“The Gastronomic Odyssey”

Optimizing the diet for Atlantic Halibut (*Hippoglossus hippoglossus*) - Investigating the effects of macronutrient composition

Master of science in Aquaculture
By Victoria Helsengreen

UNIVERSITY OF BERGEN

Supervisor: Øystein Sæle, Havforskningsinstituttet

Department of Biological Sciences (BIO)
June 2023

Front page illustration from Ichtyologie, ou, Histoire naturelle, générale et particulière des poissons (1785–1797) by Marcus Elieser Bloch (1723–1799), the German physician and naturalist. Free to download under the CC0 license. Available at: https://en.wikipedia.org/wiki/File:Hippoglossus_hippoglossus2.jpg

Acknowledgments

If a compass award existed for guiding lost thesis writers, my supervisor Øystein Sæle would be the undisputed winner. His unwavering guidance and exceptional knack for transforming complex concepts into manageable tasks have been invaluable in this journey. Thanks, Øystein, not just for your invaluable and constructive feedback, but also for our refreshing detours into conversations about everything but the master's thesis. You've made this academic endeavor feel less like a daunting task and more like a rewarding expedition.

In this grand adventure, I also had the good fortune of crossing paths with a band of unsung heroes. To the team at “Prøvemottaket” at the Marine Research Institute at Nordnes, your dedication and camaraderie turned work into an enjoyable symphony, often accompanied by an unforgettable rock soundtrack or the jazz hour on the radio. Special thanks to Joar, Nina Margrethe, Edel, and Georg, who acted as my trusty sidekicks in the data analysis saga.

And thus, the curtain closes on my five-year academic journey with this master's thesis. It's a curious sensation, trading in all-nighters and student discounts for 9-to-5s and office coffee. My journey would have been a lot less memorable without my fellow explorers at the "satsesalen". Thanks for making the ride worthwhile, filling it with laughter, shared joys, wine-soaked evenings and yes, that brave little toaster that withstood our relentless demands. These are memories etched in my heart, as permanent as ink on paper.

"I came, I saw, I researched, I cited. Rinse and repeat until thesis complete."
- Victoria Helsengreen

Abstract

The primary challenge for contemporary food fish producers of halibut is the growth rate in the growth phase, which entails high feeding costs. Research on optimizing fish feed and cultivation practices has become essential to ensure the continued growth and sustainability of the aquaculture sector, both in Norway and globally. Improved feed formulations can contribute to the reduction of environmental pollution caused by unutilized nutrients in fecal matter or unconsumed feed. Moreover, optimizing feed formulations has the potential to improve feed conversion ratios, which in turn may lead to decreased feed costs and enhanced production efficiency. Research into halibut nutrition after the juvenile phase is limited. Only a few studies have focused on the nutritional requirements of larger fish, with a particular emphasis on macronutrients. This study aimed to assess the effects of different levels of different levels of protein, carbohydrates, and lipid in the diet on the growth and health of the halibut. By analyzing muscle, liver and feces samples from individuals fed 12 distinct diets, the study aimed to evaluate macronutrient compositions, explore potential interactions between macronutrients, and identify the optimal macronutrient balance for halibut feed. The study suggests optimal diet compositions for varying sizes of fish to promote growth and performance. For fish up to 700g, a diet containing 46%-56% protein, 16%-23% lipid, and 5%-15% carbohydrates is recommended. For larger fish ranging from 700g to 900g, a balanced diet should consist of 15%-25% carbohydrates, while maintaining the same protein and lipid proportions. The findings contribute to the understanding of the nutritional requirements, and the development of optimized diets, for halibut.

Abbreviation

ADC = Apparent digestibility coefficient

CF = Condition factor

HSI = Hepatosomatic index

MU = Intermediate sampling (Mellom uttak)

SD = Standard deviation

SGR = Specific growth rate

SU = Final sampling (Slutt uttak)

Table of contents

1 Introduction

1.1 Background

The growth of the aquaculture sector has experienced rapid expansion in recent decades, transforming it into one of the most important sources of food production worldwide. Aquaculture now supplies more than half of the world´s fish for human consumption (FAO, Accessed: 2023-04-28). This growth has been driven by the increasing global demand for seafood, advances in aquaculture technology, and the need to alleviate pressure on wild fish stocks (Naylor *et al.*, 2005). In Norway, the aquaculture industry has become a significant contributor to the nation's economy, with salmon and Atlantic halibut being among the species cultivated (Regjeringen, 2021). Halibut farming in Norway began with research in the 1980´s (Haug, 1990), leading to the establishment of several companies in the following decades. Although numerous bottlenecks were challenging to address and caused many businesses to fail, today, 4-5 active companies remain, and the obstacles are no longer as restrictive. Despite the advancements made, some challenges remain. Key areas of research and development focus include nutrition, genetic improvement, and environmental considerations (Gallardo *et al.*, 2022). However, the production of Atlantic halibut in Norway has shown steady growth in recent years, reflecting the increasing demand for this species (FAO, 2021).

The expansion of the sector has brought numerous benefits, such as job creation, rural development, and the availability of high-quality, nutritious seafood for consumers. However, the rapid growth of aquaculture has also raised concerns about environmental impacts, fish health, and sustainable practices (Naylor *et al.*, 2005). As a result, research on optimizing fish feed and cultivation practices has become essential to ensure the continued growth and sustainability of the aquaculture sector, both in Norway and globally.

Fish feed plays a critical role in aquaculture as it affects fish growth, health, and overall production efficiency (Assan *et al.*, 2021). The nutritional composition of fish feed, including the proportions of fat, protein, and carbohydrates, is essential for supporting the physiological needs of the fish and promoting optimal growth (NRC, 2011). Moreover, the quality of fish feed influences the environmental sustainability of aquaculture as the efficiency of feed

utilization can impact waste production and nutrient pollution in aquatic ecosystems (Lopez-alvarado, 1997). In Norway as well as in other countries with a strong aquaculture sector, research on fish feed optimization is crucial to ensure the continued growth and sustainability of the industry. By improving feed formulations, producers can enhance fish welfare, reduce environmental impacts, and increase the profitability of their operations.

Improved feed formulations can contribute to the reduction of environmental pollution caused by unutilized nutrients in fecal matter and unconsumed feed. Moreover, optimizing feed formulations has the potential to improve feed conversion ratios, which in turn can lead to decreased feed costs and enhanced production efficiency (Boyd, 2021). Additionally, optimized fish feeds can enhance fish welfare by providing appropriate nutrition, which can lead to improved immune system function and lower susceptibility to diseases (Pohlenz and Gatlin, 2014). Ultimately, optimizing fish feed can result in higher product quality, which is essential for meeting consumer demands and maintaining the competitiveness of the aquaculture sector (Prabu *et al.*, 2017). As the industry continues to grow in Norway and globally, research on optimizing fish feed for species like Atlantic halibut will become increasingly important for ensuring the long-term sustainability and success of aquaculture. The primary challenge for contemporary food fish producers of halibut is the growth rate in the growth phase, which entails high feeding costs. There is limited research on halibut nutrition beyond the juvenile phase, with only a select few studies exploring the nutritional needs of bigger fish, predominantly focusing on macronutrients.

According to the available research, protein requirements for halibut appear to decrease as the fish grow. In general, for fish weighing between 1 to 500 grams, it's recommended to have a protein consumption constituting 51 to 63% of the feed. For fish over 500 grams, a protein consumption of 35 to 41% may be required (Table 1). However, Àrnason *et al.* (2009) conducted a study which indicated no significant impact of protein levels on fish with weights ranging between 559 - 877 g and 980 - 1493 g. The results suggested that the lowest level tested, which was 41% for smaller fish and 35% for larger fish, was sufficient. Fluctuating lipid and carbohydrate levels did not have a significant impact on the fish's health. However, in the experiment with the largest fish, the feed composition affected the final weight with a 94% confidence interval, with 43% protein performing better than 35% protein.

Regarding lipid requirements, most reports suggest that halibut can tolerate lipid intake up to 25% (Hamre *et al*., 2005, 2003). Hamre *et al*. (2003) also found that small fish could not tolerate more than 5% carbohydrates, and Aksnes *et al.* 1996 discovered that this lower carbohydrate tolerance also applied to 500-gram fish. However, only Hamre *et al*. in 2005 examined the interactions between the three macronutrients, focusing on fish that grew from 0.5 to 6.2 grams. As a result, there is limited knowledge of the interplay between protein, fat, and carbohydrates in feed for larger halibut.

Table 1 The table has been extracted from the project description put forward by The Institute of Marine Research. It provides a summary of studies on the nutritional requirements of halibut. The columns "Protein," "Lipid," and "Carbohydrate" represent the percentage of macronutrients analyzed in each study, while the column "Fish" indicates the weight of the fish in grams. The values listed in the table are expressed as a percentage of the recommended nutrient intake for halibut feed.

Protein		**Lipid**		**Carbohydrate**		**Fish**		**Commendation**			**Studies**
Min.	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Protein*	*Lipid*	*Carbohydrate*	
53	83	5	30	0	15	0.5	6.2	63	<25	< 5	Hamre et at., 2003
62	86	5	30	12	12	0.4	6.7	58	<30	< 5	Hamre *et al*., 2005
56	56	14	25	14	23.5	33	100		14> <25		Martins *et al.,* 2006
51	60	20	27	7	10	140	266	51			Berge *et al.,* 1998
38	58					34	324	58			Hjertnes *et al.,*1990
41	72	12.6	33	1.4	26.9	6	556	61.8		< 3	Aksnes *et al.,* 1996
40	53	23	25			559	877	41			Arnason *et al.,* 2009
19	21	8	20	10	10	600	1500		unknown		Berge and Storebakken. 1991
35	47	28	32	14	26.5	980	1493	35 (43)			Arnason *et al.,* 2009

1.2 Biology of Atlantic halibut (*Hippoglossus hippoglossus*)

The Atlantic halibut (*Hippoglossus hippoglossus*) is a flatfish species that is distributed throughout the North Atlantic Ocean. It is a highly migratory species that is capable of adapting to a wide range of oceanic environments (Haug, 1990). Atlantic halibut is a member of the Pleuronectidae family and is considered one of the largest flatfish species (NOAA Fisheries, 2023). They have a distinctive body shape, with both eyes located on the right side of the head, which enables them to blend in with the ocean floor. Atlantic halibut also have a high level of plasticity in their feeding behavior and can consume a wide range of prey, including invertebrates, fish, and cephalopods. In terms of reproduction, Atlantic halibut are broadcast spawners, releasing large quantities of eggs into the water column, which are fertilized externally (Haug, 1990; Glover, 2006). Overfishing in the mid-1900s caused a decline in population size, which likely contributed to reduced age at sexual maturity in both males and females. However, age at sexual maturity varies among population (Kuparinen, Kuikka and Merilä, 2009). Male halibut reach sexual maturity earlier than females and have lower growth rates after first spawning. Females of Atlantic halibut achieve a desirable market size of 4-5 kg within a span of four to five years, while male individuals, exhibit limited growth and reach only half the size (Holmyard, 2009). Consequently, in the current study, an exclusively female population is employed to ensure consistency and eliminate the potential influence of gender-related growth disparities. Spawning occurs at depths of 300-700m in Norwegian waters, where accumulations of Atlantic water with high temperature and salinity provide suitable breeding grounds (Glover, *et al*. 2006).

1.3 Digestive system

The Atlantic halibut (*Hippoglossus hippoglossus)* possesses a digestive system uniquely tailored to its dietary habits and environment. The nutrients obtained from prepared feeds undergo a process of digestion and absorption within the gastrointestinal (GI) tract. This process involves various physical, chemical, and physiological mechanisms. The GI tract includes the esophagus, stomach (which produces acid and enzymes for digestion), and intestine. The GI tract also features pyloric ceca, which are extensions located posterior to the stomach that increase the absorptive surface area (Taslimi, 2020).

Upon swallowing the whole prey, the ingested food passes through the esophagus and enters the stomach, where the digestion process begins. The stomach serves as an organ for short-term storage, mixing, and primary digestion of food. In carnivorous fish like the Atlantic halibut, the stomach is both muscular and elastic, enabling it to accommodate sizable prey items. Accessory organs such as the pancreas, liver, and gall bladder play a crucial role in the digestion and absorption of nutrients. The pancreas secretes a range of digestive enzymes, while the liver and gall bladder produce and store bile salts, which aid in the emulsification of lipids within the GI tract (Taslimi, 2020). Pancreatic tissue can be found around the pyloric caeca. As with all vertebrates, the pancreas performs two functions: (1) exocrine secretion of digestive enzymes, including proteases, lipases, and carbohydrase, into the intestine, and (2) endocrine secretion of hormones such as insulin and glucagon, which modulate blood sugar levels (Strange, 2007).

Protein digestion initiates in the stomach, where the low-pH environment created by hydrochloric acid secretion and the presence of the proteolytic enzyme pepsin facilitates the breakdown of proteins. As the ingesta (chyme) exits the stomach, it encounters neutralizing fluids in the intestine and is further acted upon by enzymes from the pancreas and intestine. These enzymes assist in the breakdown of complex proteins, carbohydrates, and lipids into smaller molecules that can be absorbed into the bloodstream (Taslimi, 2020). These smaller molecules are actively transported via ATPase pumps into the capillary network surrounding the gut. The intestinal lining features folds that significantly increase the surface area available for absorption. Although not as active as the exocrine pancreas, the intestinal wall can also secrete digestive enzymes. In carnivorous fish, the alimentary canal is typically short and S-shaped. Undigested materials are eventually expelled through the anus (Strange, 2007).

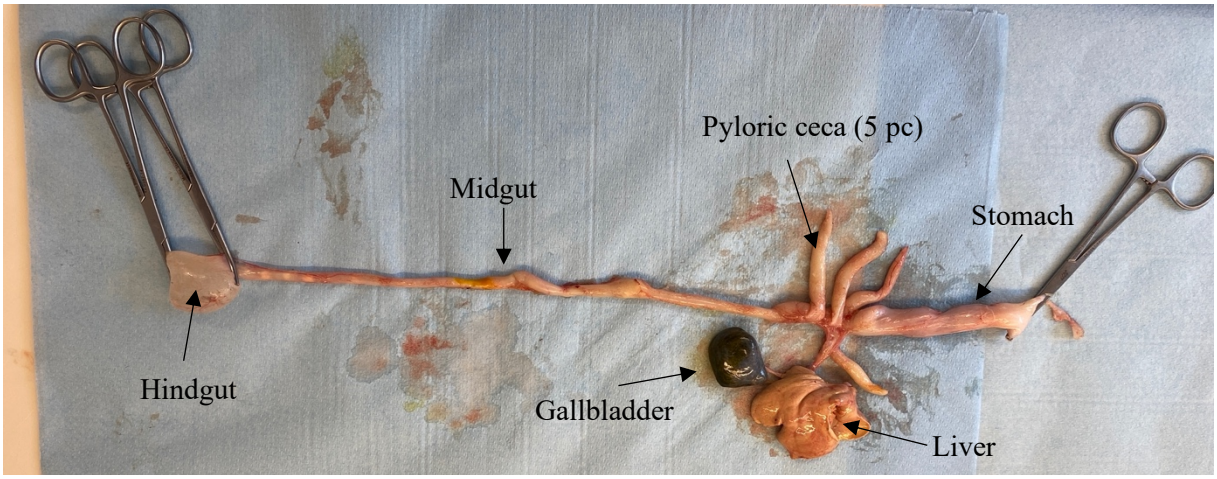

Figure 1 The picture depicts the anatomy of the gastrointestinal (GI) tract with the hindgut, midgut, gall bladder, pyloric ceca, liver, and stomach from left to right. The image was obtained from Erlend Lygre, PhD student from UiB.

1.4 Aim of study

The primary aim of this study was to assess the effects of varying levels of protein, lipid, and carbohydrates in the diet on the growth and health of Atlantic halibut (*Hippoglossus hippoglossus*). This was accomplished by analyzing muscle and liver tissue, as well as feces, from individuals fed 12 distinct diets with varying amount of protein, lipid, and carbohydrate. The study aimed to achieve the following objectives:

Assessment of various macronutrient compositions: The study evaluated the influence of different proportions of fat, protein, and carbohydrates in the diets on the growth and health of the halibut. By analyzing these macronutrient compositions, the research aimed to elucidate the nutritional requirements of the species and their impact on the overall performance on the fish.

Exploration of potential interactions between macronutrients: The study also examined the possible interactions between the macronutrients in the diets and their implications for halibut growth and health, especially from liver and muscle. This analysis provided insights into the complex relationships between the components of the diets and their influence on the tissues.

Identification of the optimal macronutrient composition for halibut feed: Based on the assessment of various macronutrient compositions and their interactions, the study aimed to

identify the optimal macronutrient balance for halibut feed. This information will contribute to the development of nutritionally balanced and efficient diets for the species.

2 Material and method

2.1 Experimental overview

The study was conducted with five distinct samplings, on October 20 (2021), February 2 (2022), April 22 (2022), August 30 (2022), and November 9 (2022). At each sampling, every individual underwent measurement of body weight and length. Several fish were removed from the study due to unwarranted welfare concerns, resulting in incomplete data sets for growth and length. During two samplings, April 22 (intermediate sampling – MU) and November 9 (final sampling – SU), fish were harvested for dissection and removal of selected organs for additional laboratory analyses.

While conducting the experiment a gill disease was discovered, which has not been further investigated. After contact with the veterinary, a speculation that the virus discovered was similar to an earlier herpes type virus found in turbot. Whether or not this had an impact on the experiment has not been investigated, and only gill health observations from the last sampling have been carried out. Hence, this has not been included.

Tank 10, which was on diet 7, was removed from the study between the MU and SU time periods due to welfare concerns. Therefore, no data or results have been collected for Tank 10 since August 30 (2022). The feces sample obtained from Tank 12 MU was insufficient for analysis and has therefore been excluded from the study results.

2.2 Sampling

2.2.1 Experimental conditions and fish characteristics

The Atlantic Halibut (*Hippoglossus hippoglossus*) utilized in the study was procured at Sterling White Halibut and transported to IMR Austevoll Research Station. The fish were separated into 15 separate tanks and had an average weight of 300 grams. Each tank was initially stocked with a density of 120 individuals and some tanks were given the same diets. The tanks were circular and arranged in a 3x6 pattern with a diameter of 2.5 meters. Each tank was equipped with an individual feeding apparatus. The water flow rate was maintained at 4000 liters per hour throughout the duration of the project, with an initial flow rate of 3000

liters per hour. The tanks were supplied with raw water at a constant temperature of 8 ° C and a stable composition throughout the year. The light regime in the tanks was synchronized with the natural light cycle using an Astor clock. Feeding was carried out using feeding machines, which were activated between 08:00 and 18:00 daily, providing equal rations of food to all fish in the tanks. The tanks were flushed daily and brushed as required to maintain optimal conditions. Sampling of the fish was carried out in conjunction with tank washing.

2.2.2 Diets

The selection of ingredients was based on industry availability. Yttrium was employed as a digestibility marker in all feed formulations. 12 distinct feed formulations were prepared, each containing varying proportions of protein, carbohydrates, and lipids. The experimental design employed a triangular mixed design model, which enabled a comparison of the three macronutrients at four different concentrations and various mixing ratios.

