscript letters are significantly different as determined by ANOVA

Table 4.3.8: Flesh amino acid composition (g/100g) of the four experimental diets (Control, SCP, IP and AP) fed to Atlantic salmon (Salmo *salar* L.).

	CONTROL	SCP	IP	AP
EAA Composition (g/100g)				
Arginine	0.95 ± 0.17	0.89 ± 0.21	1.16 ± 0.09	1.08 ± 0.16
Histidine	0.54 ± 0.11	0.41 ± 0.04	0.56 ± 0.02	0.43 ± 0.09
Isoleucine	0.75 ± 0.21	0.82 ± 0.31	0.91 ± 0.17	0.79 ± 0.18
Leucine	1.41 ± 0.07	1.63 ± 0.31	1.77 ± 0.44	1.42 ± 0.23
Lysine	1.57 ± 0.08	1.53 ± 0.24	1.88 ± 0.43	1.80 ± 0.31
Methionine	0.76 ± 0.13	0.80 ± 0.21	0.90 ± 0.23	0.81 ± 0.17
Phenylalanine	0.83 ± 0.12	0.71 ± 0.13	0.84 ± 0.07	0.76 ± 0.10
Threonine	0.83 ± 0.06	0.87 ± 0.19	1.24 ± 0.12	1.03 ± 0.34
Valine	0.78 ± 0.15	1.06 ± 0.40	1.23 ± 0.13	0.97 ± 0.12
Σ EAA	**8.40 ± 0.33^{b}**	**8.71 ± 0.39^{b}**	**10.49 ± 0.43^{a}**	**9.10 ± 0.40^{ab}**
NEAA Composition (g/100g)				
Alanine	1.53 ± 0.51	1.98 ± 0.18	2.25 ± 0.62	1.50 ± 0.53
Aspartic acid	1.83 ± 0.11	1.92 ± 0.21	2.18 ± 0.59	1.75 ± 0.22
Cysteine	0.23 ± 0.02	0.21 ± 0.19	0.11 ± 0.03	0.15 ± 0.06
Glutamic acid	2.34 ± 0.16	2.20 ± 0.38	2.89 ± 0.48	2.45 ± 0.15
Glycine	1.41 ± 0.59	1.97 ± 0.23	2.29 ± 0.51	1.54 ± 0.72
Proline	0.74 ± 0.09	0.89 ± 0.08	1.04 ± 0.20	0.86 ± 0.24
Serine	0.94 ± 0.18	1.08 ± 0.10	1.26 ± 0.35	0.99 ± 0.36
Taurine	0.08 ± 0.03	0.09 ± 0.03	0.09 ± 0.02	0.08 ± 0.00
Tyrosine	0.72 ± 0.07	0.54 ± 0.09	0.65 ± 0.15	0.69 ± 0.07
Σ NEAA	**9.81 ± 0.75**	**10.88 ± 0.83**	**12.75 ± 1.03**	**10.00 ± 0.77**
Σ TOTAL AA	**18.22 ± 1.5^{b}**	**19.60 ± 0.52^{ab}**	**23.24 ± 3.73^{a}**	**19.10 ± 2.12^{ab}**
PROTEIN %	20.67 ± 0.83	20.23 ± 0.57	20.41 ± 0.18	20.14 ± 0.53

Results are means ± SD. Values within a row with different superscript letters are significantly different as determined by ANOVA

Digestibility data

With the use of Yttrium, the apparent digestibility coefficients (ADC) of the amino acids were calculated (**Table 4.3.9**). The highest ADC was found in fish fed the SCP diet. IP appeared to have a significantly ($P<0.028$) lower digestibility levels for essential amino acids (84.22 %) compared to SCP (88.03 %). However, the ADC of total AA was unaffected, and there were no major differences for most of the individual amino acids among the control, SCP, IP and AP fish fed. Overall, SCP had the highest digestibility (92.93 %) followed by the control (91.97 %), AP (89.88 %) and the lowest not significant digestibility (89.66 %).

Table 4.3.9: Apparent Digestibility Coefficient (ADC %) of amino acid composition (g/100g) of the four experimental diets (control, SCP, IP and AP) fed to Atlantic salmon (Salmo *salar* L.).

