Future of Fish Feed

F3 Krill Replacement Challenge

Competitor Information Packet

This packet provides information on the F3 Krill Replacement Challenge, including guidance on the Challenge trial methodology and winner determination for Entrants. For more details on qualifying criteria, see the F3 Krill Replacement Challenge Official Rules on the F3 Challenge website (F3Challenge.org).

Challenge Overview

The goal of this F3 Challenge is to create a substitute for krill in aquaculture feeds. Krill is a popular ingredient in aquaculture feeds where it is often used as an attractant, palatant and potentially for its nutritional benefits. However, mismanagement of this crucial organism at the base of the food chain could lead to disastrous consequences for wild ecosystems, commercial fisheries and aquaculture. Encouraging alternatives to krill can reduce demand on wild fisheries and future-proof global food security. With more sustainable alternatives to wild-caught ingredients, the aquaculture feed industry will have alternatives that can make supply chains more robust and enable aquaculture to continue to grow.

Background

The F3 team needed to target aquacultured species that are finicky about feed. To meet that requirement, the F3 Team conducted a preliminary comparative feeding study of Atlantic Salmon to determine the viability of the Challenge. Two prerequisites were needed to hold the Challenge:

1. A plant-based feed with krill (positive control) that performed as well as a fishmeal control.
2. That the plant-based feed WITHOUT krill did not perform as well as the two controls above.

If the two prerequisites were met, then it would be clear that there existed a base feed on top of which we could add each of the different Entrant's Krill Replacements so that each Entrant's feed performance could be compared against each other and against the controls.

This comparative feeding trial demonstrated that a Challenge was viable. The results, methodology and experimental design of this trial are shown in the Appendix A: Summary - F3 Atlantic Salmon Trial.

Feed Manufacturability Requirements

Prior to registering for the Challenge or sending the F3 Krill Replacements to be entered into the Challenge, Entrants need to verify that their F3 Krill Replacements not only meet the requirements outlined in the Challenge Rules, but that they are shippable, storable, and manufacturable into a feed using the processes outlined in this section.

Shipping and Storage: The Entrant is responsible for making sure that the F3 Krill Replacement can withstand shipping and arrive intact. Typically, feed products should last for one year if they are stored in a cool, dry place ideally below 25 ℃ (77 ℉), and sheltered from sun and rain exposure. The Challenge Host's storage capacity is detailed below.

- Dry storage
- Freezer maintained at -20°C
- Refrigerator maintained at -2 to -8°C

If Entrants have any additional storage requirements, they need to contact f3krillreplacement@gmail.com to determine if the Challenge Host can accommodate special storage requirements onsite. Entrants may proceed to register for the Challenge and ship their product and storage instructions only after the Challenge Host has confirmed they can accommodate any special storage requests. Storage instructions should also be shared with the F3 Team via email at f3krillreplacement@gmail.com.

Safety: The Entrant is responsible for including any instructions related to safe handling and manufacturing of the product accompany the F3 Krill replacement samples and are also transmitted to f3krillreplacement@gmail.com.

Manufacturability: The F3 Krill Replacement needs to be easily incorporated into the feed manufacturing process to create Experimental Treatment Diets according to the following process:

All Experimental Treatment Diets for the Challenge will be extruded utilizing a Brabender twin-screw extruder, with the lipid component vacuum-coated after the extrusion process, where the ingredients will be subjected to a time/temperature regime of <15 minutes at 120°C. As such, the krill replacement candidates must be able to withstand one of the two following conditions:

1. If the product is heat stable and able to withstand the above time/temperature regimes, then it will be included in the "mash" with the remainder of the ingredients and subjected to a hammermill step in the feed manufacturing process. This process grinds all the ingredients to a suitable and consistent particle size prior to feed extrusion. While the heat generated during a hammermill process is not as high as those experienced in the feed extrusion process, each Entrant must ascertain if their product will be harmed and/or altered by the hammermill process, which might impact its potential impact on the efficacy of their product.

2. If the product is not heat stable and cannot withstand the extrusion time/temperature regime as described above, then the Entrant must supply their product in a form ground to less than 500 microns and it must be fat-soluble so that it can be applied post-extrusion in the top-coating of the feed with canola and algal oils (thereby skipping the time/temperature regime of extrusion). If the product is NOT fat soluble, then we will have to top-coat the product (again, ground to <500 microns) in a water-soluble vector, most likely with a binder to ensure complete adhesiveness to the feed pellets, as well as to minimize the potential for product leeching once the pellets hit the water, and before it is ingested by the fish, and then top-coat the feed pellets with the appropriate level of canola and algal oils to finalize the feed manufacturing process.