Table 2 The table presents data of the proportions of protein, lipid, carbohydrate, and starch components within the various diets.

Diet	Tank nr	Protein	Lipid	Carbohydrate	Starch
1	15	64	18	8	5
2	2, 5, 7	56	16	19	15
3	12	69	10	11	8
4	4	51	23	17	13
5	6, 18	45	23	24	19
6	8	57	25	8	5
7	10	50	30	11	7
8	11	66	5	19	16
9	3	77	5	7	5
10	13	46	16	30	25
11	14	53	9	29	25
12	1	60	5,0	26	22

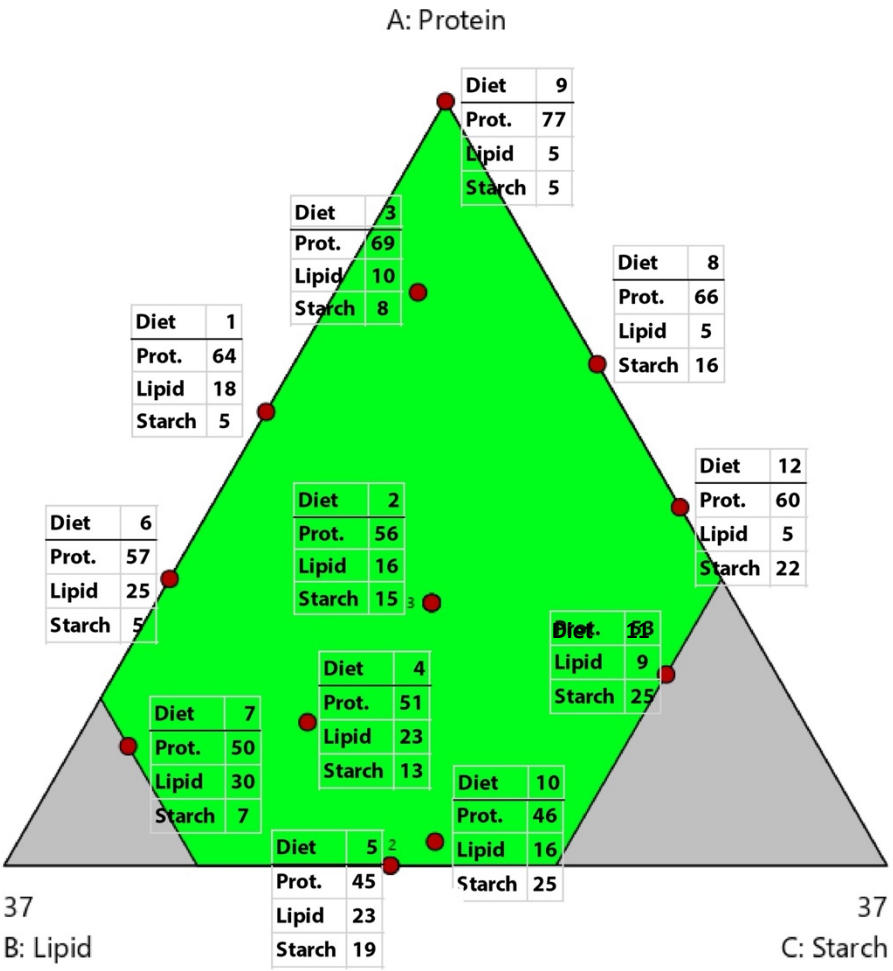

Figure 2: The triangular figure indicates the location of a particular diet based on its fat, protein, and carbohydrate composition.

2.2.3 Bodyweight, length, and welfare

During each sampling, measurements of body weight and length were quantified for every fish. All fish were transferred from a clean seawater source to an anesthetizing solution containing 20 g/L of FINQUEL. The duration of anesthesia varied but was typically 3-5 minutes until the fish exhibited sufficient calmness for further handling. The fish underwent PIT tag reading and were identified. Additionally, the body weight (g) and body length (cm) of each fish were quantified. An external health assessment was also performed, which included examination of ventral and dorsal wounds, emaciation, as well as fin damage. Each condition was assigned a health index ranging from 0 - 3, with 0 representing minimal impact and 3 indicating severe impact. Finally, the fish were returned to their rearing tank where oxygen levels were maintained between 80-100%. An excel spreadsheet was employed to

record all data, which were then used to calculate the condition factor (Fulton's factor) and specific growth rate (SGR) for each tank during the measurement periods.

Period 1 - SGR 1: Oct21 (2021) - Feb22 (2022)
Period 2 - SGR 2: Feb22 (2022) - Apr22 (2022)
Period 3 - SGR 3: Apr22 (2022) - Aug22 (2022)
Period 4 - SGR 4: Aug22 (2022) - Nov22 (2022)

Formula 1 Fulton's condition factor (K)

$$K = \left(\frac{weight}{length^3}\right) * 100$$

Formula 2 SGR (Specific growth rate)

$$SGR = \left(\frac{\ln final\ weight - \ln initial\ weight}{time}\right) * 100$$

2.2.5 Dissections

During the intermediate (MU) and final (SU) sampling stages, a random selection of six and eight fish, respectively, were picked for further analyses. Blood samples were drawn from the posterior dorsal aorta of each fish. The fish were then dissected, and the abdominal cavity was clamped to separate the foregut, midgut, and hindgut to prevent cross-contamination. The heads were then removed. The contents of each gut section were squeezed into individual 12.5 mL test tubes. Muscle tissue samples were collected from each fish at a standardized location, dorsal side posterior to the gill cover. A scalpel was utilized during the sample collection process. To ensure statistical relevance in this study, muscle samples obtained from fish from the same tank were combined into a single pooled sample (120 mL). Specifically, test tube 1 contained the 6/8 fish from tank 1, test tube 2 contained the 6/8 fish from tank 2, and so on. A similar procedure was performed for liver tissue samples, but each liver was weighed individually before being pooled in a single sample. An index was assigned to each

liver based on its weight relative to the weight of the fish. All tissue- and feces samples was stored at -20 °C until analyzed.

2.3 Laboratory analyses

The analyses were conducted in the Nordnes facilities of the Norwegian Institute of Marine Research (IMR) after the samples had been collected. The research methodologies have been developed by the Institute of Marine Research (IMR) and can be accessed from IMR's online database using the provided reference ID within the descriptions.

2.3.1 Weight

Prior to the homogenization procedure, the weight of all samples was determined using a TOLEDO MS6002TS scale. The average weight of 12 empty sample vessels of varying volumes (120 mL, 50 mL, 12.5 mL) was determined to account for deviations.

Formula 3 Average weight empty vessels

$$W_{tissue} = W_{tc} - W_m$$

$$W_{tc} = Weight\ of\ test\ tube\ with\ content$$
$$W_m = Weight\ of\ mean\ tube$$

Individual feces samples were collected from each fish using 12.5 mL sample vessels during sampling. These samples were weighed and pooled based on their origin tank to create 50 mL pooled samples. The samples were pooled in the same manner as the muscle and liver tissue samples, resulting in a total of 15 pooled samples of feces. This resulted in a total of 30 sample tubes of liver, 30 sample tubes of muscle, and 30 sample tubes of feces, with 15 from the MU sampling and 15 from the SU sampling. All samples were recorded and weighed in an Excel spreadsheet. Hepatosomatic index (HSI) was derived from the liver weight.

Formula 4: Hepatosomatic index (HSI)

$$HSI = \frac{Liver\ weight}{Body\ weight} * 100$$

2.3.2 Homogenization and freeze drying

Both muscle and liver samples were treated similarly under the homogenization procedure. After being thawed for approximately 30 minutes, the samples were homogenized using a Braun MQ 9 hand mixer with a cylinder and cutter. The resulting samples aliquots were divided into matrices for further analysis, which included determining total nitrogen, total fat, and ash content. The muscle samples were also freeze-dried for total nitrogen analysis.

Feces samples underwent homogenization using a Polytron MR2100 homogenizer and were then allocated into matrices for protein, ash, fatty acid composition, and yttrium + freeze-drying analysis.

For the 12 fish feed samples, a Retsch GM200 homogenizer was used for the homogenization process. The number of rotations per minute was adjusted based on the degree of pulverization and fat content. The homogenization was repeated 3-4 times in 5-second intervals, with rotational speed ranging from 3.5k to 10k RPM. The resulting sample materials were then distributed into the designated matrices for subsequent analysis.

Freeze-drying was performed using a LABCONCO FreeZone freeze dryer with 18-liter capacity.

2.3.3 Crude protein determination using a nitrogen analyzer

The crude protein determination method aimed to quantify the total protein, and it has been accredited by the Institute of Marine Research with reference ID: MET.UORG.01-04, method 171. The method was to quantify nitrogen content in tissue samples, feces and fish feed matrices using the Leco FP 628 instrument. The instrument is validated for analysis in the concentration range of 0.1-16 g N/100g sample. Prior to analysis, it was crucial that the samples were homogenized and in the appropriate form (wet matter, dry matter, or freeze-dried) for that specific sample.

Prior to the analysis of the sample material, ten blank samples were run to ensure the instrument was calibrated properly. Furthermore, four samples of EDTA (article no. 502-092), two biological control materials (fish feed) and two samples of sulfanilamide (article no. 05 001 726) were analyzed for validation purposes.

0,15 – 0,25 g of the sample material was weighed and placed in a metal foil that was closed in a drop shape. This was then placed in the instrument's sample carousel and underwent primary combustion in a tube with a temperature of 950 ° C in the presence of O^2. The combustion resulted in the oxidation of nitrogen, carbon and hydrogen to NO_x, CO_2 and H_2O, respectively. The combustion gases were carried through to a secondary combustion tube at 850 ° C for further oxidation and removal of particles.

$$C_xH_yN_z + \left(\frac{x+y}{4}\right)O^2 \rightarrow x\,CO_2 + \left(\frac{y}{2}\right)H_20 + z\,NO_x$$

The combustion gases then passed through a pre-cooler and a thermoelectric cooler to remove water vapor. They were equilibrated in a ballast tank before being carried through an aliquot loop using helium as a carrier gas. The helium was chosen due to its low reactivity properties. The gases then underwent reduction in a tube filled with copper reagent at a temperature of 700 ° C, which resulted in the reduction of NO_x to N^2 and capture of excess oxygen from the combustion. Finally, the gases passed through a tube with Lecosorb and Anhydrone to remove CO_2 and H_2O that were generated during the process.

$$NO_x + Cu -> N^2 + CuO$$

The amount of N^2 was detected in a thermal conductive cell, known as a temporal conductivity detector (TCO). The result was expressed as % total nitrogen.

2.3.4 Fat determination – ethyl acetat

The aim was to determine the fat content of organically extractable material in fish feed and muscle- and liver tissue samples, and the method has been accredited by Institute of Marine Research with reference ID: MET.UORG.01-01, method 091. The method is based on the principle of extraction of fat, defined as the fraction soluble in ethyl acetate, primarily consisting of non-polar lipids, using a solution of 30% isopropanol in ethyl acetate. The method has been validated and quality-assured for fat concentrations above 0.1 g/100 g. It should be noted that the method for fat determination by this method was not recommended for feces due to the limited sample material available. Instead, **2.3.5 fatty acid composition of total fatty acids by GC** was used for feces analysis.

Prior to analysis, it was crucial to ensure proper homogenization and thawing of wet material, such as liver and muscle tissue. In addition to the sample material, two control samples were analyzed as part of the validation process to ensure the accuracy and reliability of the results. A pre-prepared solution of 30% isopropanol in ethyl acetate was used for extraction. Approximately 1 g of the homogenized sample was weighed into a 50 mL glass bottle, followed by the addition of 30 mL of the extraction solution using a dispenser. The sample-extraction solution mixture was then shaken for 2 hours in a shaking machine (IKA HS 501 digital).

The resulting solution was filtered through a S&S 597 1/2 (Ø 150 mm) filter into a 100 mL Erlenmeyer flask. A portion of the filtrate, 10 mL for muscle tissue and 5 mL for liver tissue, was pipetted with Thermo scientific fine pipette with fixed volume 5ml and 10 ml, into a tared evaporation dish and left overnight in a fume cupboard to evaporate the ethyl acetate. The dishes were then placed in a heating oven at 70 ° C for 1 day, followed by cooling to room temperature in a desiccator and re-weighing to obtain the final fat content.

The content of fat was determined through an automated calculation process in the Laboratory Information Management System (LIMS) using the following formula:

Formula 5 Total content of fat %

$$\% \, fat = \frac{A * B * 100}{C * (D - (1{,}1 * B))}$$

$$A = mL\ ethyl\ acetate\ added\ sample$$
$$B = grams\ of\ fat\ in\ evaporation\ pan$$
$$C = grams\ of\ sample\ weighed$$
$$D = mL\ pipetted\ filtrate$$

2.3.5 Fatty acid composition of total fatty acids using GC

The aim of the method was to determine the fatty acid composition of total fatty acids using gas chromatography (Trace 1310) in feces and fish feed, and it has been accredited by the Institute of Marine Research with reference ID: MET.NÆR.01-02, method 041. The method was tested for concentrations above 0.01 mg of fatty acid per gram of wet material. Due to the limited availability of feces, this method was also utilized to determine the total amount of fat.

Firstly, the samples were homogenized to ensure uniformity. Reagents, including chloroform:methanol, 0.5 M NaOH, and the internal standard (Methyl 19:0 and chloroform:methanol) were prepared. The samples were then extracted using internal standard with a dilution factor of 20 (formula 2.2). The extracted solution was then mixed and kept in a freezer overnight.

Formula 6 Amount of internal standard

$$\frac{A * 20}{100} = B$$

$$A: fat\ in\ sample\ (mg)$$
$$B: Amount\ of\ internal\ standard$$

The following day, the samples were thawed, mixed again using a whirl mixer, and filtered using a vacuum block and sovirel tube at a pressure between -0.5 and 0 bar. The filtered samples were then steamed into a rapid vapor system with a speed of 42%, a heat of 40 ° C, and a pressure of approximately 300 mbar for 3-4 minutes. The pressure was then lowered to 100 mbar and left for an additional 15 minutes.

Chloroform:methanol was then evaporated and 1 ml of 0.5 M NaOH was added to the samples. The samples were then boiled on a block heater at 100 ° C for 15 minutes, cooled, and mixed with 2 ml of BF_3. The samples were then boiled again on the block heater for 5 minutes and cooled. 2 ml of hexane and 2 ml of water were added to the samples and mixed using a whirl mixer. The samples underwent a centrifugation at 3,000 revolutions per second

for 30 seconds. The upper layer (hexane phase) was pipetted out and stored in a freezer for later analysis on a gas chromatograph (GC).

When run on the GC (Trace1310), the samples were diluted to a concentration of 0.2 mg/ml. 0.5 µl of the sample was then injected with an "On column injector" and the results were presented in the institute's program (including Chromeleon) after 45 minutes per test.

The content of fatty acids was determined through an automated calculation process in the Laboratory Information Management System (LIMS) using the following formulas:

Formula 7 Area of fatty acid

$$a = b\left(\frac{c * d}{e}\right)$$

$$a = concentrated\ fatty\ acid$$
$$b = retention\ factor = 1$$
$$c = internal\ standard$$
$$d = area\ of\ fatty\ acid$$
$$e = area\ of\ internal\ standard$$

Formula 8 Fatty acid mg/g

$$fatty\ acid\ \left(\frac{mg}{g}\right) = \frac{a(concentraed\ fatty\ acid)}{sample\ weight}$$

2.3.6 Total determination of Yttrium

The purpose of this analysis was to determine the concentration of yttrium in fish feed and feces for the purpose of evaluating digestibility. The method has been accredited by the Institute of Marine Research with reference ID: MET.UORG.01-06, method 197. Standard solutions were prepared prior to the analysis, including a 5% nitric acid solution, a stock solution (0.05 ml yttrium standard diluted with 5% nitric acid) for constructing a standard curve, and a 0.5 mg/l Rh solution as an internal standard.

For the series of experiments, sample blanks and control samples were included, and five stock solutions were prepared with starting concentrations of 5, 10, 20, 50, and 100 µg/l, which were then diluted to 10 ml with 5% nitric acid. A standard blank consisting of only 5% nitric acid was also included in the study.

To begin the analysis, 0.20-0.25 grams of freeze-dried sample material were weighed into a 15ml digestion tube and 2ml of concentrated nitric acid (HNO_3) were added. Deionized water (0.5 ml) was also added to the sample tube, which was then plugged with a cork. After digestion, the samples were diluted to 25ml with water and transferred to a 50ml centrifuge tube.

UltraCave was set up by filling the Teflon container called "Baseload" with 300ml of water and 10ml of hydrogen peroxide. The samples were then placed in the carousel and the program was initiated from the computer. The program ran for 62 minutes, including a 25-minute cooling period for the samples.

To determine the concentration of trace elements in the samples, the resulting supernatant was analyzed using ICP-MS (Thermo iCapQ). However, since the machine provided results in micrograms per liter (µg/L), a dilution factor was calculated to convert the concentrations of the samples to milligrams per kilogram (mg/kg) for analysis. This conversion was necessary to ensure that the results were consistent with the units used for the rest of the study.

Formula 9 Dilution factor

$$df = \frac{v * n}{w * 1000}$$

$$df = dilution\ factor$$
$$v = volume$$
$$n = number\ of\ dilutions$$
$$w = weight\ sample$$

To ensure that the concentrations of the samples were reported in the appropriate units for the research, a dilution factor was calculated and entered the computer program. This factor enabled the conversion of the concentrations from micrograms per liter (µg/L) to milligrams per kilogram (mg/kg) for subsequent analysis. This method was employed to ensure the consistency and accuracy of the results obtained. The samples were injected at a volume of 2.5 ml and introduced into an inductively coupled plasma (ICP) torch, which ionized argon gas with a radiofrequency coil, generating a high-temperature plasma (~10,000 K) that desolated, vaporized, and atomized the sample, breaking it down into constituent atoms. The resulting atoms in the plasma were ionized by the loss or gain of one or more electrons, forming ions that were typically in a high-energy state and could fragment further. The ions were then separated based on their mass-to-charge ratio (m/z) using a mass spectrometer. The ions were accelerated using an electric field and passed through a magnetic field, which deflected them in a curved path based on their mass-to-charge ratio. Ions with the same mass-to-charge ratio were detected by a detector located at the end of the magnetic field. The resulting signal was recorded and processed by a computer, which converted it into a mass spectrum that represented the relative abundance of ions at each mass-to-charge ratio.

2.3.7 Inorganic residue and ash content

The analysis aimed to determine the inorganic residue and ash content of a sample using LECO TGA 801. The machine was newly acquired, and this was the first application of the method using halibut samples, rendering it non-accredited at the time of experimentation. The procedure involved complete combustion of the organic compounds in the sample, leaving only the ash and inorganic residues, which could be used to calculate the ash and solids content and provide valuable information about the sample's composition.