	CONTROL	SCP	IP	AP
EAA Composition (%)				
Arginine	96.59 ± 0.37	96.30 ± 0.31	95.99 ± 0.94	94.32 ± 2.49
Histidine	93.85 ± 0.76	94.25 ± 0.44	89.60 ± 2.35	91.76 ± 4.52
Isoleucine	91.98 ± 1.39	92.32 ± 0.49	88.97 ± 2.01	88.73 ± 5.87
Leucine	93.24 ± 1.41	93.22 ± 0.40	91.88 ± 1.86	90.74 ± 4.36
Lysine	93.89 ± 1.26	93.41 ± 0.59	90.42 ± 2.20	92.68 ± 2.96
Methionine	93.25 ± 1.16	94.02 ± 0.39	92.53 ± 1.52	92.97 ± 2.41
Phenylalanine	94.09 ± 0.74	94.24 ± 0.31	92.11 ± 2.21	92.29 ± 2.94
Threonine	89.04 ± 3.09	87.69 ± 0.86	92.11 ± 2.38	89.50 ± 6.05
Valine	90.92 ± 2.24	90.89 ± 0.47	88.09 ± 1.89	85.18 ± 8.39
Σ EAA	86.60 ± 1.07^{ab}	88.03 ± 1.20^{a}	84.22 ± 2.35 ^{b}	88.50 ± 7.82^{ab}
NEAA Composition (%)				
Alanine	88.92 ± 4.48	88.13 ± 1.32	89.74 ± 1.56	91.11 ± 4.06
Aspartic acid	87.34 ± 3.30	89.24 ± 1.01	84.80 ± 4.34	89.36 ± 2.33
Cysteine	90.94 ± 3.16	90.01 ± 3.56	89.41 ± 3.51	91.59 ± 3.40
Glutamic acid	96.37 ± 0.66	96.34 ± 0.40	95.45 ± 1.10	95.12 ± 1.18
Glycine	84.91 ± 7.65	81.98 ± 3.12	87.44 ± 3.38	92.24 ± 4.18
Proline	93.88 ± 1.59	93.94 ± 0.75	93.23 ± 1.58	94.41 ± 2.69
Serine	92.12 ± 1.47	90.85 ± 1.50	92.47 ± 1.64	92.27 ± 2.73
Taurine	93.96 ± 0.82	94.13 ± 0.64	91.27 ± 2.63	90.53 ± 3.05
Tyrosine	88.54 ± 0.92	89.07 ± 1.09	87.04 ± 1.93	89.54 ± 1.14
Σ NEAA	80.25 ± 1.58	81.77 ± 1.82	78.03 ± 3.28	81.41 ± 2.03
Σ TOTAL AA	91.97 ± 1.36	92.93 ± 0.72	89.66 ± 2.39	89.88 ± 1.38
PROTEIN %	96.59 ± 0.37	96.30 ± 0.31	95.99 ± 0.94	94.32 ± 2.49

Consumer acceptance study

The testing of raw samples on their appearance and odour showed no significant differences in the scoring between diets (**Table 4.3.10**). Of the three novel proteins, SCP performed closest to the control for both attributes. The results for the cooked samples, showed more significant differences within the scoring for flavour (p=0.014), texture (p=0.001) and overall liking (p=0.002). SCP samples scored consistently highest (at statistical parity with the control), while AP scored consistently lowest. There were no significant differences in the groups for scoring on appearance, odour and aftertaste.

Table 4.3.10: The mean scores presented for the key sensory attributes tested in the consumer acceptance study. Salmon samples were scored on a 1–9-point hedonic scale where 1 = Dislike extremely and 9 =Like extremely.

Sensory Attribute	CONTROL	SCP	IP	AP
Raw Appearance	7.69 ± 0.97	7.63 ± 1.01	7.5 ± 1.10	7.36 ± 1.35
Raw Odour	6.84 ± 1.84	6.81 ± 2.16	6.63 ± 1.92	6.49 ± 1.88
Cooked Appearance	5.76 ± 1.26*	5.81 ± 1.40*	5.65 ± 1.52*	5.71 ± 1.41*
Cooked Odour	6.96 ± 1.37	6.86 ± 1.25	6.98 ± 1.36*	6.89 ± 1.38*
Cooked Flavour	7.23 ± 1.45^{ab}	6.96 ± 1.59^{a}	6.83 ±1.68^{ab}	6.56 ± 1.40^{b}
Cooked Texture	6.91 ± 0.61^{a}	6.88 ± 0.68^{a}	6.34 ± 0.62^{ab}	6.11 ± 0.76^{b}
Cooked Aftertaste	6.94 ± 1.66	6.76 ± 1.72	6.65 ± 2.04	6.44 ± 1.77
Cooked Overall	7.13 ± 1.50^{ab}	6.86 ± 1.59^{a}	6.49 ± 1.55^{b}	6.34 ± 1.52^{b}