NOTE: The feed manufacturer has the ability to accept or reject F3 Krill Replacements should they prove not manufacturable based on practical feasibility of incorporation during feed fabrication. If the F3 Krill Replacement's corresponding feed is not manufacturable, the reason for failure to incorporate will be provided.

Challenge Logistics

Entrants will send their F3 Krill Replacements to the F3 Representative specified in the Challenge Rules or as directed by the F3 Team by the deadline specified in the Challenge Rules. The F3 Representative will repackage and blind-label the Entrants F3 Krill Replacement and send the F3 Krill Replacement Products to the Challenge Host, and create a key that will be shared to facilitate logistics with the F3 administration but not with the F3 Judges.

The Challenge Host will add F3 Krill Replacement products into the F3 plant-based diet as specified below to create Experimental Treatment Diets.

All the products will be blind-labeled by an F3 Representative, so neither the Challenge Host making the feed and performing the experiment nor the F3 Judges will know the identity of the F3 Krill Replacements during the conduct of the Challenge. The experimental design and methodology are described below.

Methodology and Experimental Design

The Challenge Host will be responsible for the proper conduct of the experiments in consultation with the F3 Team, Judges, and the Scientific Review Committee. The Trial will consist of up to twelve treatments: two controls and up to ten Entrant Experimental feeds. If there are four or fewer Qualified Entrants, then there will be a single trial. If there are greater than four Qualified Entrants then there will be a second trial.

All treatments will be fed to Atlantic Salmon either in four replicate tanks for 12 weeks or in two replicate tanks for 12 weeks, and then another two replicate tanks for a second 12-week trial. Throughout the trial(s) weight gain, survival and feed conversion ratio will be measured. The treatments have the following labels and composition:

Control Diets:

1. Krill Negative Control (KNeg) = F3 plant-based feed* + 0% krill (5% wheat flour added)
2. Krill Positive Control (KPos) = F3 plant-based feed* + 5% krill

*The F3 (fish-free feed) plant-based feed includes 6% poultry blood meal as an essential amino acid source. The F3 Team defines “plant-based” as consisting mostly of plant materials, with a small amount of (non-marine) animal protein included.

Experimental Treatment diets each based on Entrant’s F3 Krill Replacement:

F3 plant-based feed* + up to 5% F3 Krill Replacement from Entrants. If <5% inclusion is requested by the Entrant, the F3 Chief Scientific Officer will work with the Entrant and feed manufacturer to determine how to make up the remaining composition, likely with wheat flour. The formula for the Experimental Treatment diets is below.

Ingredient (%)	Experimental Treatment
Krill Meal	0
F3 Krill Replacement Product*	5
Menon Pro50FF	13
Soy Protein Concentrate (Selecta 60)	27.1
Corn Protein Concentrate (E 75)	7.7
Blood Meal	6
Wheat Gluten Meal	2.15
Wheat Flour	10.134
Canola Oil	16.95
Algae Oil	4.5
Monoammonium Phosphate	3.25
Vitamin Mineral Premix	1.7
Lysine HCl	1.2
Taurine	0.5
DL-Methionine	0.65
Threonine	0.16
Asta, pink	0.006

All treatments will undergo the same protocols, including feeding regime, water quality monitoring, growth and survival measurements. Protocols and growing conditions for the

Challenge Trial will be similar to those implemented in the trial described in Appendix A: F3 Atlantic Salmon Trial. The detailed diet formulas found in Appendix A: F3 Atlantic Salmon Trial will be precisely followed for the Challenge Trial. The top-performing competitor will win as specified in the Winner Determination section below.

Winner Determination

The prize will be awarded to the Entrant(s) whose F3 Krill Replacement demonstrates the best "Performance" during the Challenge Trial based on differences that are statistically significant. "**Performance**" is defined by a combination of weight gain, feed-conversion ratio and survival observed during the Challenge Trial. Performance will be measured by assessing statistical rather than numerical differences across treatments using the following metrics :

a. ***Weight Gain:*** Fish for all treatments will have an initial weight that is not statistically different.[1] Fish weight gain will be expressed in terms of percentage weight gain with respect to the Krill Positive group, e.g. 90% of KPos, 110% of KPos.

   Weight gain is defined as the percentage increase of the animal's initial body weight.

   The experimental treatment that has the greatest increase in fish biomass is likely to win. Any mortality events are factored into this method.

b. ***Feed Conversion Ratio & Survival:*** In the case of a statistical tie in weight gain, the winner will be selected by the Challenge judges factoring in the best (lowest) Feed Conversion Ratio (FCR) and (highest) survival rate.