To begin the procedure, the sample was weighed and placed in a crucible, which was then inserted into the furnace of the TGA 801 machine. The temperature was gradually increased to 750 °C in the presence of oxygen (O2) to eliminate any volatile organic compounds, followed by a further temperature increase to ensure complete combustion of the sample.

Throughout the combustion process, the TGA 801 machine monitored and recorded the sample's mass continuously. Upon completion of the combustion process, the final mass of the sample was determined, representing the ash and solids content, which was calculated as the difference between the initial and final masses.

To ensure reliable results, the procedure was conducted in a controlled and consistent manner. The parameters of the procedure, including the temperature ramp and atmosphere, were rigorously controlled, and standardized to ensure consistency in the results.

2.3.8 Digestibility

From the findings of freeze-drying-, protein-, total fatty acid-, and yttrium analysis, the apparent digestibility coefficient (ADC) was determined as a measure of digestibility. To calculate the ADC, the dry weight of the samples was first obtained, and subsequently, formula **11** was employed for the calculations.

Formula 10 Dry weight

$$dw = \frac{ww * 100}{dm}$$

$$dw = dry\ weight$$
$$ww = wet\ weight$$
$$dm = dry\ matter$$

Formula 11 ADC %

$$ADC\ \% = 100 - \left(100 * \left(\frac{YF}{YI}\right) * \left(\frac{NI}{NF}\right)\right)$$

$$YF = Yttrium\ in\ feed$$
$$YI = Yttrium\ in\ intenstine$$
$$NF = Nutrient\ in\ feed\ (Protein, Fat)$$
$$NI = Nutrient\ in\ intestine\ (Protein, Fat)$$

2.4 Data analyses

Data processing in this study was carried out using LIMS, Excel, and Design Expert version 12. LIMS (Laboratory Information Management System) is a proprietary processing system employed by the Marine Research Institute, where all data is utilized and stored during the analyses. Data extracted from LIMS was subsequently processed in Excel for further handling and was also imported into Design Expert version 12 for additional data analysis. Excel were utilized for creating graphs that were not generated by Design Expert version 12, as well as for performing certain calculations.

Based on the length and weight data from the five samples, condition factor and specific growth rate (SGR) were determined using the formulas **1** and **2** respectively. From Excel a trend analysis was performed on condition factor and SGR.

For data analysis, design of experiments (DOE) analysis was conducted in Design-Expert version 12 (DX). A Mixture design with numeric components and responses was utilized. The experiment consisted of 15 runs, corresponding to the number of tanks. The software subsequently analyzed the raw data and identified the most suitable statistical model. If the data's variation (ratio) exceeded 3, a transformation was applied to normalize the data. Transformations included square root, natural log, base 10 log, inverse square root, inverse, power, logit, or arcsine square root. The software generated a fit summary, providing an overview of calculated p-values for different models, and flagging any potentially aliased models. Design Expert then conducted an ANOVA analysis using the selected model, displaying the results in a triangular design format. The ANOVA analyses were set with a confidence interval of 95%, and a p-value of less than 0.05 was considered statistically significant.

Each result in the study is presented with a figure or table. Although only the figures are presented in the results section, the tables containing the ANOVA test results can be found in Appendix table A3-A28 for reference.

3 Results

Initially, the results obtained during the MU period is presented, encompassing overall health parameters, alongside the assessment of condition factor and SGR. Subsequently, analysis of tissue samples, including liver, muscle, and fecal specimens, is presented. Transitioning to the SU period, the investigation continues with a continuation of the aforementioned analyses, aiming to elucidate any alterations or trends observed during this specific phase. Finally, the development of weight, SGR, and condition factor is presented.

3.1 Intermediate sampling (MU)

3.1.1 Health parameters

In chapter 2.1.3, it was mentioned that an operational welfare score was conducted externally. Health indices were assigned to each condition on a scale of 0 to 3, where 0 denoted minimal influence and 3 signified a severe impact. Generally, health scores were low, yet no apparent pattern emerged concerning the relationship between dietary composition and health indicators. Tanks with distinct diets exhibited varying scores for all health aspects. Furthermore, these differences were typically minor and might not hold clinical importance.

Table 3 Summary of average overall external health indicators based on tank and diet from MU, rated on a scale of 0 to 3, with 0 signifying minimal impact and 3 denoting significant impact.

Tank	*Diet*	*Emaciation*	*Wounds dorsal*	*Wounds ventral*	*Fins*	*HSI MU*
15	1	1.47	1.78	1.12	1.08	1.38
2	2	1.33	1.00	1.19	1.08	1.80
5	2	1.43	1.00	1.16	1.02	1.27
7	2	1.20	1.29	1.15	1.04	1.64
12	3	1.49	1.11	1.16	1.06	1.26
4	4	1.41	1.00	1.13	1.10	1.55
6	5	1.27	1.43	1.06	1.07	2.05
18	5	1.48	1.40	1.11	1.26	1.92
8	6	1.16	1.44	1.17	1.03	1.35
11	8	1.48	1.50	1.18	1.08	1.32
3	9	1.14	1.50	1.07	1.05	1.05
13	10	1.49	1.11	1.16	1.06	1.76
14	11	1.36	1.13	1.15	1.03	1.49
1	12	1.51	0.00	1.15	1.09	0.95

3.1.2 Condition factor

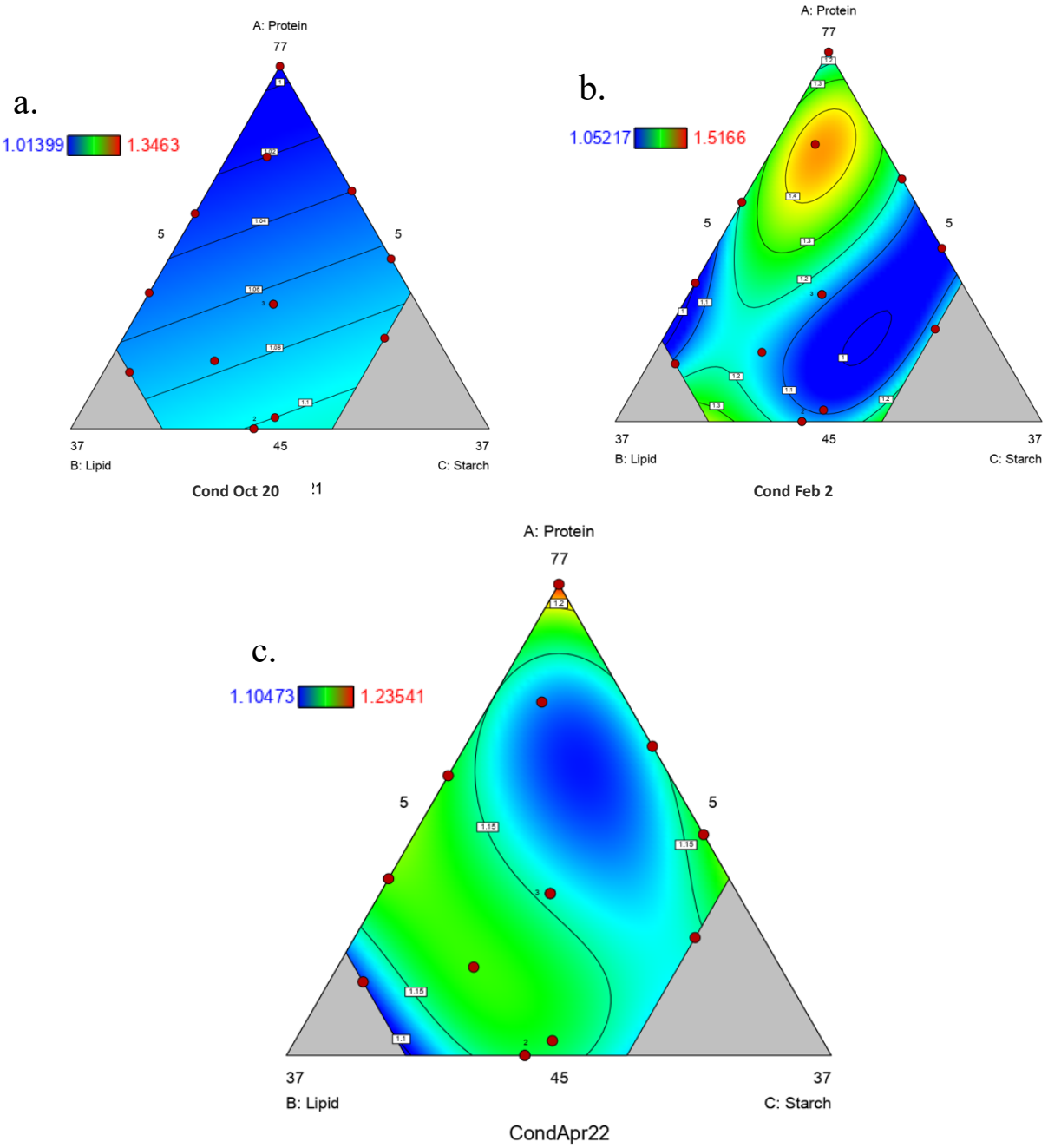

Figure 3 The figure displays a panel which present the results of the condition factor conducted in the DX design from October 20 (2021) to April 22 (2022). The boxes to the left of each figure indicate the minimum and maximum condition factor for the figure, and the colors represent the corresponding conditions. a: Shows the condition factor results from October 20 (2021), presented in a linear model, with a p-value of 0.3861. b: Shows the condition factor results from February 2 (2022), presented in a cubic model, with a p-value of 0.1984. c: Shows the condition factor results from April 22 (2022), presented in a linear model, with a p-value of 0.1451.

The condition factor data obtained on October 20 followed a linear model, but the fit was not statistically significant (P-value of 0.3861), and the values ranged from 1.01 - 1.3 (Appendix table A3 for reference). On February 2 and April 22 (MU), the data showed a best fit to a cubic model, but neither of these fits were statistically significant, with p-values of 0.1984 and 0.1451, respectively (Appendix table A4 and A5 for reference). The values varied from 1.05 - 1.5 on February 2 and from 1.04 - 1.2 on April 22. Although these results were not statistically significant, they approached the significance threshold, indicating some discernible trends.

From February 2 to April 22, there was a shift in the effect of high protein content on the condition factor, initially resulting in a favorable condition factor but later leading to a lower condition factor during MU. Additionally, high lipid content was associated with a lower condition factor during MU. Diets with high carbohydrate levels generally exhibited slightly lower condition factors. The condition factors observed in the February 2 measurements were slightly higher than those on October 20 and April 22.

In summary, while the statistical significance was not achieved, there were indications of trends in the condition factor data. The effects of protein, lipid, and carbohydrate content on the condition factor varied depending on the sampling period.

3.1.3 SGR

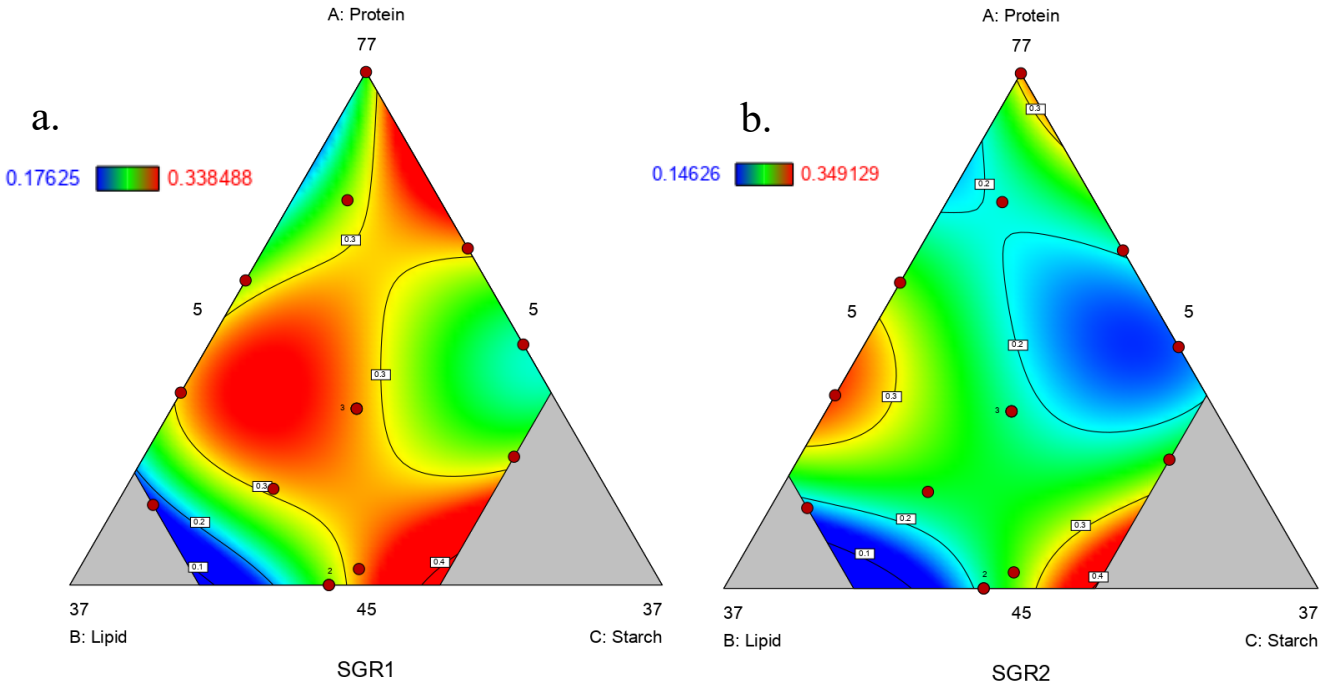

Figure 4 The figure displays a panel which present the results of the SGR conducted in the DX design from SGR 1 (Oct21 (2021) - Feb22 (2022)) and SGR 2 (Feb22 (2022) - Apr22 (2022)). Boxes to the left of each figure indicate the minimum and maximum SGR for the figure, and the colors represent the corresponding conditions. a.: SGR 1 with a p-value 0,0462 and values ranging from 1,176-0,338. b.: SGR 2 with a p-value 0,4582 and values ranging from 0,146-0,349.

The SGR results obtained from DX design analyses showed that both SGR 1 and SGR 2 data were best fit by a cubic model, but only SGR 1 demonstrated statistical significance, with a p-value of 0.0462 (Appendix table A8 for reference). SGR 2 had a p-value of 0.462, indicating no statistical significance, and the values ranged from 0.146 - 0.349 (Appendix table A9 for reference).

SGR 1 analysis revealed that diets with high fat content resulted in lower SGR compared to the other diets. Conversely, diets with a combination of high protein and low lipid content, low carbohydrate content, or high carbohydrate and low protein content exhibited the most favorable SGR values, ranging from 0.17625 - 0.338488.

3.1.4 Tissue analyses

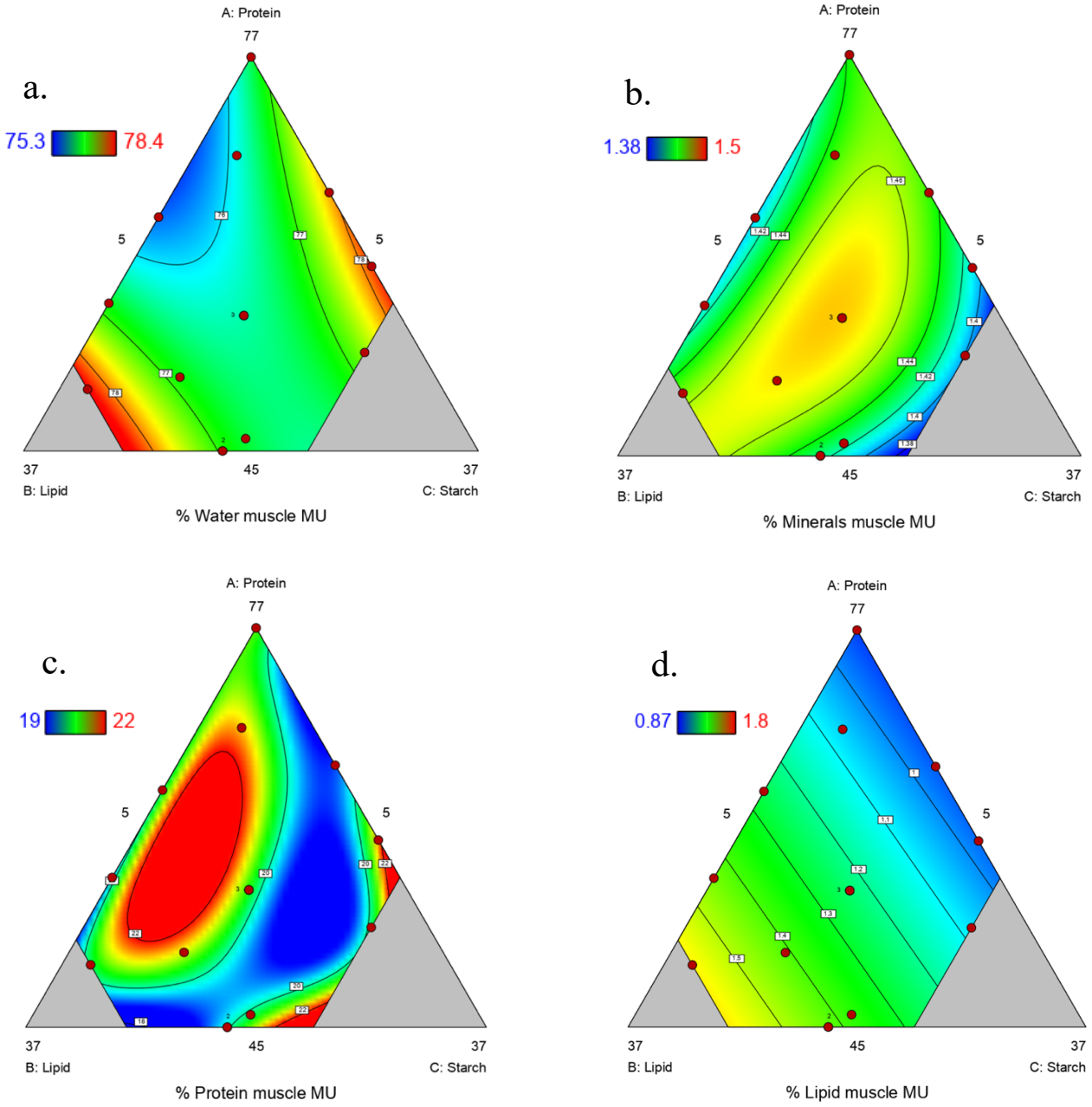

Figure 5 The figure displays a panel which present of the results obtained from muscle tissue analysis, as outlined in Chapter 2, utilizing DX design version 12 at the intermediate sampling (MU). The left boxes show the % range for the measured nutrient, while the colors indicate the corresponding conditions. The following sections present the findings from each analysis. a: The figure displays freeze drying analysis results, quantifying water percentage in muscle tissue samples. P-value 0.0213. b: The figure presents the results from ash analysis which quantifies the percentage of minerals in muscle tissue. P-value 0.2443. c: The figure presents the results from total nitrogen analysis which quantifies the percentages of protein in muscle tissue. P-value 0.0904 d: The figure presents the results from total fat analysis which quantifies the percentages of lipid in the muscle tissue. P-value 0.0020.