Results are mean ± SD. Values within a row with different superscript letters are statistically significant as determined by ANOVA. * indicates statistically significant difference as determined by t-test between raw and cooked attribute within a treatment group

Comparing scores for samples pre and post cooking, the results showed that the cooking process had a greater impact on appearance than odour. Cooking the samples caused a statistically significant negative impact on appearance liking, whereas cooking significantly enhanced the odour for both the IP and AP groups.. The liking of appearance for cooked samples decreased by nearly 2 points (on a 1-9 point scale) for each of the 4 diets. There was no statistically significant difference between raw and cooked odour for the Control or SCP samples.

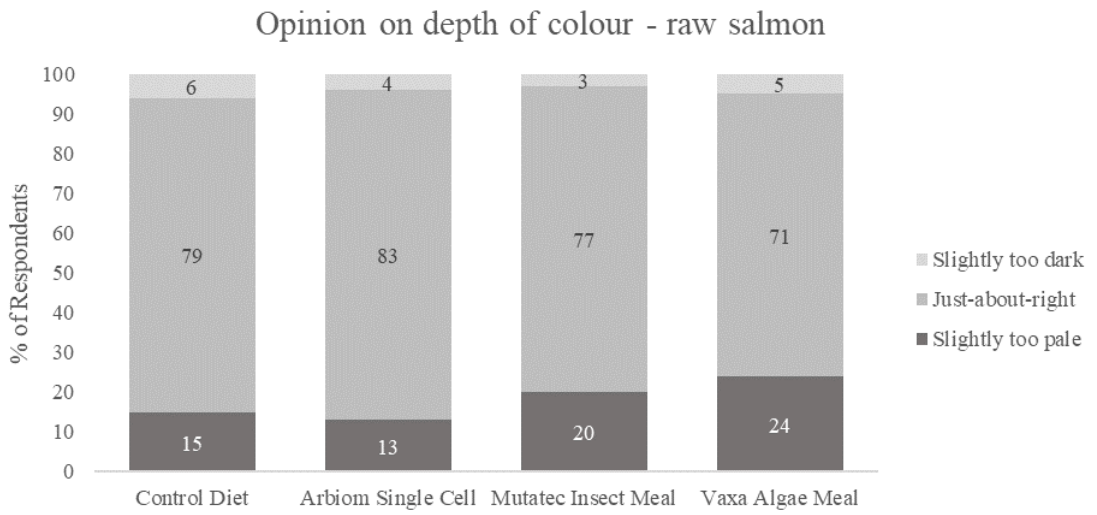


Figure 4.3.1: Consumers scoring of depth of colour of the raw salmon samples using a 5- point Just-About-Right or JAR scale.

With the exception of AP, there was little variation between diets on the scoring of depth of colour in raw samples (**Figure 4.3.2**). IP samples performed similarly to the Control (77% satisfaction vs 79%) and SCP slightly outperformed both (83% satisfaction). AP was significantly penalised on depth of colour (71% satisfaction) which resulted in a 1-point (on a 1–9-point scale) reduction in its mean overall liking score with one in four respondents complaining that the colour was slightly too pale.