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Total\ weight\ of\ feed\ consumed\ (g)}{Total\ weight\ gain\ (g)}\ x\ 100\%$$

Statistical Analysis:

a. All test diets will be compared to KPos using one way ANOVA to find the best weight gain among the candidate diets. Distinctly different and poorer performers can be eliminated. Then, if there is overlap among the top 2-3 performers, distinctly different and poorer performers can be eliminated, and the weight gain comparison among the smaller group of test diets and Kpos can be rerun. If there is still overlap, rerun the comparison among the smaller group and Kpos using FCR as the response.
b. The other two diets, FM Control and KNeg will be included as additional controls.
c. Sidaks or Tukey's multiple comparison techniques will be deployed in all cases.

In the case that a tie still remains, the prize money will be equally divided between the winners.

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[1] https://journals.lww.com/jbjsjournal/Citation/2012/03070/_Not_Statistically_Different__Does_Not_Necessarily.13.aspx#:~:text=The%20Orthopaedic%20Forum-,%E2%80%9CNot%20Statistically%20Different%E2%80%9D%20Does%20Not%20Necessarily%20Mean%20%E2%80%9Cthe%20Same,Between%20Difference%20and%20Equivalence%20Studies&text=Researchers%20often%20want%20to%20evaluate,equivalent%20to%20an%20existing%20treatment.

For questions regarding the F3 Krill Replacement Challenge, please visit our website (f3challenge.org) or contact the F3 Team at f3krillreplacement@gmail.com.

APPENDIX A: Summary: F3 Atlantic Salmon Trial

June 4-September 1, 2022

Objective

To evaluate whether a krill replacement challenge could be designed based on the performance of *Salmo salar* during 90 days of feeding.

Methodology & Experimental Design

The trial was carried out at a commercial research facility for salmon aquaculture. At the trial start, sixty fish of an approximate initial weight of 148.4 g ± 12.9 g and a condition index equal to or greater than 1.2 were randomly distributed into each 500-L tank. Each experimental group comprised four tanks which were randomly distributed.

Fish were subjected to a 10-day acclimatization period before starting the trial. The acclimatization period began with a 24-hour fast for all tanks, after which time a commercial diet for Atlantic salmon was fed for the remainder of the period. After acclimatization and following a second 24-hour fasting period, feeding of the experimental diets began. Automatic feeders were programmed for a 14-hour cycle with 3-4 second pulses every 6-8 minutes, and pellets not consumed were recovered twice daily and weighed to estimate feed consumption rates.

Fish length and weight were measured for each individual fish at the beginning and end of the trial. Proximate analysis of the fish was also analyzed at the beginning and end of the trial. Mid-way through the trial (day 40) and after fasting, the average fish weight per tank was quantified by volumetric sampling.

Growing Conditions

Water Flow	Water renewal rate of 1-1.5 changes per hour. System operated with 30% fresh water input and 70% reuse. Seawater supplied by Quillaipe Bay, filtered by 60 um rotary filters and disinfected with UV light.
Light Regime	24 hour
Initial Culture Density	17.88 kg/m3
Average Water Temperature	11.6 °C
Average Oxygen Saturation	102%
Salinity	32%

Experimental Diets (4 mm pellet)

Ingredient (%)	Feed 1 FM (FMFO Control)	Feed 2 KNEG (Krill Negative Control)	Feed 3 KPOS (Krill Positive Control)
Fish meal	25.27	0	0
Poultry meal	0	0	0
Krill meal	0	0	5
Menon Pro50FF	13	13	13
Soy Protein Concentrate Selecta 60	11.88	27.1	27.1
Corn Protein Concentrate E75	1.87	11.9	7.7
Blood meal	6	6	6
Wheat gluten meal	2.15	2.15	2.15
Wheat flour	15.944	10.004	11.134
Fish oil	19.79	0	0
Canola oil	0	17.7	16.95
Algae oil	0	4.5	4.5
Monoammonium Phosphate	1.85	4.3	3.25
Vitamin mineral premix	0.7	0.7	0.7
Lysine HCl	0.65	1.45	1.2
Taurine	0.5	0.5	0.5
DL-methionine	0.39	0.6	0.65
Threonine	0	0.09	0.16
Asta, pink	0.006	0.006	0.006

Performance Indices

- Survival Rate

$$Survival\ Rate\ (\%) = \frac{Number\ of\ initial\ fish - Number\ of\ harvested\ fish}{Number\ of\ initial\ fish}\ x\ 100\%$$