The analyses conducted on muscle tissue samples demonstrated distinct model fits for different parameters. The freeze-drying analysis, which assessed water percentages in muscle tissue, yielded a quadratic model that exhibited statistical significance (p-value: 0.0213) (Appendix table A12 for reference). The ash analysis, examining mineral percentages, best fit a special cubic model but did not reach statistical significance (p-value: 0.2443) (Appendix table A13 for reference). The total nitrogen analysis, measuring total protein percentages, showed the best fit with a special quartic model, approaching statistical significance (p-value: 0.0904) (Appendix table A14 for reference). Lastly, the total fat analysis, quantifying lipid percentages in muscle tissue, displayed the best fit with a linear model, indicating statistical significance (p-value: 0.0020) (Appendix table 15 for reference).

Regarding water content, the graphical representation highlights that diets with high lipid content or minimal lipid presence contribute to increased water retention in muscle tissue. Conversely, diets low in carbohydrate content are associated with reduced water accumulation in the muscle tissue. The water content ranged from 75,3% - 78,4%.

Furthermore, the linear model emphasizes the significant impact of different dietary compositions on lipid deposition in the muscle during the MU period. Diets with elevated fat content result in greater fat deposition compared to diets with lower fat content. The lipid ranged from 0,87% - 1,8%.

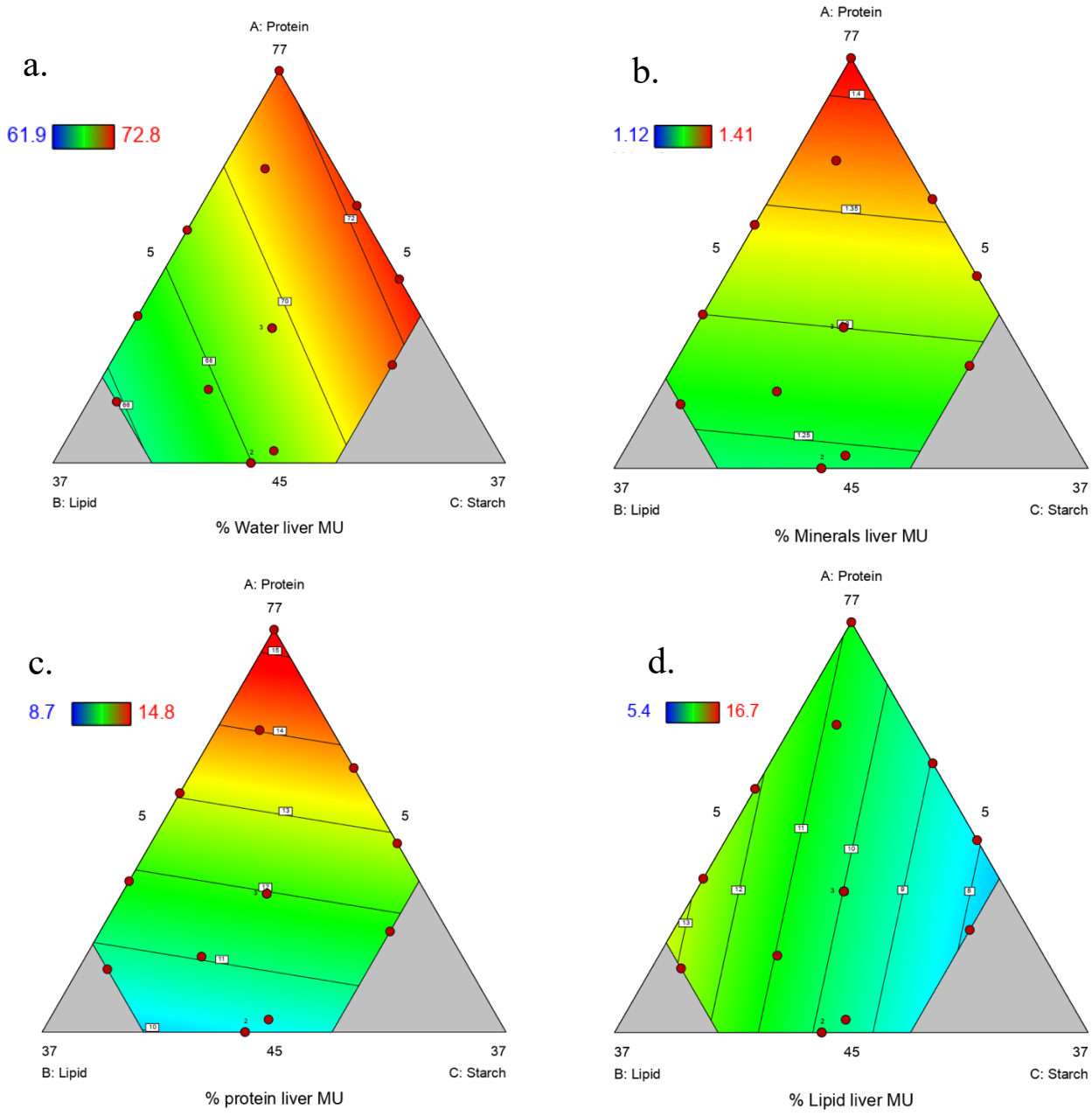

Figure 6 The figure displays a panel which present the results obtained from liver tissue analyses, as outlined in Chapter 2, utilizing DX design version 12 at the intermediate sampling (MU). The left boxes show the % range for the measured nutrient, while the colors indicate the corresponding conditions. The following sections present the findings from each analysis. a: The figure displays freeze drying analysis results, quantifying water percentage in muscle tissue samples. P-value 0.0442. b: The figure presents the results from ash analysis which quantifies the percentage of minerals in liver tissue. P-value 0.0150. c: The figure presents the results from total nitrogen analysis which quantifies the percentages of protein in liver tissue. P-value 0.0003 d: The figure presents the results from total fat analysis which quantifies the percentages of lipid in the liver tissue. P-value 0.2715

The analyses conducted on liver tissue samples revealed distinct model fits for different parameters. The freeze-drying analysis, assessing water percentages in liver tissue, showed a linear model with statistical significance (p-value: 0.00442) (Appendix table A16 for reference). The ash analysis, quantifying mineral percentages, best fit a special linear model that exhibited statistical significance (p-value: 0.0150) (Appendix table A17 for reference). The total nitrogen analysis, measuring total protein percentages, demonstrated the best fit with a linear model that reached statistical significance (p-value: 0.0003) (Appendix table A18 for reference). Lastly, the total fat analysis, quantifying lipid percentages in liver tissue, displayed the best fit with a linear model, although it did not exhibit statistical significance (p-value: 0.2715) (Appendix table A19 for reference).

Regarding water content, the analysis suggests that diets with reduced fat content are associated with increased water content in the liver. The water accumulation ranged from 61.9% - 72.8%. In terms of mineral content, the linear gradient indicates that protein is the primary determinant, as a higher protein content leads to increased mineral content in the liver. The mineral ranged from 1.12% - 1.41%. Furthermore, the linear model for total nitrogen analysis indicates that protein deposition in the liver is primarily influenced by protein levels in the diet. A higher protein diet results in greater protein deposition in the liver. The lipid accumulation ranged from 5.4% - 16.7%.

3.2 Final sampling (SU)

3.2.1 Health parameters

Table 4 Summary of average overall external health indicators based on tank and diet from SU, rated on a scale of 0 - 3, with 0 signifying minimal impact and 3 denoting significant impact.

Tank	*Diet*	*Emaciation*	*Wounds dorsal*	*Wounds ventral*	*Fins*	*HSI SU*
15	1	0.63	0.13	1.38	0.75	1.38
2	2	1.38	0.00	1.38	1.13	1.29
5	2	1.25	0.00	1.25	0.88	1.41
7	2	1.00	0.00	1.50	0.88	1.48
12	3	1.00	0.00	1.50	1.00	1.81
4	4	1.13	0.38	1.75	0.88	1.60
6	5	1.25	0.13	1.63	1.25	1.60
18	5	1.13	0.00	1.00	1.13	1.57
8	6	1.25	0.13	1.25	1.13	1.58
11	8	0.63	0.63	1.63	0.75	1.55
3	9	0.38	0.38	1.38	0.75	1.23
13	10	0.38	0.50	1.38	0.88	1.92
14	11	0.75	0.13	1.00	0.63	1.92
1	12	1.13	0.00	1.13	0.88	1.17

3.2.2 Condition factor

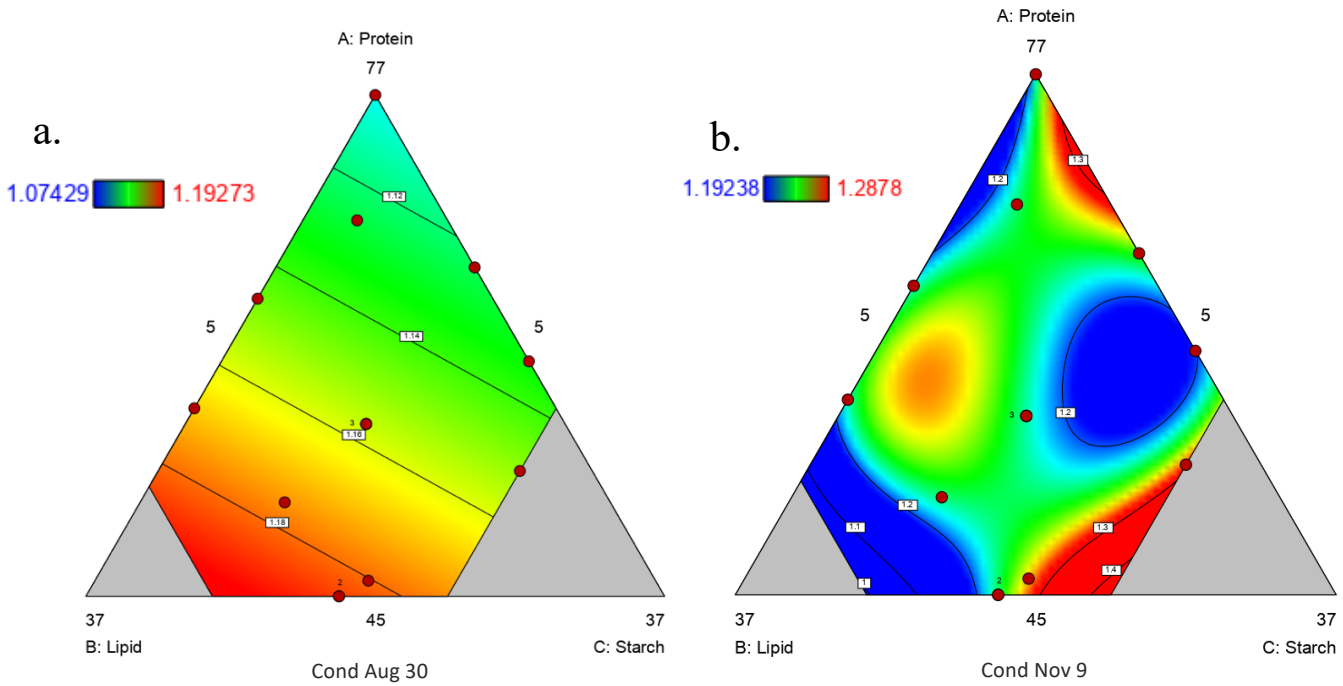

Figure 7 The figure displays a panel which present the results of the condition factor conducted in the DX design from August 30 (2022) to November 9 (2022). The boxes to the left of each figure indicate the minimum and maximum condition factor for the figure, and the colors represent the corresponding conditions. a.: Shows the condition factor results from August 30 (2022), presented in a linear model, with a p-value of 0.0136. b.: Shows the condition factor results from November 9 (2022), presented in a cubic model, with a p-value of 0.1140.

The condition factor data obtained on August 30 exhibited a significant linear correlation, with a P-value of 0.0136, and values ranging from 1.07 - 1.19 (Appendix table A6 for reference). On November 9, the data showed the best fit to a cubic model, although it was not statistically significant, with a p-value of 0.1140 (Appendix table A7 for reference).

In August there was a significant correlation between the condition factor and the amount of lipid in the diet varying between 1.07 - 1.19. Higher lipid content was associated with a higher condition factor. In November, although the model did not reach statistical significance, it approached the threshold. There was a trend suggesting that both high and low lipid content in the diet resulted in slightly lower condition factors, while diets with high carbohydrate and low protein content exhibited higher condition factors. The condition factor varied between 1.19 and 1.28.

3.2.3 SGR

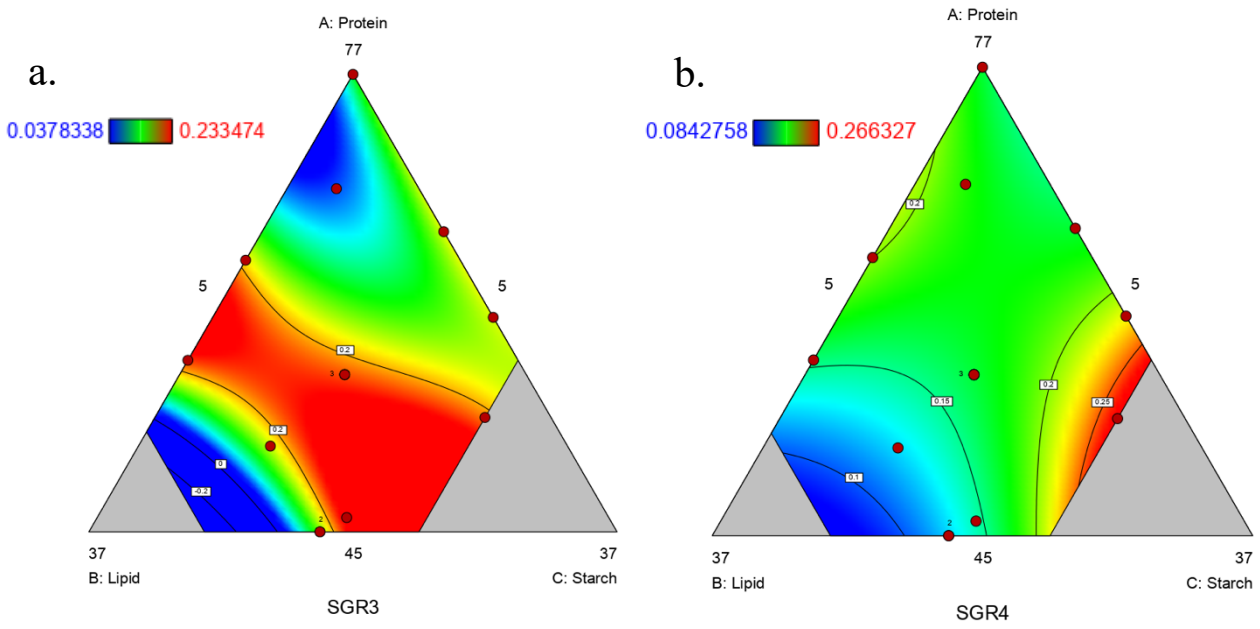

Figure 8 The figure displays a panel which present the results of the SGR conducted in the DX design from SGR 3 (Apr22 (2022) - Aug22 (2022)) and SGR 4 Aug22 (2022) - Nov22 (2022)). Boxes to the left of each figure indicate the minimum and maximum SGR for the figure, and the colors represent the corresponding conditions. a.: SGR 3 presented in a cubic model with a p-value 0,0711 and values ranging from 0.037 - 0.233. b.: SGR 4 presented in a cubic model with a p-value 0.2054 and values ranging from 0.084 - 0.266.

The SGR results obtained from DX design analyses indicated that both SGR 3 and SGR 4 data exhibited the best fit with a cubic model. However, neither of them demonstrated statistical significance, with p-values of 0.00711 and 0.2054, respectively (Appendix table A10 and A11 for reference). SGR 3 had values ranging from 0.037 - 0.233, while SGR 4 had values ranging from 0.0842 - 0.266.

The absence of statistical significance suggests that the findings related to SGR 3 and SGR 4, and the variables being examined, may not possess strong reliability. Nonetheless, there are discernible patterns within the data. SGR 3 demonstrates a negative correlation between high lipid content in diets and SGR. Additionally, varying carbohydrate content shows indications of improved SGR.

3.2.4 Tissue analyses

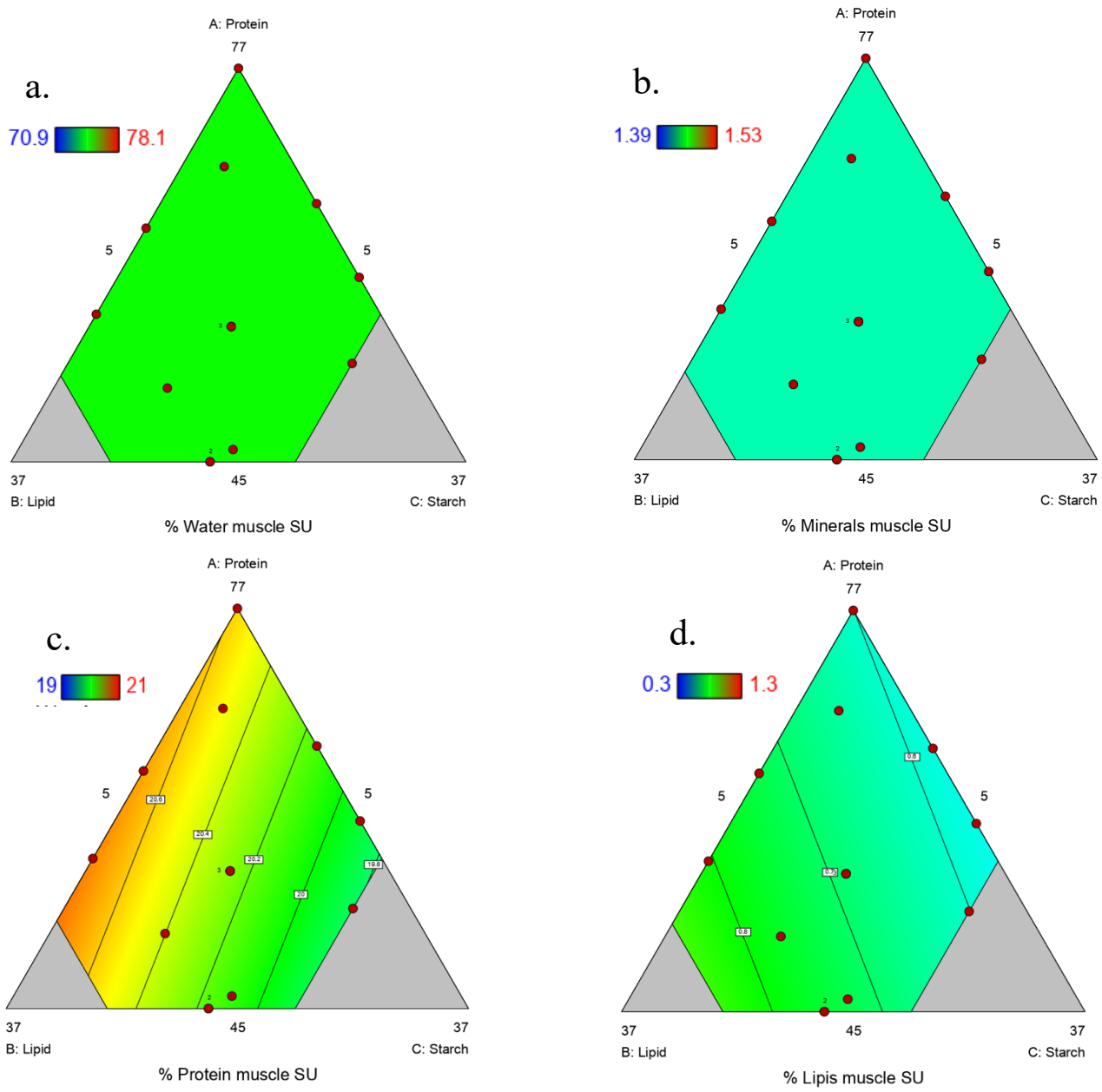

Figure 9 The panel provides an overview of the results obtained from muscle tissue analyses, as outlined in Chapter 2, utilizing DX design version 12 at the final sampling (SU). The left boxes show the % range for the measured nutrient, while the colors indicate the corresponding conditions. The following sections present the findings from each analysis. a.: The figure presents the results from freeze drying analysis, which quantifies the percentage of water present in the muscle tissue samples, fitted to a mean model. No statistical significance was observed. b.: The figure presents the results from ash analysis which quantifies the percentage of minerals in muscle tissue, fitted to a mean model. No statistical significance was observed. c: The figure presents the results from total nitrogen analysis which quantifies the percentages of protein in muscle tissue, fitted in a linear model. P-value 0.2979. d: The figure presents the results from total fat analysis which quantifies the percentages of lipid in the muscle tissue, fitted in a linear model. P-value 0.6210

The analyses performed on samples of muscle tissue revealed distinct model fits for various parameters. The freeze-drying analysis, which aimed to determine the water percentages in the muscle tissue, demonstrated a mean model fit, but no statistically significant results were observed (Appendix table A20 for reference). Similarly, the ash analysis, which examined the mineral percentages in the samples, showed the best fit with a mean model, but no statistical significance was observed (Appendix table A21 for reference). On the other hand, the total nitrogen analysis, which measured the total protein percentages, exhibited the best fit with a linear model. However, this model did not yield statistical significance (p-value: 0.2979) (Appendix table A22 for reference). Lastly, the total fat analysis, which quantified the lipid percentages in the muscle tissue, displayed the best fit with a linear model, but no statistical significance was indicated (p-value: 0.6210) (Appendix table A23 for reference).