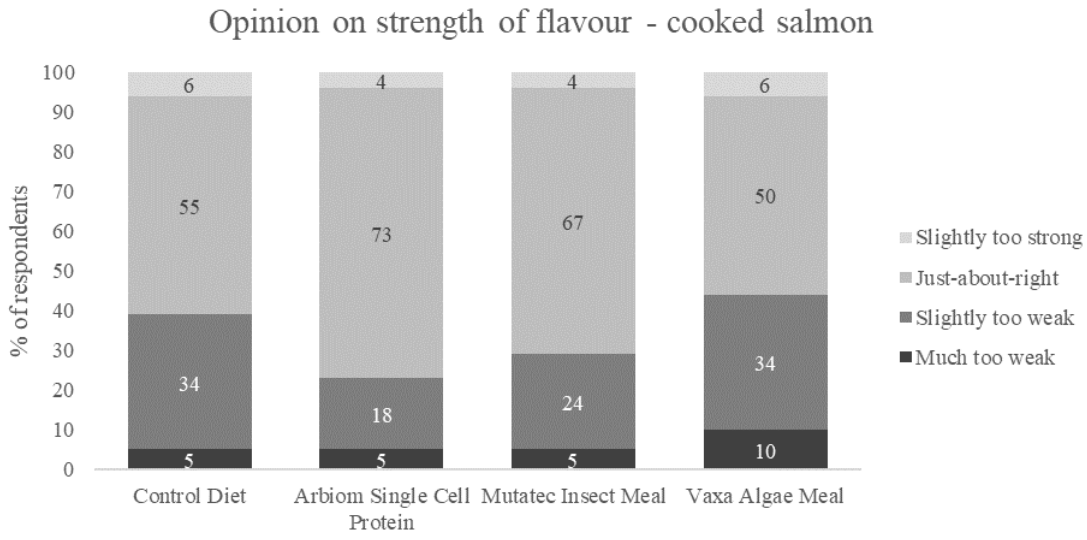


Figure 4.3.2: Consumers scoring of strength of flavour of the salmon samples using a 5- point Just-About-Right or JAR scale.

The results for strength of flavour of the cooked samples found SCP scoring higher than all three of the other diets (**Figure 4.3.2**). The control did not perform well on cooked flavour (55% satisfaction) with AP scoring just below at a level of 50% satisfaction. SCP and IP performed fairly well at 73% and 67% satisfaction. Samples from all four diets were significantly penalised for having too weak a flavour which resulted in a 2-point reduction (on a 1–9-point scale) in their mean overall liking scores. Approximately one in four respondents complained that SCP (23%)

and IP (29%) samples were too weak in flavour and more than one in three respondents complained about the Control (39%) and Vaxa (44%).

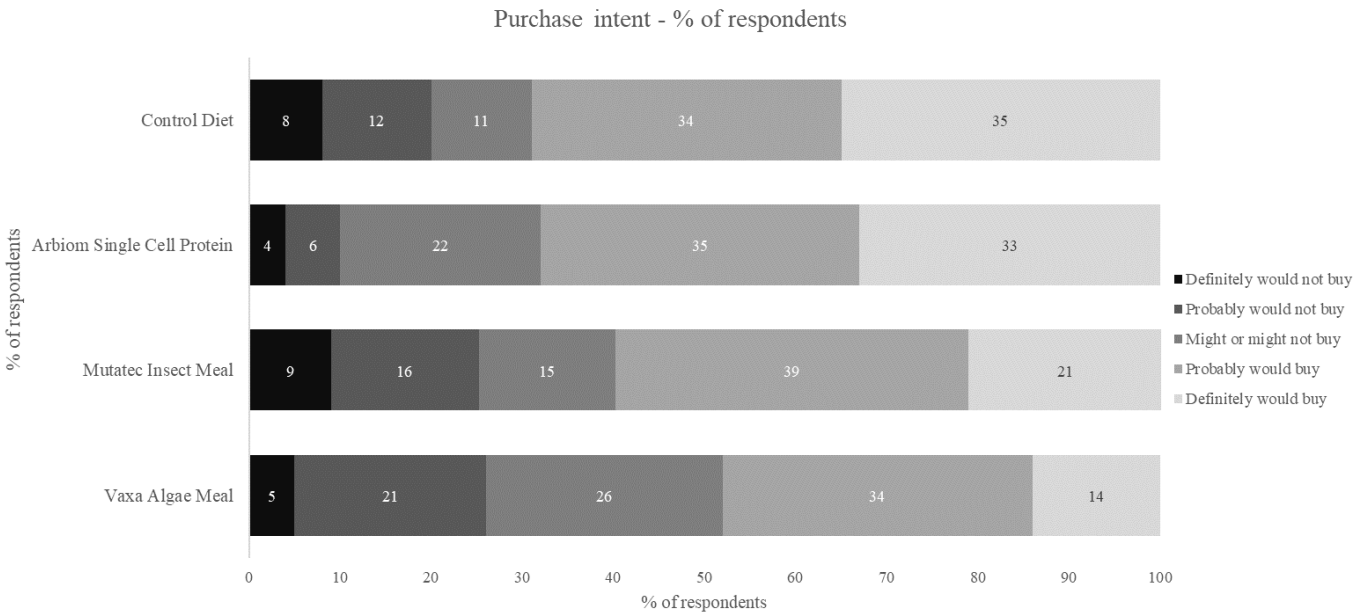


Figure 4.3.3: Consumers scoring of overall purchase intent for salmon samples using a 5- point Just-About-Right or JAR scale.