- Weight Gain

$$Percent\ Weight\ Gain\ (PWG) = \frac{Final\ weight\ (g) - Initial\ weight\ (g)}{Initial\ weight\ (g)}\ x\ 100\%$$

- Specific Growth Rate (NOTE: For F3 Krill Replacement Trial, Relative Weight Gain will be measured instead of Specific Growth Rate)

$$Specific\ Growth\ Rate\ (SGR\%) = \frac{log\ Final\ weight\ (g) - log\ Initial\ weight\ (g)}{Number\ of\ days}\ x\ 100\%$$

- Feed Conversion Ratio

$$Feed\ Conversion\ Ratio\ (FCR) = \frac{Total\ weight\ of\ feed\ consumed\ (g)}{Total\ weight\ gain\ (g)}\ x\ 100\%$$

- Condition Factor (K)

$$Condition\ Factor\ (K) = \frac{Final\ weight\ (g)}{Final\ Length^3\ (cm)}\ x\ 100\%$$

- Specific Feeding Rate (SFR)

$$Specific\ Feeding\ Rate\ (SFR) = \frac{Feed\ fed\ (g)}{mean\ fish\ weight\ (g)\ X\ days}\ x\ 100\%$$

Laboratory Analyses

NOTE: The following tests were performed for all treatment groups in the trial. For the F3 Krill Replacement Challenge, all diets at the end of the trial will be tested for peroxide values to ensure rancidity has not occurred.

- Feed analysis
  - Proximate Composition
  - Amino Acid Profile
  - Peroxide Value
- Fish analysis
  - Proximate Composition

Results

Proximate Analysis of Feeds

Diet	Moisture (%)	Lipid (%)	Ash (%)	Protein (%)	Peroxide Index (T1)
Feed 1	4.9	22.9	7.7	46.0	Detected (<2)
Feed 2	6.1	21.7	6.1	46.4	Detected (<2)
Feed 3	3.3	23.1	6.2	46.0	Detected (<2)

Amino Acid Analysis of Feeds

Amino Acids (g / 100 g)	Feed 1	Feed 2	Feed 3
Aspartic Acid	4.12	3.55	3.63
Glutamic Acid	6.86	6.78	6.57
Alanine	2.58	2.23	2.13
Arginine	2.39	2.07	2.05
Phenylalanine	2.06	2.06	2
Glycine	2.29	1.56	1.58
Hydroxyproline	0.247	<0.2 (LOQ)	<0.2 (LOQ)
Histidine	1.47	1.18	1.13

Isoleucine	1.6	1.32	1.39
Leucine	3.68	3.87	3.65
Lysine	3.67	3.16	3.19
Ornithine	<0.05 (LOQ)	<0.05 (LOQ)	<0.05 (LOQ)
Proline	2.22	2.21	2.16
Serine	1.9	1.84	1.81
Tyrosine	1.31	1.29	1.27
Threonine	1.7	1.43	1.55
Valine	2.41	2.03	2.01
Tryptophan (Total)	0.537	0.469	0.512
Cysteine + Cystine	0.585	1.534	0.515
Methionine	1.26	1.21	1.26

Productive Data for Fish

Metric	**Feed 1**	**Feed 2**	**Feed 3**	**P value (*)**
Initial weight (g)	155.4 ± 0.11	155.5 ± 1.19	154.6 ± 0.89	0.87
Final Weight (g)	542.5 ± 3.84	496.9 ± 17.7	549.6 ± 8.95	0.05*
SGR	1.39 ± 0.009	1.29 ± 0.038	1.41 ± 0.018	0.02*
FCRb	0.803 ± 0.01	0.890 ± 0.036	0.870 ± 0.05	0.01*
SFR	1.12 ± 0.010	1.15 ± 0.015	1.19 ± 0.047	<0.001***
Growth (%)	249.0 ± 2.4	219.5 ± 11	241.8 ± 18	0.03*
Survival (%)	100	99.44	100	-

(*) Ordinary one-way ANOVA

Proximate Analysis of Fish

Diet	**Protein (%)**	**Lipid (%)**	**Ash (%)**	**Moisture (%)**
Trial Start Sample	19.0	4.6	1.4	74.1
Feed 1 (Trial End)	18.7 ± 0203	11.3 ± 0.282	2.36 ± 0.295	68.5 ± 0.306
Feed 2 (Trial End)	18.4 ± 0.404	11.1 ± 0.661	2.42 ± 0.214	68.8 ± 1.06
Feed 3 (Trial End)	18.5 ± 0.227	13.1 ± 0.615	2.03 ± 0.05	66.7 ± 0.342