Considering the lack of a significant p-value or a very high p-value, the obtained models cannot be used effectively to analyze the results.

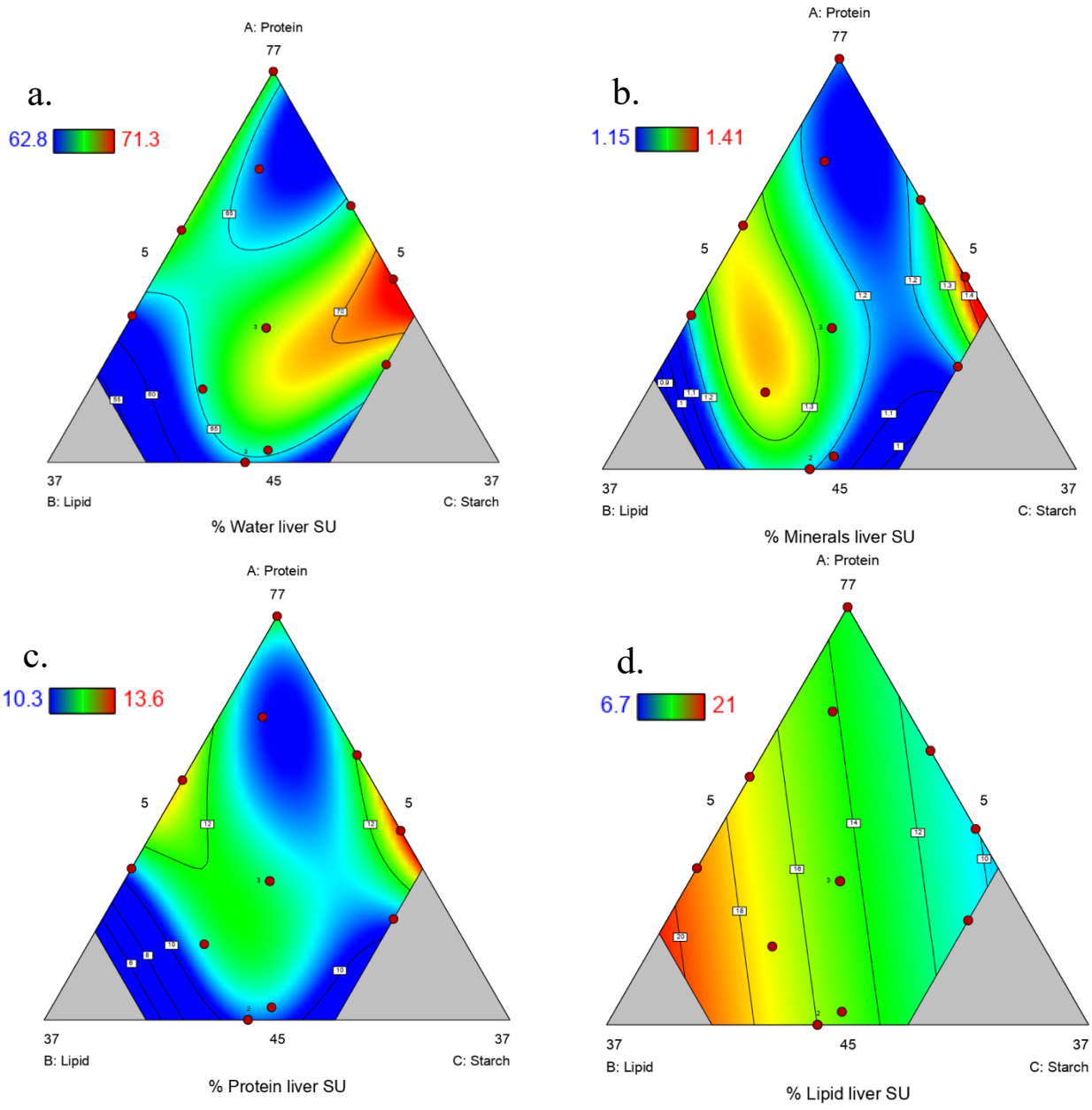

Figure 10 The panel provides an overview of the results obtained from liver tissue analyses, as outlined in Chapter 2, utilizing DX design version 12 at the final sampling (SU). The left boxes show the % range for the measured nutrient, while the colors indicate the corresponding conditions. The following sections present the findings from each analysis. a: The figure presents the results from freeze drying analysis, which quantifies the percentage of water present in the liver tissue samples, fitted to a cubic model. P-value 0.2385. b: The figure presents the results from ash analysis which quantifies the percentage of minerals in liver tissue, fitted to a cubic model. P-value 0.0025. c: The figure presents the results from total nitrogen analysis which quantifies the percentages of protein in liver tissue, fitted to a cubic model. P-value 0.0021 d: The figure presents the results from total fat analysis which quantifies the percentages of lipid in the liver tissue, fitted to a linear model. P-value 0.0297.

The analyses conducted on liver tissue samples revealed distinct model fits for different parameters. The freeze-drying analysis, assessing water percentages in liver tissue, showed a cubic model with not a statistical significance (p-value 0.2385) (Appendix table A24 for reference). The ash analysis, quantifying mineral percentages, best fit a special cubic model that exhibited statistical significance (p-value 0.0025) (Appendix table A25 for reference). The total nitrogen analysis, measuring total protein percentages, demonstrated the best fit with cubic model that reached statistical significance (p-value 0.0021) (Appendix table A26 for reference). Lastly, the total fat analysis, quantifying lipid percentages in liver tissue, displayed the best fit with a linear model, and exhibited statistical significance (p-value 0.0297) (Appendix table A27 for reference).

The analysis conducted on liver samples using ash analysis to determine water and mineral percentages showed significant results. High-fat diets exhibited a negative correlation with water content in the muscle, where increased dietary lipid content led to lower water content in the liver. Additionally, fish fed low-fat diets had higher water content in the liver. High-protein diets also showed lower water content in the muscle. Mineral content was influenced by the levels of carbohydrate and fat, with moderate levels of lipid and carbohydrate resulting in slightly higher mineral content. Moreover, there was an increasing amount of mineral deposition in the liver of fish fed lipid-deficient diets. The water content ranged from 62.8% - 71.3%, and the mineral content ranged from 1.15% - 1.41%.

Protein content in the liver was also affected by the lipid content in the diet. Additionally, diets high in protein and high in carbohydrate had a negative effect. Low-carbohydrate and low-fat diets exhibited slightly higher protein deposition in the liver. The protein content varied between 10.3% and 13.6%.

The lipid content in the liver was also dependent on the dietary lipid content, with higher lipid content in the diet resulting in increased lipid deposition in the liver. The lipid content in the liver ranged from 6.7% - 21%.

3.4 Apparent digestibility coefficient (ADC)

Upon examination of the digestive analysis, the dry matter percentages for the MU sample were found to be exceedingly high, which led to the generation of invalid data. Consequently, these findings are not reported. In contrast, the results for the SU sample are displayed in figure 11.

In the majority of the tested diets, the ADC percentages for these nutrients were relatively high, indicating that halibut effectively digests and absorbs them from various dietary levels. A comparison of the ADC percentages for fatty acids and protein within each diet reveals a trend wherein the ADC for fatty acids is typically higher than that for protein, with the exception of diet 9, which is characterized by a high protein content.

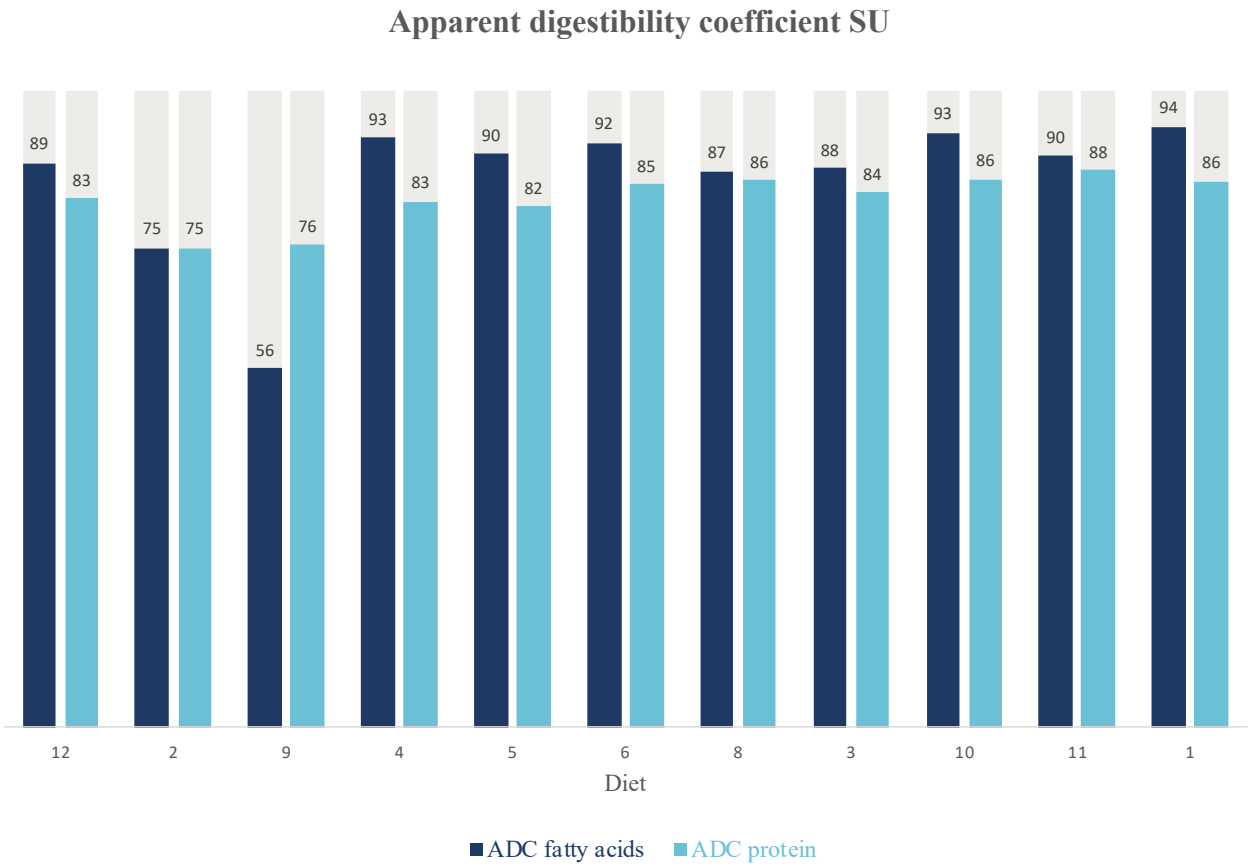

Figure 11 Results from apparent digestibility coefficient calculation extracted from Yttrium, total fatty acids and crude protein analysis of feces conducted in excel by formula 11.

3.3 Development of weight, SGR and condition factor

Table 5 Summary of the statistical analysis of the mean weight (M.w) calculated for each distinct tank. The standard deviation, derived on the premise of the entire population of measured weights. The data was collected from 5 distinct sampling, October 20 (2021), February 2 (2022), April 22 (2022) and November 9 (2022).

	Date	*October 20, 2021*	*February 2, 2022*	*April 22, 2022*	*August 30, 2022*	*November 9, 2022*
Diet	**Tank**	**M.w ± SD**	**M.w ± SD**	**M.w ± SD**	**M.w ± SD**	**M.w ± SD**
1	15	311 ± 89	426±149	529 ± 211	729 ± 356	835 ± 449
2	2	327 ± 74	489 ± 159	602 ± 234	837 ± 339	921 ± 425
2	5	321 ± 84	482 ± 417	518 ± 205	739 ± 328	852 ± 389
2	7	313 ± 73	443 ± 140	564 ± 205	760 ± 328	822 ± 409
3	12	313 ± 80	446 ± 159	525 ± 204	573 ± 232	675 ± 330
4	4	314 ± 78	459 ± 162	557 ± 242	733 ± 326	799 ± 386
5	6	316 ± 78	434 ± 136	519 ± 202	705 ± 298	748 ± 488
5	18	321 ± 79	440 ± 159	556 ± 231	739 ± 338	816 ± 395
6	8	322 ± 82	458 ± 156	608 ± 232	854 ± 378	948 ± 462
7	10	311 ± 81	422 ± 157	452 ± 168		
8	11	304 ± 71	438 ± 161	522 ± 215	707 ± 329	806 ± 421
9	3	313 ± 67	417 ± 123	564 ± 340	672 ± 285	765 ± 358
10	13	311 ± 77	455 ± 154	553 ± 203	771 ± 329	879 ± 388
11	14	317 ± 203	431 ± 139	531 ± 202	748 ± 309	888 ± 382
12	1	330 ± 82	448 ± 173	517 ± 232	671 ± 272	774 ± 339

Table 6 Summary of mean condition factor and the R^2 value generated from excel linear trend for each diet over the 5 samplings from October 20 (2021) to November 9 (2022).

Tank	*Diet*	*Oct 20*	*Feb 2*	*Apr 22*	*Aug 30*	*Nov 9*	*R^2*
15	1	1.0140	1.1374	1.1495	1.1487	1.2251	0.8113
2	2	1.0436	1.1366	1.1537	1.1774	1.2171	0.9019
5	2	1.0403	1.2021	1.1195	1.1363	1.2363	0.4573
7	2	1.0407	1.0522	1.1632	1.1525	1.2236	0.8928
12	3	1.0475	1.5166	1.1397	1.0743	1.2233	0.0057
4	4	1.0439	1.1412	1.1705	1.1552	1.2266	0.817
6	5	1.0557	1.1403	1.1674	1.1927	1.2269	0.9273
18	5	1.3463	1.1239	1.1646	1.1899	1.2481	0.0571
8	6	1.0401	1.0536	1.1984	1.1901	1.2208	0.8293
10	7	1.0402	1.1097	1.1047	-	-	-
11	8	1.0161	1.1240	1.1307	1.1623	1.2497	0.9072
3	9	1.0527	1.1040	1.2354	1.1219	1.2216	0.5112
13	10	1.0174	1.1230	1.1567	1.1783	1.2574	0.9354
14	11	1.1021	1.1229	1.1407	1.1569	1.2878	0.7672
1	12	1.0291	1.0837	1.1804	1.1133	1.1924	0.6853

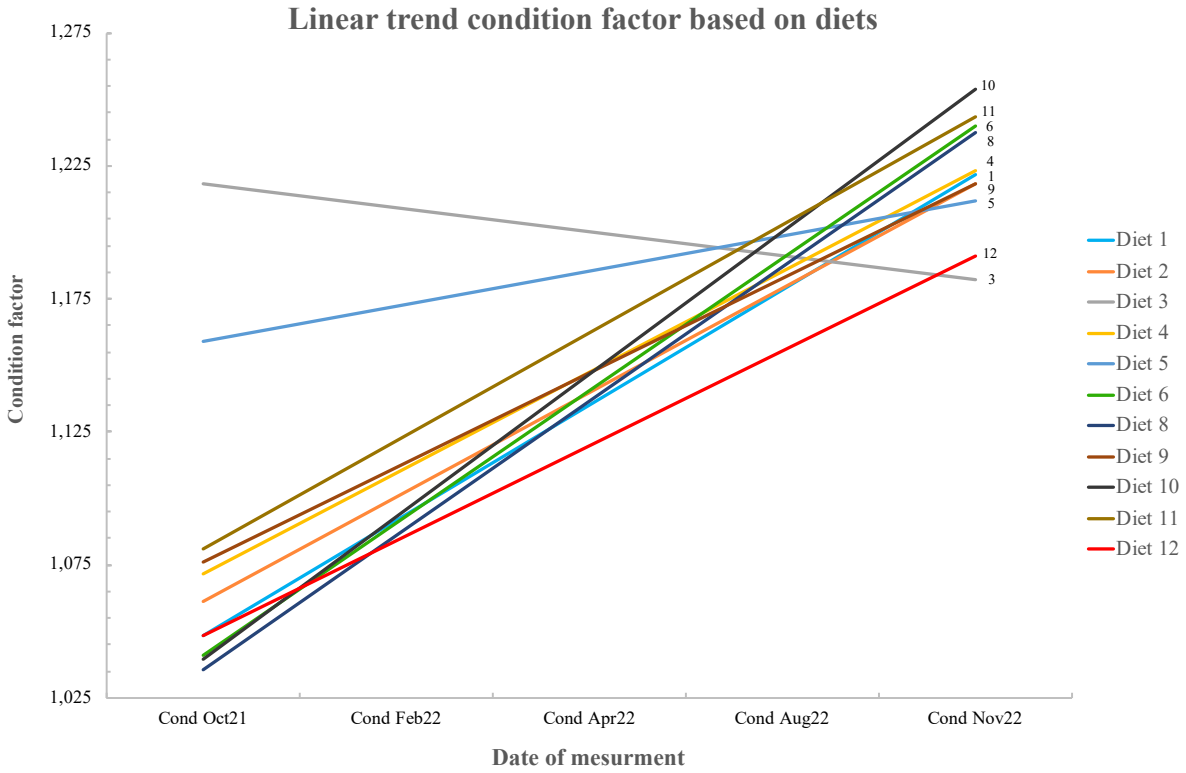

Figure 12 The figure presents the graphs which illustrates the average SGR for each diet in the period 1-4. A linear trend analysis was conducted in Excel to calculate the predictive values of Y for a given array of X values. The analysis utilized the least square method based on the provided two data series.