Evaluation of both raw and cooked samples contributed to the overall purchase intent score which saw slight differences between the diets (**Figure 4.3.3**). There was no statistically significant difference between the scores, however AP scored the lowest with the minority (48%) of respondents who would purchase compared to 60% respondents voting to purchase Ip fed salmon. SCP performed better, with 68% of respondents voting that they would purchase which is close to the Control with 69%.

Cooked Nutritional Analysis

The results from the cooked nutritional analysis showed a significant difference in carbohydrate and starch levels but no difference in any of the other nutrients (**Table 4.3.11**). Samples of salmon fed the Control diet, showed significantly higher quantities of carbohydrate and starch when compared to the other three novel ingredients. There were no significant differences on any nutritional component between the three novel proteins.

Table 4.3.11: Results from the nutritional analysis performed on a mash of three cooked fillets from each diet.

	CONTROL	SCP	IP	AP
Energy (kj)	861.66 ± 65.58	945 ± 25.12	880.67 ± 77.42	937.00 ± 72.91
(kcal)	206.00 ± 15.87	226.66 ± 5.77	211.00 ± 19.08	224.67 ± 17.93
Fat (g)	11.93 ± 1.96	14.03 ± 0.86	12.2 ± 2.28	13.63 ± 2.37
of which saturates (g)	2.00 ± 0.35	2.32 ± 0.15	2.07 ± 0.39	2.27 ± 0.37
mono-unsaturates (g)	5.74 ± 0.99	6.79 ± 0.45	5.92 ± 1.20	6.60 ± 1.21
polyunsaturates (g)	3.39 ± 0.50	3.92 ± 0.22	3.44 ± 0.56	3.84 ± 0.64
Carbohydrate (g)	0.8 ± 0.17^{a}	0.5 ± 0.00^{b}	0.50 ± 0.00^{b}	0.50 ± 0.00^{b}
Starch (g)	0.73 ± 0.19^{a}	0.38 ± 0.08^{b}	0.45 ± 0.00^{b}	0.42 ± 0.0^{b}
of which sugars (g)	0.07 ± 0.03	0.12 ± 0.08	0.05 ± 0.00	0.08 ± 0.03
Fibre (g)	0.4 ± 0.00	0.79 ± 0.98	0.47 ± 0.12	0.83 ± 0.40
Protein (g)	24.4 ± 0.17	24.7 ± 0.45	25.00 ± 0.62	25.06 ± 1.01
Sodium (g)	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Salt Equivalent (g)	0.08 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
Declared Omega 3 (mg)	934.67 ± 105.63	1002.33 ± 34.12	926.33 ± 108.19	997.00 ± 116.09
Omega 6 (mg)	1606.67 ± 263.50	1926.66 ± 126.62	1653.33 ± 300.22	1870.00 ± 346.41
Trans Fatty Acids (g)	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.084 ± 0.00
Moisture (g)	62.10 ± 1.48	59.93 ± 0.06	61.47 ± 1.88	60.53 ± 1.79
Ash (g)	1.3 ± 0.00	1.36 ± 0.06	1.30 ± 0.00	1.27 ± 0.06
EPA (mg)	369.33 ± 43.47	427.00 ± 19.97	393.6 ± 49.90	428.67 ± 53.59
DHA (mg)	565.33 ± 64.53	575.33 ± 17.01	532.67 ± 58.35	568.33 ± 62.66
Total Omega 3 (mg)	1786.67 ± 235.44	2006.66 ± 95.04	1790.00 ± 251.59	1973.33 ± 288.85
Nitrogen	3.90 ± 0.03	3.96 ± 0.07	4.00 ± 0.10	4.01 ± 0.16

Results are mean ± SD. Values within a row with different superscript letters are statistically significant as determined by ANOVA.