The linear trend shows some variation across the diets. There is a positive overall trend in condition factor from October 2021 to November 2022, except diet 3 (high protein). Diet 10 (low protein) exhibited a markedly low initial CF yet displayed a notably robust positive trend. In contrast, diet 11 (high starch) evinced an overall strong positive trend, boasting both high starting and concluding CF values. Diet 5 (Tank 18) commenced with the highest CF value but exhibited a negative trend over time. Among tanks that received the same dietary regimen, tanks 2, 5, and 7 on diet 2, as well as tanks 6 and 18 on diet 5, yielded the aforementioned outcomes from table 6. In the case of diet 2, although there were some discrepancies in the CF values, they eventually converged to similar final values. Tanks 18 and 6 exhibited converse trends, with the former displaying a declining trend and the latter displaying an increasing trend. While diet 5 (low protein) displays a comparatively high starting value, figure 12 demonstrates that it undergoes one of the most minimal alterations in condition factor in relation to the other diets.

Table 7 The table provides a summary of mean specific growth rate and the R^2 value generated from excel linear trend for each diet.

Tank	*Diet*	*SGR1*	*SGR2*	*SGR3*	*SGR4*	*R^2*
15	1	0.2901	0.2548	0.1973	0.0689	0.9192
2	2	0.3385	0.2396	0.2225	0.0769	0.921
5	2	0.3003	0.1463	0.2335	0.1215	0.4988
7	2	0.2993	0.2826	0.2068	0.0573	0.8791
12	3	0.2708	0.2058	0.0378	0.1901	0.2882
4	4	0.3124	0.2291	0.1704	0.0271	0.9655
6	5	0.2865	0.1888	0.1869	-0.0185	0.8507
18	5	0.2538	0.2710	0.1546	0.0422	0.8458
8	6	0.2933	0.3491	0.2199	0.0640	0.7282
10	7	0.1762	0.1721	-	-	-
11	8	0.3169	0.2051	0.1917	0.1224	0.9156
3	9	0.2579	0.3199	0.1336	0.0979	0.6822
13	10	0.3269	0.2308	0.2183	0.0827	0.9166
14	11	0.2866	0.2573	0.2193	0.1822	0.9968
1	12	0.2230	0.1617	0.1642	0.1158	0.8792

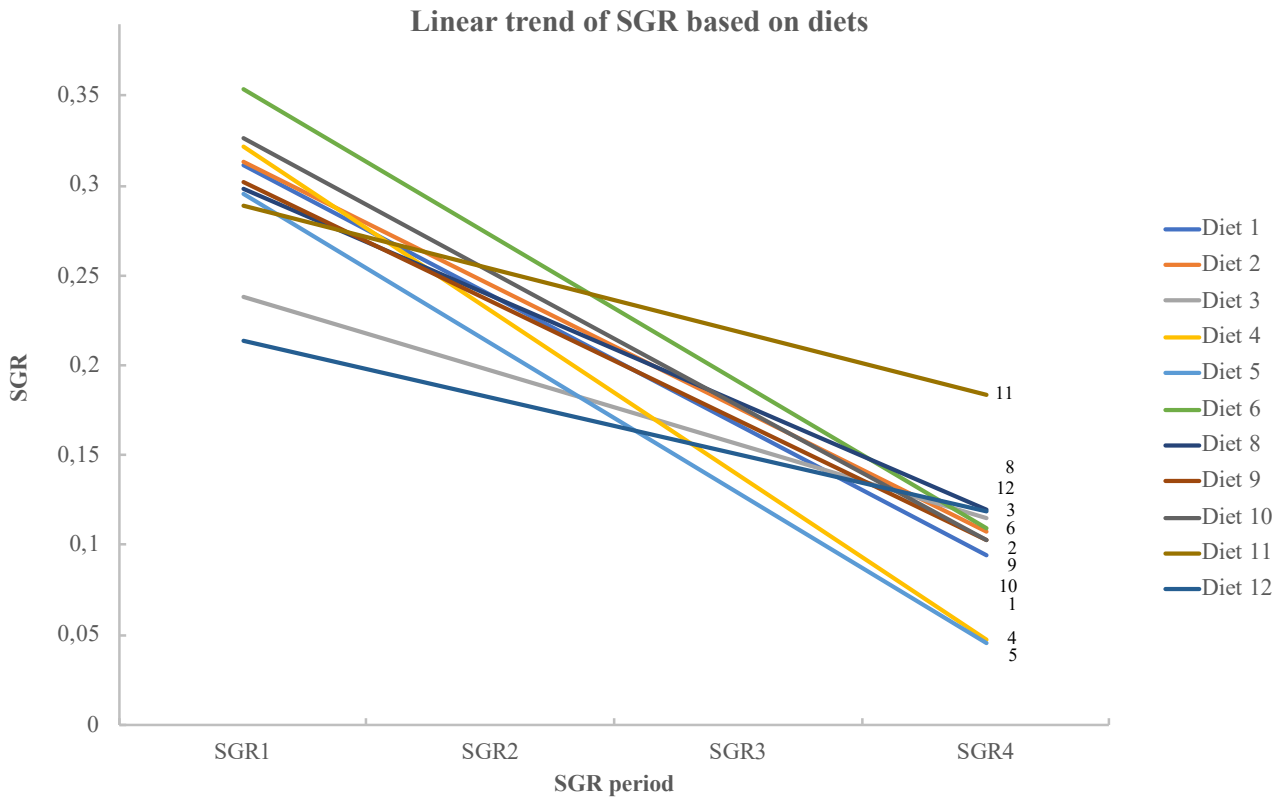

Figure 12 The figure presents the graphs which illustrates the average SGR for each diet in the period 1-4. A linear trend analysis was conducted in Excel to calculate the predictive values of Y for a given array of X values. The analysis utilized the least square method based on the provided two data series.

The linear trend of SGR values in halibut shows variability across diets. An overall negative trend was observed from October 2021 to November 2022, with diet 12, 3, and 11 (all low lipid) displaying a comparatively low negative trend. Diet 11 displayed consistently high values for both the initial and final measurements. In contrast, diet 4 (low protein), and 5 (low protein) exhibited more pronounced negative trends. Tanks that received the same dietary regimen, diet 2 (middle diet) and diet 5 (low protein), showed different levels of SGR development. From table 7, diet 2 Tank 7 exhibited the lowest trend of SGR development, followed by tanks 2 and 5, while tank 5 had the least negative trend of development. Tanks 6 and 18 exhibit a pronounced decline and are the most impacted. However, tank 6 demonstrates a more substantial downward trend than tank 18.

4 Discussion

4.1 Discussion of methods

The presence of the gill disease identified during the project has caused significant statistical noise in the data, particularly in the SGR and weight measurements. This noise affects not only each individual tank but also introduces variability between tanks with the same diet. During the sampling process, a potential correlation between fish size and gill health was observed. However, it is noteworthy that gill health assessments were solely conducted during the final sampling and have not been integrated into the current analysis. Exploring the potential correlation between fish size and gill complications would be of interest for future investigations. Although a correlation between fish size and gill complications might exist, no association between diet type and these gill complications has been observed. Nevertheless, it is plausible that the presence of gill disease has introduced statistical noise into the dataset.

The discussion that does not pertain to the results obtained from DX design and Excel analysis is solely based on raw data. Furthermore, errors and gaps were discovered in the raw dataset from the final weight measurements. This particularly affected diet 5 in tank 6, resulting in limited data for the final weight, which impacted the average. Consequently, this had an influence on diet 6 in tank 8, as it followed diet 5 in the spreadsheet file. These values were inflated, further affecting tank 6. As a result, the average final weight for diet 5 changed from 913g - 781g, and for diet 6 from 1102g - 947g. Despite this adjustment, diet 6 still resulted in the largest fish based on the final weight, as the weight of 947g surpasses that of the other diets. A new DX analysis was performed on final weight and SGR 4 and is presented in the results chapter. The issue arose during the sampling process, where problems with PIT tag reading occurred, leading to gaps in the data files.

Between MU and the SU, there were notable differences in fecal concentration, with SU exhibiting a significantly higher water content compared to MU. As a result, the calculation of ADC was not valid for MU because of errors in the freeze-drying process. Conversely, SU encountered some challenges during the freeze-drying process, with the feces starting to foam. This may have influenced the results by potentially introducing contamination or loss of material. In addition, in the results, there are some anomalies where certain samples were

empty, such as fecal samples from tank 5 in diet 2 during the final sampling. This weakens the strengths of the feces analyses as it excludes one measurement from the overall calculation.

4.2 Discussion MU

4.2.1 Growth

It has been demonstrated that marine fish, including Atlantic halibut, generally have a high protein requirement (Aksnes, Hjertnes and Opstvedt, 1996). However, studies on protein requirements in Atlantic halibut have primarily focused on smaller individuals (<300 g) (Árnason *et al.*, 2009). Nonetheless, the protein requirement of Atlantic halibut is size-dependent (Hatlen, Grisdale-Helland and Helland, 2005). Consequently, the protein content in the diet decreases as the fish grows (Árnason *et al.*, 2009). According to the findings of Árnason *et al.* (2009) and Hatlen, Grisdale-Helland and Helland (2005), previous studies conducted by Aksnes, Hjertnes and Opstvedt (1996), and Hjertnes and Opstvedt (1989), suggested that a protein content of 62% in the diet was required for optimal growth in halibut weighing between 7 g and 500 g. However, when comparing the results of these publications, the most favorable growth within the weight range of 150 g - 500 g appeared to occur in the group fed the diet containing 51% protein. Moreover, SGR exhibited similar levels in the diets containing 41% and 52% protein within the same weight interval.

Thus, their assertion of 62% protein as optimal appears applicable to the initial weight interval of 7 g - 150 g. These findings align with the results of Hjertnes *et al*. (1993), which demonstrated no detrimental effects on growth when utilizing a 40% protein diet compared to graded levels up to 60% for fish weighing approximately 100 g or more. In this study, it was found that the fish with protein content from 46% - 56% had better SGR than lower protein level when the weight interval was between 300g and 450g (SGR 1). Conversely, diets with protein levels ranging from 66% - 77% demonstrated improved SGR and increased weight during this stage. This observation provides further support for the theory that smaller fish have higher protein requirements. However, the situation changes in SGR 2, where diets rich in protein show a decline in SGR. These also had a higher average weight in the period compared to remaining diets. From the period SGR 2-3 when MU was recorded and the fish grew from 450 g – 700 g, it was observed that diets with protein levels ranging from 46% - 56% still yielded favorable outcomes. However, it was also noted that certain diets with higher protein content (60%) resulted in elevated SGR, condition factor, and overall growth.

A study conducted on rainbow trout revealed that fish weighing approximately 233g exhibited an optimal dietary protein composition of 45% (Ma *et al.*, 2019). In this particular study, diets ranging from 46% - 56% protein demonstrated a notable increase in the condition factor. According to Ma *et.al* (2019), the utilization of proteins and amino acids in the diet depends on the processes of digestion and absorption. The breakdown of nutrients in the digestive tract of fish depends largely on digestive enzymes. It has been observed in fish that the activities of digestive enzymes change with the variation of dietary protein levels (Huang *et al.*, 2016; Sagada *et al.*, 2017; Adorian *et al.*, 2018). Fish are susceptible to oxidative damage when fed an unsuitable diet, which can lead to poor growth and reduced health. Oxidative damage can occur either due to the overproduction of reactive oxygen species (ROS) or due to the reduction in cellular antioxidant levels (Matés, 2000). Some studies have shown that dietary protein levels can affect the antioxidant capacity of fish (Xu *et al.*, 2016; Yan *et al.*, 2017). The excessive amount of protein in the diets might explain why the fish that received higher protein content did not attain better growth, as it appears that the protein quantity was excessive. (Ma *et al.*, 2019). The findings from this study further confirm that fish fed diets with high protein content (66%-77%) had lower average weights compared to those fed diets with lower protein levels. For instance, fish fed a diet containing 69% protein had an average weight of 572g, while those on a diet with 77% protein had an average weight of 670g. In contrast, fish fed a diet with 53% protein achieved a higher average weight of 888g during the same period. This trend was consistent in SU as well, where fish on high protein diets still displayed lower weights.

In terms of the influence of lipid and carbohydrate on SGR and condition factor, only the condition factor measured on August 30 between SGR 3 and SGR 4 was significant. Diets high in lipids exhibited significantly improved condition factors. However, the range of variation in condition factors was relatively moderate, ranging from 1,07 – 1,19. Nevertheless, a trend emerged from SGR 1- SGR 3, indicating that diets with higher lipid content (approximately 30%) generally resulted in lower SGR values throughout the SGR periods 1-3.

Conversely, all levels of carbohydrates present in the diets showed a slightly more favorable impact on SGR. However, it is worth noting that diets with lower carbohydrate content appear to rely more on the combination of fats and proteins. For instance, a diet with higher lipid content (25%) and lower protein content (57%) exhibited a higher SGR compared to a diet with lower lipid content (18%) and higher protein content (64%), despite both diets having the same

amount of carbohydrates (5%). This trend is also evident in SGR 2 and SGR 3, although with higher uncertainty (statistically not significance). In terms of weight, there was no observable effect of fat on the diet. However, diets that were low in carbohydrates (5%-15%) showed somewhat higher weight during the period of MU. However, a shift in the pattern occurs in the final sampling (SU), where diets high in carbohydrates (25%) also demonstrate higher final weight. This phenomenon can be attributed to the fact that smaller fish tend to be more sensitive to carbohydrates compared to larger ones (Hatlen, Grisdale-Helland and Helland, 2005). This is also present in Hamre *et al.* (2003) and Aksnes, Hjertnes and Opstvedt (1996), where it was reported that small fish (<500 g) could not tolerate more than 5% carbohydrates.

4.2.2 Muscle and liver deposition

In a previous study conducted on rainbow trout by Suárez *et al.* (2014) it was observed that trout fed a lower lipid diet exhibited higher water and protein content in comparison to those fed a higher lipid diet. This finding aligns with this study, where diets with lower lipid (5%) content demonstrated slightly higher water content in the muscle compared to diets with higher lipid (±23%) content. However, it is important to highlight that both high and low lipid diets displayed elevated levels of water content in muscle tissue. The range between the minimum and maximum content averaged a narrow range of only 75% - 78% across all analyses.

The protein content in muscle tissue did not exhibit significant variations, ranging from 19% to 22%. Although the test did not yield statistical significance ($p=0.09$), there was an observed trend suggesting that diets with lower carbohydrate content tended to promote higher protein deposition in the muscles. This phenomenon could be attributed to the limited carbohydrate digestion capacity typically found in fish. Consequently, excessive carbohydrate intake may give rise to potential nutritional challenges (Jauncey, 1982; Roberts and Bullock, 1989; Lall, 1991). Excess carbohydrates can diminish the rate of growth and result in inefficient feed utilization (Hemre, Mommsen and Krogdahl, 2002). In essence, the fish's limited ability to absorb nutrients from the diet, caused by the high carbohydrate content, may have hindered the storage of protein in the muscle tissue.

It is evident that there is greater variability in the amount of lipid present in the muscle compared to the variation in water, protein, and mineral content. The quantity of lipid in the diet exerts a significant influence on the accumulation of fat in the muscle. This observation can be correlated with the physiological characteristic of halibut, where it tends to accumulate

fat reserves in different regions, especially around the fins, ventral valve and next to the lateral line. (Mannan, Fraser and Dyer ,1961). Accordingly, the incorporation of a high fat diet apparently results in an enhanced deposition of adipose tissue in the muscle structure.

In the liver, however, the correlation between dietary lipid content and liver lipid content is not as strong. While a higher lipid content in the diet tends to result in slightly more lipid in the liver, this relationship is also influenced by the carbohydrate content in the feed. Although not statistically significant, it becomes evident in the final sampling (SU) that lipid is a crucial factor in determining the amount of fat present in the liver. Similar findings have been reported in other studies conducted on large yellow croaker (*Larimichthys crocea*), turbot (*Psetta maxima*), cod (*Gadus Morhua*), and juvenile cobia (*Rachycentron canadum*) (Regost *et al.*, 2001; Hansen *et al.*, 2008; Yan *et al.*, 2015).

4.2.3 ADC

Regarding the calculation of ADC from MU, complications arose during the freeze dry analysis, resulting in the inability to calculate the ADC accurately. Therefore, the ADC values for this particular sampling unit could not be determined.

4.3 Discussion SU

4.3.1 Growth

During the final period (SGR 4), diets with lower protein content (45% - 57%) exhibited higher final weight and superior SGR. It is noteworthy that the considered "low" protein content in this study was relatively higher compared to other conducted studies. The variation in condition factor could not be effectively modeled based on dietary composition. However, the average condition factor of diets with low protein content remained comparable to those with high protein content. Interestingly, despite fish fed high protein diets showing the highest SGR and condition factor, they paradoxically displayed lower overall weight. Árnason *et al.* (2009) conducted previous research and estimated optimal protein contents of 40.5% for fish weighing 560g and 35% for fish weighing 970g to achieve maximal growth. In this study, the observed protein contents were slightly higher. Diets ranging from 45% - 57% protein content resulted in better growth, with final weights ranging from 888g - 947g. In contrast, diets ranging from 64% - 77% protein content resulted in lower weights, ranging from 675g - 765g. Within the range of 45% - 57% protein content, there was no significant impact on final weight, aligning with the findings of Hatlen, Grisdale-Helland, and Helland (2005). The results from this study and Hatlen, Grisdale-Helland, and Helland (2005) demonstrated slightly higher protein contents compared to the findings of Árnason *et al.* (2009).

The present investigation, conducted on halibut, reveals distinct results compared to earlier research performed on salmonids (Martins, Valente and Lall, 2007), marking a discrepancy in how energy, in the form of fat, is stored in these diverse species. Contrary to the salmonids study, significant growth or overall performance advantages were not detected in halibut when fed a diet with elevated lipid levels (30%). The incorporation of higher lipid content (30%) in the diet led to diminished specific growth rates (SGR), even reflecting in the condition factor wherein halibut on high-lipid diets exhibited lower condition factor. Nevertheless, statistical analysis did not indicate substantial differences in SGR and condition factor.

Harmonizing with the findings of Martins, Valente, and Lall (2007), it was observed that diets composed of approximately 16% - 25% lipid content appeared to facilitate enhanced SGR. This tendency aligns with the outcomes of numerous other studies including those undertaken by Berge and Storebakken (1991), Nortvedt and Tuene (1998), Helland and Grisdale-Helland (1998), and Hamre et al. (2003). Collectively, these studies offer evidence of limited or no

significant benefits when the dietary lipid content ranged from 20% - 39% in terms of fish growth and performance. This underscores the importance of species-specific nutritional requirements and metabolic responses to dietary composition.

Berge and Storebakken (1991) conducted a study on halibut and reported no significant effect of increasing dietary fat content (8%, 12%, 16%, or 18%) on weight gain. However, halibut fed a diet with higher lipid content (20%) exhibited a slightly higher mean growth rate compared to those fed a diet with 8% lipid, although these differences were not statistically significant. In the present study, distinct differences in final weight were observed, with values of 764g, 865g, and 938g for diets averaging 7%, 16%, and 23% lipid, respectively. Additionally, previous research by Boujard *et al.*, 2004) and Martins *et al.*, 2007 has indicated that an increase in dietary lipid can promote growth. Moreover, SGR observed in both high and low-fat diets in the current study surpassed the mean growth rate reported by Berge and Storebakken (1991) (0.58% vs. 0.27%). This suggests that there were other factors with that study that were not optimal for the fish.

It is evident from the findings that halibut fed diets with lipid levels around 20±3% achieved the best growth. The lack of effectiveness observed with higher lipid levels could potentially be attributed to the upper feeding limit of the fish's capacity to utilize dietary lipids, resulting in diminished growth due to reduced feed consumption values, as highlighted by Katsika *et al.*, 2021.

In Aksnes, Hjertnes and Opstvedt (1996) a decrease in growth rate was reported when carbohydrate was increased from 3% to 8%, but Helland and Grisdale-Helland (1998) found no negative effects of using 7% or 10% carbohydrate. The diets in this study, with varying carbohydrate content (5% - 25%), while lipid and protein remain relatively constant, exhibit similar SGR (Figure 8a.). Interestingly, the final weight of the fish showed variation in relation to carbohydrate content in the diets. The final weights ranged from 835g for diets with 5% carbohydrate, 865g for diets with 15% carbohydrate, to 888g for diets with 25% carbohydrate. Observations of enhanced growth in halibut with elevated dietary carbohydrate levels appear at odds with established research, which suggests a decrease in feed utilization in carnivorous species such as halibut when dietary carbohydrate exceeds 10% (Hemre, Lie and Sundby, 1993; Hemre *et al.*, 1995; Helland and Grisdale-Helland, 1998). However, several factors could potentially explain these observations. A possible influencing factor

could be the phenomenon of compensatory feed intake, whereby fish amplify their total food intake when starch represents an increasing portion of the available energy (Bergot, 1979; Bergot and Breque, 1983; Beamish *et al.*, 1986). Essentially, the halibut in this study have consumed more food to meet their energy requirements, leading to increased growth. Additionally, the literature highlights that carnivorous fish can exhibit growth promotion as long as protein intake is kept within a minimum adequate level (Beamish *et al.*, 1986; Degani, Viola and Levanon, 1986). Hence, it can be hypothesized that despite the elevated carbohydrate levels in the diets, the protein levels remained adequate to support growth. This observation aligns with the overall high protein content maintained in this experiment, which could potentially contribute to the underlying cause of the observed phenomenon. As feed collectors were not utilized in this study, it becomes challenging to definitively confirm these suppositions.

4.3.2 Muscle and liver deposition

The investigation conducted by Hatlen, Grisdale-Helland, and Helland (2005) found no significant changes in the whole-body composition of the 800g fish. Additionally, regarding protein deposition in both muscle and liver, no distinct patterns were observed among the various dietary groups in this study. There were no significant differences in protein content between groups with high or low levels of specific nutrients, as the values were relatively similar across all groups. However, when examining fat deposition, a notable trend emerged. Diets high in lipid content led to higher levels of fat deposition in both muscle and liver tissues. This finding aligns with previous research by Aksnes, Hjertnes and Opstvedt (1996) and Martins, Valente and Lall (2007), who reported that higher dietary lipid content resulted in increased lipid deposition in halibut. A regression analysis was also performed between HSI and lipid content in the liver, but the results suggested that there is no significant relationship between the lipid content in the liver and the HIS (See Appendix table A29 for reference). The research conducted by Martins, Valente, and Lall (2007) on halibut yielded similar results, indicating that the higher lipid intake in various dietary treatments did not have a significant impact on the HSI. This finding remained consistent even when fish were fed a diet containing 25% lipid. Previous studies, such as those conducted by Berge and Storebakken (1991) and Helland and Grisdale-Helland (1998), also reported similar outcomes. In contrast to findings from studies on cod and haddock, the HSI of halibut was not influenced by an increase in dietary lipid (Santos, Burkowb and Jobling, 1993; Nanton, Lall and McNiven, 2001),. However, the HSI of halibut did show a notable increase when the carbohydrate level in the diet was elevated.

This effect may be attributed to a higher accumulation of liver glycogen in halibut, as observed in the research conducted by Hamre *et al.* (2003). This was also seen in this study when comparing diets with 5% carbohydrate and 22-25% carbohydrate, where 5% carbohydrate had an HSI of 1,38 while 22%-25% carbohydrate had 1,49-1,92. The same effect was found in rainbow trout (Callet *et al.*, 2020). Hatlen, Grisdale-Helland and Helland (2005) also conducted experiments using smaller (60 g) and larger (800 g) Atlantic halibut.

4.3.3 ADC

When considering the ADC, the study found no significant effects of the diet on ADC for total fatty acids or protein. Nevertheless, the diet with the highest protein content (77%) had the lowest ADC of 56% for fatty acids and 75% for protein. The average ADC values for protein and fatty acids were 86% and 83%, respectively, while the diet with the highest protein content showed lower values, suggesting poorer digestion. This finding aligns with previous research by Windell, Foltz and Sarokon (1978) on Rainbow trout, which also observed poorer digestion in fish fed high protein diets despite lower body weight. This indicates that the feed may not have been efficiently assimilated and utilized for growth (Taslimi, 2020). Helland and Grisdale-Helland (1998) reported that replacing fish meal with fish oil to increase dietary fat content had no effect on fat digestibility when carbohydrate levels were constant. However, in the present study, diets with varying levels of fat while maintaining relatively constant carbohydrate levels showed differences in digestibility. Significant reductions in fat and protein digestibility were observed in fish fed diets containing 16% lipid, in comparison to the other diets. The fat and protein digestibility values for this particular diet were measured at 75% and 75%, whereas in the other diets, the values were 90% and 84%, respectively. Moreover, the diet composition consisting of 16% lipid, 56% protein, and 15% carbohydrate exhibited lower SGR and condition factor compared to the other diets with varying levels of protein, carbohydrates, and fat. Notably, this particular diet falls within the mean range of each macronutrient.

Dietary carbohydrate content had no effect on protein digestibility, consistent with previous studies conducted on cod (Hemre, Lie and Sundby, 1993) and Atlantic salmon (Arnesen and Krogdahl, 1993; Aksnes, 1995; Arnesen, Krogdahl and Sundby, 1995; Hemre *et al.*, 1995; Grisdale-Helland and Helland, 1997).

5. Conclusion

5.1 Identifying optimal diet formulations for enhanced fish performance

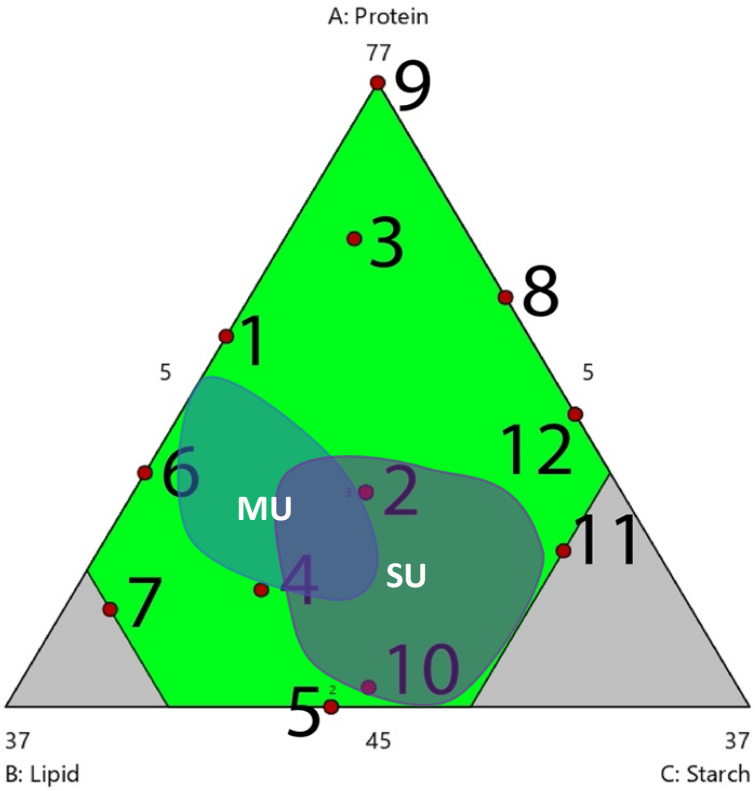

Figure 13 The figure illustrates the area which, according to the findings, can be characterized as the optimal diet from MU (300g-700g fish) and SU (700g-900g fish).

Proper diet formulation plays a crucial role in maximizing the growth and performance of farmed fish. In the case of Atlantic halibut, understanding the ideal composition of protein, lipid, and carbohydrate in the diet is essential for achieving optimal growth rates and body composition. This study aimed to identify the optimal diet formulations for enhanced performance in Atlantic halibut based on a comprehensive evaluation of various dietary parameters.

In accordance with earlier findings, the present study observed no detrimental effects of diets containing protein levels between 40% and 60% for fish weighing over 100g. However, it is important to note that the protein requirements of fish can vary depending on their size. In this

study, diets comprising 46% - 56% protein demonstrated improved SGR and weight during the period leading up to the measurement of MU. Conversely, diets with protein levels exceeding 60% exhibited inferior overall performance, suggesting that protein levels above 57% are not favorable for fish weighing between 300g and 700g. Notably, there were no significant differences observed among diets ranging from 46% - 56% protein content, indicating that protein levels within this range adequately meet the performance needs of fish weighing between 300g and 900g.

The determination of optimal lipid levels in the MU stage proved somewhat challenging due to the negative influence of higher lipid levels on SGR. However, no discernible impact of lower SGR on final weight was observed. On the contrary, diets with higher lipid content (30%) yielded inferior results in terms of overall performance. Conversely, diets with lower lipid content (5%) did not elicit any significant effects. In the SU stage, diets with moderate lipid levels demonstrated favorable overall performance. In sum, diets with lipid levels around 16% - 23% appeared to provide the most optimal outcomes in terms of growth and performance.

Regarding dietary carbohydrates, it was evident that fish in the MU stage may exhibit heightened sensitivity to higher carbohydrate content, although this sensitivity diminished as the fish reached the SU stage. This observation can be attributed to the increased feed intake by fish with higher carbohydrate diets, leading to a higher final weight. In the MU stage fish fed diets with lower carbohydrate content displayed higher weight. However, there was minimal variation in final weight when the carbohydrate levels in the SU stage varied. These findings suggest that fish weighing up to approximately 700g tolerate lower carbohydrate levels ranging from 5% - 15%, while fish in the weight range of 700g - 900g can tolerate carbohydrate levels up to 25%.

References

Adorian, T. J. *et al.* (2018) 'Effect of sex and protein level on the intermediary metabolism, growth, deposition of nutrients and profile of volatile compounds of silver catfish (*Rhamdia quelen*)', *Aquaculture Nutrition*, 24(2), pp. 793–801. doi: 10.1111/ANU.12608.

Aksnes, A. (1995) 'Growth, feed efficiency and slaughter quality of salmon, *Salmo salar* L., given feeds with different ratios of carbohydrate and protein', *Aquaculture Nutrition*, 1(4), pp. 241–248. doi: 10.1111/J.1365-2095.1995.TB00050.X.

Aksnes, A., Hjertnes, T. and Opstvedt, J. (1996) 'Effect of dietary protein level on growth and carcass composition in Atlantic halibut (*Hippoglossus hippoglossus* L)', *Aquaculture*, 145(1–4), pp. 225–233. doi: 10.1016/S0044-8486(96)01347-6.

Árnason, J. *et al.* (2009) 'Optimum feed formulation for Atlantic halibut (*Hippoglossus hippoglossus* L.): Minimum protein content in diet for maximum growth', *Aquaculture*, 291(3–4), pp. 188–191. doi: 10.1016/J.AQUACULTURE.2009.03.025.

Arnesen, P. and Krogdahl, Å. (1993) 'Crude and pre-extruded products of wheat as nutrient sources in extruded diets for Atlantic salmon (*Salmo salar*, L) grown in sea water', *Aquaculture*, 118(1–2), pp. 105–117. doi: 10.1016/0044-8486(93)90284-6.

Arnesen, P., Krogdahl, A. and Sundby, A. (1995) 'Nutrient digestibilities, weight gain and plasma and liver levels of carbohydrate in Atlantic salmon (*Salmo salar*, L.) fed diets containing oats and maize', *Aquaculture Nutrition*, 1(3), pp. 151–158. doi: 10.1111/J.1365-2095.1995.TB00039.X.

Assan, D. *et al.* (2021) 'Fish Feed Intake, Feeding Behavior, and the Physiological Response of Apelin to Fasting and Refeeding', *Frontiers in Endocrinology*, 12, p. 1676. doi: 10.3389/fendo.2021.798903.

Beamish, F. W. H. *et al.* (1986) 'Dietary carbohydrate and growth, body composition and heat increment in rainbow trout (*Salmo gairdneri*)', *Fish Physiology and Biochemistry*, 1(2), pp. 85–91. doi: 10.1007/BF02290208/METRICS.

Bergot, F. (1979) 'Carbohydrate in rainbow trout diets: Effects of the level and source of carbohydrate and the number of meals on growth and body composition', *Aquaculture*, 18(2), pp. 157–167. doi: 10.1016/0044-8486(79)90028-0.

Bergot, F. and Breque, J. (1983) 'Digestibility of starch by rainbow trout: Effects of the physical state of starch and of the intake level', *Aquaculture*, 34(3–4), pp. 203–212. doi: 10.1016/0044-8486(83)90203-X.

Boujard, T. *et al.* (2004) 'Regulation of feed intake, growth, nutrient and energy utilisation in European sea bass (*Dicentrarchus labrax*) fed high fat diets'. doi: 10.1016/j.aquaculture.2003.11.010.

Boyd, C. (2021) *A low feed conversion ratio is the primary indicator of efficient aquaculture*, *2021*. Available at: https://www.globalseafood.org/advocate/a-low-feed-conversion-ratio-is-the-primary-indicator-of-efficient-aquaculture/ (Accessed: 30 April 2023).

Callet, T. *et al.* (2020) 'Exploring the Impact of a Low-Protein High-Carbohydrate Diet in

Mature Broodstock of a Glucose-Intolerant Teleost, the Rainbow Trout', *Frontiers in Physiology*, 11(May), pp. 1–21. doi: 10.3389/fphys.2020.00303.

Degani, G., Viola, S. and Levanon, D. (1986) 'Effects of dietary carbohydrate source on growth and body composition of the European eel (*Anguilla anguilla* L.)', *Aquaculture*, 52(2), pp. 97–104. doi: 10.1016/0044-8486(86)90029-3.

FAO (2021) *FAO Yearbook. Fishery and Aquaculture Statistics 2019/FAO annuaire. Statistiques des pêches et de l'aquaculture 2019/FAO anuario. Estadísticas de pesca y acuicultura 2019*, *FAO Yearbook. Fishery and Aquaculture Statistics 2019/FAO annuaire. Statistiques des pêches et de l'aquaculture 2019/FAO anuario. Estadísticas de pesca y acuicultura 2019*. doi: 10.4060/cb7874t.

FAO (no date) *Fisheries and Aquaculture - Fisheries and Aquaculture - Aquaculture*. Available at: https://www.fao.org/fishery/en/aquaculture (Accessed: 28 April 2023).

Gallardo, P. *et al.* (2022) 'Status of Atlantic halibut (*Hippoglossus hippoglossus*) aquaculture production technology in Chile', *Aquaculture Reports*, 22, p. 100958. doi: 10.1016/J.AQREP.2021.100958.

Grisdale-Helland, B. and Helland, S. J. (1997) 'Replacement of protein by fat and carbohydrate in diets for Atlantic salmon (*Salmo salar*) at the end of the freshwater stage', *Aquaculture*, 152(1–4), pp. 167–180. doi: 10.1016/S0044-8486(97)00003-3.

Grisdale-Helland, B. and Helland, S. J. (2002) ' Atlantic halibut, *Hippoglossus hippoglossus* . ', *Nutrient requirements and feeding of finfish for aquaculture*, (June 2014), pp. 103–112. doi: 10.1079/9780851995199.0103.

Hamre, K. *et al.* (2003) 'Macronutrient composition of formulated diets for Atlantic halibut (*Hippoglossus hippoglossus*, L.) juveniles', *Aquaculture*, 227(1–4), pp. 233–244. doi: 10.1016/S0044-8486(03)00506-4.

Hansen, J. Ø. *et al.* (2008) 'Apparent digestion and apparent retention of lipid and fatty acids in Atlantic cod (*Gadus morhua*) fed increasing dietary lipid levels', *Aquaculture*, 284, pp. 159–166. doi: 10.1016/j.aquaculture.2008.07.043.

Hatlen, B., Grisdale-Helland, B. and Helland, S. J. (2005) 'Growth, feed utilization and body composition in two size groups of Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in protein and carbohydrate content', *Aquaculture*, 249(1–4), pp. 401–408. doi: 10.1016/j.aquaculture.2005.03.040.

Haug, T. (1990) 'Biology of the Atlantic Halibut, *Hippoglossus hippoglossus* (L., 1758)', *Advances in Marine Biology*, 26(C), pp. 1–70. doi: 10.1016/S0065-2881(08)60198-4.

Helland, S. J. and Grisdale-Helland, B. (1998) 'Growth, feed utilization and body composition of juvenile Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in the ratio between the macronutrients', *Aquaculture*, 166(1–2), pp. 49–56. doi: 10.1016/S0044-8486(98)00273-7.

Hemre, G. -I *et al.* (1995) 'Carbohydrate nutrition in Atlantic salmon, *Salmo salar* L.: growth and feed utilization', *Aquaculture Research*, 26(3), pp. 149–154. doi: 10.1111/J.1365-2109.1995.TB00896.X.

Hemre, G. I., Lie, Ø. and Sundby, A. (1993) 'Dietary carbohydrate utilization in cod (*Gadus morhua*): metabolic responses to feeding and fasting', *Fish Physiology and Biochemistry*, 10(6), pp. 455–463. doi: 10.1007/BF00004600.

Holmyard, N. (2009) *Halibut farming's uncertain future*, *SeafoodSource*. Available at: https://www.seafoodsource.com/news/aquaculture/halibut-farming-s-uncertain-future (Accessed: 21 May 2023).