Conclusions

The dose-response trial performed was intended to suggest what the best performing inclusion rate would be for use in the semi commercial scale trial. No statistically significant differences were found in initial body weight, final body weight and SGR. For FCR slight but significant effect was shown for Mutatec (FCR, $R^2 = 0.54$). This shows that a substitution with insect meal, SCPs meal from Arbiom and Processum, and Vaxa algae meal up to 21% does not affect salmon performance negatively in a dose-response trial on a pilot scale.

The semi commercial scale trial demonstrates that fish performance, as measured through a suite of routine production metrics, was comparable between the control diets and the test diets where all three alternative protein sources were included at 10% fixed inclusion. Even though the inclusion of the alternative ingredients was not targeting the substitution of any particular ingredient, the nutritional profile, (*i.e* the protein content and digestibility) of the testing ingredients, led to a substitution of Soya protein concentrate in all the three experimental diets. Broadly flesh composition analysis was comparable across treatment groups with the exception of flesh pigmentation with there being a reduction in total carotenoid levels due to a reduction in Astaxanthin concentrations in the Algal protein fed group compared to all other treatments. The root cause of the reduced astaxanthin concentration in the algal protein fed fish was due to an overabundance of Lutein, Beta Carotene and Astacene within the pigment quotient to the diet which originates from the algal protein concentrate. It is conceivable that due to the similarity in structural form, these alternative carotenoids could interfere and ultimately inhibit astaxanthin absorption, though this hypothesis requires further research to validate. The consumer acceptance testing of the salmon samples demonstrates that there was a perceived difference in the fillet samples with the pale ness of the algal protein fed salmon being a principle contributing factor to the overall reduction in purchase intent observed in the algal protein fed salmon compared to the control and SCP fed salmon. Ultimately this body of work has demonstrated that all three alternative protein sources can be used to substitute plant-based proteins in salmon feed formulations with no measurable impact on fish growth performance. However, the study has also clearly demonstrated that inclusion of algal protein can have a negative impact on flesh pigmentation which in turn is a major contributing factor to consumer acceptance of such fed fish. Future work with this protein source must look to resolve this pigmentation conflict through refined secondary processing before it can be further considered as a viable feedstock in salmon feed formulations. With respect to the single cell and insect protein sources, while the current study demonstrates their potential for incorporation in salmon feed in substitution of plant proteins, clarity is required on the impacts of their production (*i.e.* full life cycle analysis) as well as availability and cost of the raw materials. This context is vital to position the two protein sources within the basket of existing available raw materials and will determine further application of either protein source in salmon aquaculture.

5 References

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37 (8), 852–857. https://doi.org/10.1038/s41587-019-0252-6.

Borrel, A., Castelló-Orvay, F., & González-García, E. (2019). Potential of black soldier fly larvae meal as partial fishmeal replacement in aquafeeds: a review. Journal of the World Aquaculture Society, 50(1), 1-19. doi: 10.1111/jwas.12568

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Parma L., Busti, S., Ciulli, s., Volpe, E., Errani, F., Oterhals, Å., Romarheim, O.H., Aspevik, T., Dondi, F., Gatta, P.P., Bonaldo, A., 2023. Growth, plasma biochemistry and immune-related gene expression of European sea bass (Dicentrarchus labrax) fed bioactive peptides from farmed salmon by-products. Aquaculture, 563, art. No. 738982.

Pelusio, N.F., Schicchitano, D., Parma, L., Dondi, F., Brini, E., D'Amico, F., Candela, M., Yúfera, M., Gilannejad, N., Moyano, F.J., Gatta, P.P., Bonaldo, A., 2021. Interaction between dietary Lipid level and Seasonal Temperature Changes in Gilthead sea bream Sparus aurata: Effects on growth, fat deposition, Plasma biochemistry, Digestive enzyme activity, and Gut bacterial community. Frint. Mar. Sci. 8, 664701.

Quast, C., Klindworth, A., Pruesse, E., Schweer, T., Horn, M., Glo, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, 1–11. https://doi.org/ 10.1093/nar/gks808.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mah´e, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 2016, e2584. https://doi.org/10.7717/ peerj.2584.

Tacon, A. G. J., & Metian, M. (2015). Feed matters: satisfying the feed demand of aquaculture. Reviews in Fisheries Science & Aquaculture, 23(1), 1-10.