Huang, Y. S. *et al.* (2016) 'Effects of protein levels on growth, feed utilization, body composition, amino acid composition and physiology indices of juvenile chu's croaker, Nibea coibor', *Aquaculture Nutrition*, 23(3), pp. 594–602. doi: 10.1111/ANU.12426.

Katsika, L. *et al.* (2021) 'Understanding the Interaction Effects between Dietary Lipid Content and Rearing Temperature on Growth Performance, Feed Utilization, and Fat Deposition of Sea Bass (*Dicentrarchus labrax*)', *Animals : an Open Access Journal from MDPI*, 11(2), pp. 1–14. doi: 10.3390/ANI11020392.

Kuparinen, A., Kuikka, S. and Merilä, J. (2009) 'Estimating fisheries-induced selection: Traditional gear selectivity research meets fisheries-induced evolution', *Evolutionary Applications*, 2(2), pp. 234–243. doi: 10.1111/J.1752-4571.2009.00070.X.

Lopez-alvarado, J. (1997) 'Aquafeeds and the environment', (November 1997).

Ma, R. *et al.* (2019) 'Protein nutrition on sub-adult triploid rainbow trout (1): Dietary requirement and effect on anti-oxidative capacity, protein digestion and absorption'. doi: 10.1016/j.aquaculture.2019.03.069.

Martins, D. A. *et al.* (2007) 'Effects of moderately oxidized dietary lipid and the role of vitamin E on the stress response in Atlantic halibut (*Hippoglossus hippoglossus* L.)', *Aquaculture*, 272, pp. 573–580. doi: 10.1016/j.aquaculture.2007.08.044.

Martins, D. A., Valente, L. M. P. and Lall, S. P. (2007) 'Effects of dietary lipid level on growth and lipid utilization by juvenile Atlantic halibut (*Hippoglossus hippoglossus*, L.)', *Aquaculture*, 263(1–4), pp. 150–158. doi: 10.1016/J.AQUACULTURE.2006.10.017.

Matés, J. M. (2000) 'Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology', *Toxicology*, 153. Available at: www.elsevier.com/locate/toxicol (Accessed: 29 May 2023).

Nanton, D. A., Lall, S. P. and McNiven, M. A. (2001) 'Effects of dietary lipid level on liver and muscle lipid deposition in juvenile haddock, *Melanogrammus aeglefinus* L', *Aquaculture Research*, 32, pp. 225–234. doi: 10.1046/J.1355-557X.2001.00019.X.

Naylor, R. *et al.* (2005) 'Fugitive salmon: Assessing the risks of escaped fish from net-pen aquaculture', *BioScience*, 55(5), pp. 427–437. doi: 10.1641/0006-3568(2005)055[0427:FSATRO]2.0.CO;2.

NOAA Fisheries (2023) *Atlantic Halibut*, *12.01.2023*.

NRC (no date) *Nutrient Requirements of Fish and Shrimp - National Research Council, Division on Earth and Life Studies, Board on Agriculture and Natural Resources, Committee on the Nutrient Requirements of Fish and Shrimp - Google Books*, *2011*. Available at:

https://books.google.no/books?hl=en&lr=&id=H8tABAAAQBAJ&oi=fnd&pg=PT24&ots=a8deof85Rq&sig=XfhtoiTCLxC0lcHQseGGg-mjJp4&redir_esc=y#v=onepage&q&f=false (Accessed: 28 April 2023).

Pohlenz, C. and Gatlin, D. M. (2014) 'Interrelationships between fish nutrition and health', *Aquaculture*, 431, pp. 111–117. doi: 10.1016/J.AQUACULTURE.2014.02.008.

Prabu, E. *et al.* (2017) 'An overview on significance of fish nutrition in aquaculture industry', 5(6), pp. 349–355.

Regjeringen (2021) 'Norsk havbruksnæring', pp. 1–5. Available at: https://www.regjeringen.no/no/tema/mat-fiske-og-landbruk/fiskeri-og-havbruk/1/oppdrettslaksen/Norsk-havbruksnaring/id754210/.

Regost, C. *et al.* (2001) 'Dietary lipid level, hepatic lipogenesis and flesh ž / quality in turbot Psetta maxima'. Available at: www.elsevier.nlrlocateraqua-online (Accessed: 27 May 2023).

Sagada, G. *et al.* (2017) 'Optimizing protein and lipid levels in practical diet for juvenile northern snakehead fish (*Channa argus*)'. doi: 10.1016/j.aninu.2017.03.003.

Santos, J. Dos, Burkowb, I. C. and Jobling, M. (1993) 'Patterns of growth and lipid deposition in cod (*Gadus morhua* L.) fed natural prey and fish-based feeds', *Aquaculture*, 110, pp. 173–189.

Strange, R. J. (2007) *WFS 450/550 Fish Physiology Online*, *2007*. Available at: http://web.utk.edu/~rstrange/wfs550/html-con-pages/v-digest-sys.html.

Suárez, M. D. *et al.* (2014) 'Influence of dietary lipids and culture density on rainbow trout (*Oncorhynchus mykiss*) flesh composition and quality parameter', *Aquacultural Engineering*, 63, pp. 16–24. doi: 10.1016/j.aquaeng.2014.09.001.

Taslimi, S. (2020) *Fish digestion and metabolsim*. Available at: https://www.cavendish.ac.uk/fish-digestion-and-metabolism/.

Windell, J. T., Foltz, J. W. and Sarokon, J. A. (1978) 'Effect of fish size, temperature, and amount fed on nutrient digestibility of a pelleted diet by Rainbow Trout, *Salmo gairdneri*', *American Fisheries Society*, 107(4), pp. 613–616. doi: 0.1577/1548-8659(1978)107<613:EOFSTA>2.0.CO;2.

Xu, J. *et al.* (2016) 'Optimal dietary protein level improved growth, disease resistance, intestinal immune and physical barrier function of young grass carp (*Ctenopharyngodon idella*)'. doi: 10.1016/j.fsi.2016.05.021.

Yan, J. *et al.* (2015) 'Dietary Lipid Levels Influence Lipid Deposition in the Liver of Large Yellow Croaker (*Larimichthys crocea*) by Regulating Lipoprotein Receptors, Fatty Acid Uptake and Triacylglycerol Synthesis and Catabolism at the Transcriptional Level OPEN ACCESS'. doi: 10.1371/journal.pone.0129937.

Yan, J. *et al.* (2017) 'Effects of dietary protein and lipid levels on growth performance, fatty acid composition and antioxidant-related gene expressions in juvenile loach *Misgurnus anguillicaudatus*', *Aquaculture Research*, 48(10), pp. 5385–5393. doi: 10.1111/ARE.13352.

Appendix

Table A1 The table provides an overview of the ingredients used in the diets.

Diet no	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
	%	%	%	%	%	%	%	%	%	%	%	%
FM 999	59.1	50.5	63.3	46	40.2	52.9	45.7	60.9	71.6	41.7	48.7	55.5
Wheat gluten	15	12.82	16.07	11.68	10.2	13.43	11.6	15.46	18.17	10.58	12.36	14.09
Wheat 18/20	9.49	14.09	9.32	15.06	15.61	9.3	13.24	3.83	2.82	9.04	4.37	1.44
Tapioca starch		8.4	3.3	5.4	12.1			16.25	4	23.4	26.5	24.9
FO 20/20	8	7.15	2.95	11.5	11.7	12.8	15.6	0.2		7.5	3.1	0.46
Marine lecithin	4.6	3.95	1.8	6.5	6.6	6.9	9.3	0.2		4.2	1.75	0.35
NaH2PO4	1.4	1.28	1.2	1.5	1.4	1.91	1.7	1.3	1.3	1.5	1.5	1.45
CaCO3	0.45			0.5	0.5	0.9	1			0.4		
Stay-C	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin mix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lys	0.5	0.45	0.55	0.45	0.35	0.45	0.45	0.5	0.6	0.35	0.4	0.45
Thr	0.25	0.2	0.25	0.2	0.18	0.2	0.2	0.2	0.25	0.17	0.16	0.2
Met	0.05		0.05	0.05		0.05	0.05		0.05			
His	0.1	0.1	0.15	0.1	0.1	0.1	0.1	0.1	0.15	0.1	0.1	0.1
Yttrium oxide	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
sum	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table A2 Average health measures throughout the project. The table provides a summary of average overall external health indicators based on tank and diet from October 20 (2021) to November 9 (2022), rated on a scale of 0 to 3, with 0 signifying minimal impact and 3 denoting significant impact. HSI calculated as descripted in formula 2.4

Tank	Diet	Wounds ventral	Emaciation	Wounds dorsal	Fins	Gills	HSI
15	1	1.180	1.500	1.400	1.129	1.627	1.164
2	2	1.329	1.368	1.273	1.051	1.547	1.799
5	2	1.169	1.385	1.000	1.043	1.689	1.270
7	2	1.053	1.473	1.211	1.019	1.839	1.644
12	3	1.316	1.500	1.375	1.044	2.024	1.263
4	4	1.356	1.524	1.154	1.058	2.183	1.551
6	5	1.160	1.586	1.125	1.023	1.919	2.050
18	5	1.196	1.545	1.375	1.100	2.024	1.921
8	6	1.167	1.441	1.200	1.041	1.824	1.347
11	8	1.267	1.328	1.533	1.185	1.367	1.322
3	9	1.237	1.372	1.100	1.000	1.522	1.052
13	10	1.324	1.367	1.750	1.059	1.767	1.756
14	11	1.159	1.156	1.444	1.143	1.756	1.490
1	12	1.275	1.368	1.188	1.053	1.237	0.954

Table A3 ANOVA for linear model for condition factor 20 October (2021).The table provides the output summary from DX design.

ANOVA for Linear model

Response 1: Cond Oct 21

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0136	2	0.0068	1.03	0.3861	not significant
[1]Linear Mixture	0.0136	2	0.0068	1.03	0.3861	
Residual	0.0792	12	0.0066			
Lack of Fit	0.0370	9	0.0041	0.2918	0.9341	not significant
Pure Error	0.0422	3	0.0141			
Cor Total	0.0928	14				

[1] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A4 ANOVA for cubic model for condition factor 2 February (2022).The table provides the output summary from DX design.

ANOVA for Cubic model

Response 2: Cond Feb22

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1335	9	0.0148	2.21	0.1984	not significant
[(1)]Linear Mixture	0.0146	2	0.0073	1.09	0.4048	
AB	0.0003	1	0.0003	0.0473	0.8365	
AC	0.0203	1	0.0203	3.03	0.1425	
BC	0.0304	1	0.0304	4.53	0.0866	
ABC	0.0373	1	0.0373	5.55	0.0651	
AB(A-B)	0.0123	1	0.0123	1.84	0.2333	
AC(A-C)	0.0246	1	0.0246	3.67	0.1137	
BC(B-C)	0.0121	1	0.0121	1.80	0.2376	
Residual	0.0336	5	0.0067			
Lack of Fit	0.0221	2	0.0111	2.90	0.1988	not significant
Pure Error	0.0114	3	0.0038			
Cor Total	0.1670	14				

[(1)] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III – Partial**

Table A5 ANOVA for cubic model for condition factor April 22 (2022). The table provides the output summary from DX design.

ANOVA for Cubic model

Response 3: CondApr22

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0118	9	0.0013	2.68	0.1451	not significant
[(1)]Linear Mixture	0.0015	2	0.0007	1.49	0.3103	
AB	0.0017	1	0.0017	3.43	0.1231	
AC	6.667E-07	1	6.667E-07	0.0014	0.9720	
BC	0.0003	1	0.0003	0.6918	0.4434	
ABC	0.0008	1	0.0008	1.62	0.2589	
AB(A-B)	0.0025	1	0.0025	5.20	0.0714	
AC(A-C)	0.0002	1	0.0002	0.3090	0.6022	
BC(B-C)	0.0002	1	0.0002	0.4165	0.5471	
Residual	0.0024	5	0.0005			
Lack of Fit	0.0014	2	0.0007	1.96	0.2850	not significant
Pure Error	0.0011	3	0.0004			
Cor Total	0.0142	14				

[(1)] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A6 ANOVA for linear model for condition factor August 30 (2022). The table provides the output summary from DX design.

ANOVA for Linear model

Response 4: Cond Aug22

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0080	2	0.0040	6.52	0.0136	significant
[1]Linear Mixture	0.0080	2	0.0040	6.52	0.0136	
Residual	0.0067	11	0.0006			
Lack of Fit	0.0059	8	0.0007	2.56	0.2370	not significant
Pure Error	0.0009	3	0.0003			
Cor Total	0.0147	13				

[1] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A7 ANOVA for cubic model for condition factor November 9 (2022). The table provides the output summary from DX design.

ANOVA for Cubic model

Response 5: Cond Nov22

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0059	9	0.0007	3.62	0.1140	not significant
[(1)]Linear Mixture	0.0013	2	0.0006	3.54	0.1303	
AB	0.0008	1	0.0008	4.40	0.1040	
AC	0.0020	1	0.0020	10.84	0.0302	
BC	0.0008	1	0.0008	4.45	0.1026	
ABC	0.0004	1	0.0004	2.37	0.1982	
AB(A-B)	0.0010	1	0.0010	5.68	0.0756	
AC(A-C)	0.0022	1	0.0022	11.90	0.0260	
BC(B-C)	0.0007	1	0.0007	3.96	0.1174	
Residual	0.0007	4	0.0002			
Lack of Fit	0.0003	1	0.0003	2.24	0.2317	not significant
Pure Error	0.0004	3	0.0001			
Cor Total	0.0066	13				

[(1)] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A8 ANOVA for cubic model for SGR 1. The table provides the output summary from DX design.

ANOVA for Cubic model

Response 6: SGR1

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0219	9	0.0024	4.96	0.0462	significant
[1]Linear Mixture	0.0010	2	0.0005	1.01	0.4293	
AB	0.0078	1	0.0078	15.92	0.0104	
AC	0.0010	1	0.0010	2.10	0.2067	
BC	0.0000	1	0.0000	0.0309	0.8674	
ABC	0.0002	1	0.0002	0.4817	0.5186	
AB(A-B)	0.0059	1	0.0059	11.99	0.0180	
AC(A-C)	0.0021	1	0.0021	4.36	0.0911	
BC(B-C)	0.0004	1	0.0004	0.8678	0.3943	
Residual	0.0025	5	0.0005			
Lack of Fit	0.0009	2	0.0005	0.9006	0.4939	not significant
Pure Error	0.0015	3	0.0005			
Cor Total	0.0244	14				

[1] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A9 ANOVA for cubic model for SGR 2. The table provides the output summary from DX design.

ANOVA for Cubic model

Response 7: SGR2

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0310	9	0.0034	1.16	0.4582	not significant
[(1)]Linear Mixture	0.0058	2	0.0029	0.9696	0.4407	
AB	0.0063	1	0.0063	2.11	0.2063	
AC	0.0016	1	0.0016	0.5298	0.4993	
BC	0.0001	1	0.0001	0.0272	0.8755	
ABC	6.920E-09	1	6.920E-09	2.333E-06	0.9988	
AB(A-B)	0.0101	1	0.0101	3.41	0.1243	
AC(A-C)	0.0010	1	0.0010	0.3308	0.5901	
BC(B-C)	0.0002	1	0.0002	0.0507	0.8308	
Residual	0.0148	5	0.0030			
Lack of Fit	0.0017	2	0.0009	0.1993	0.8294	not significant
Pure Error	0.0131	3	0.0044			
Cor Total	0.0458	14				

[(1)] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A10 ANOVA for cubic model for SGR 3. The table provides the output summary from DX design.

ANOVA for Cubic model

Response 8: SGR3

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0309	9	0.0034	4.86	0.0711	not significant
[1]Linear Mixture	0.0073	2	0.0036	5.15	0.0782	
AB	0.0036	1	0.0036	5.04	0.0881	
AC	6.124E-06	1	6.124E-06	0.0087	0.9303	
BC	0.0065	1	0.0065	9.16	0.0389	
ABC	0.0066	1	0.0066	9.31	0.0380	
AB(A-B)	0.0047	1	0.0047	6.62	0.0617	
AC(A-C)	1.800E-06	1	1.800E-06	0.0025	0.9621	
BC(B-C)	0.0000	1	0.0000	0.0365	0.8579	
Residual	0.0028	4	0.0007			
Lack of Fit	0.0019	1	0.0019	6.59	0.0828	not significant
Pure Error	0.0009	3	0.0003			
Cor Total	0.0337	13				

[1] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Table A11 ANOVA for quadratic model for SGR 3.The table provides the output summary from DX design.

ANOVA for Quadratic model

Response 9: SGR4

Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1241	5	0.0248	1.87	0.2054	not significant
[(1)]Linear Mixture	0.0561	2	0.0281	2.11	0.1832	
AB	0.0232	1	0.0232	1.75	0.2228	
AC	0.0211	1	0.0211	1.59	0.2433	
BC	0.0072	1	0.0072	0.5391	0.4838	
Residual	0.1061	8	0.0133			
Lack of Fit	0.0337	5	0.0067	0.2786	0.8987	not significant
Pure Error	0.0725	3	0.0242			
Cor Total	0.2303	13				

[(1)] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:
10

Table A12 ANOVA for quadratic model for SGR 3. The table provides the output summary from DX design.

ANOVA for Quadratic model

Response 1: % Water muscle MU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8.52	5	1.70	4.74	0.0213	significant
[(1)]Linear Mixture	0.8714	2	0.4357	1.21	0.3418	
AB	5.14	1	5.14	14.29	0.0043	
AC	0.1967	1	0.1967	0.5474	0.4782	
BC	2.22	1	2.22	6.18	0.0347	
Residual	3.23	9	0.3593			
Lack of Fit	2.39	6	0.3979	1.41	0.4192	not significant
Pure Error	0.8467	3	0.2822			
Cor Total	11.75	14				

[(1)] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A13 ANOVA for special cubic model from minerals in muscle analysis MU. The table provides the output summary from DX design.

ANOVA for Special Cubic model

Response 2: % Minerals muscle MU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0104	6	0.0017	1.67	0.2443	not significant
[(1)]Linear Mixture	0.0004	2	0.0002	0.1691	0.8474	
AB	0.0010	1	0.0010	0.9251	0.3643	
AC	0.0016	1	0.0016	1.52	0.2530	
BC	0.0008	1	0.0008	0.7576	0.4094	
ABC	0.0028	1	0.0028	2.75	0.1359	
Residual	0.0083	8	0.0010			
Lack of Fit	0.0036	5	0.0007	0.4692	0.7851	not significant
Pure Error	0.0047	3	0.0016			
Cor Total	0.0187	14				

[(1)] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A14 ANOVA for spesial quartic model from protein in muscle analysis MU. The table provides the output summary from DX design.

ANOVA for Special Quartic model

Response 3: % Protein muscle MU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.13	8	0.7667	3.14	0.0904	not significant
[(1)]Linear Mixture	0.6091	2	0.3046	1.25	0.3527	
AB	0.0944	1	0.0944	0.3861	0.5572	
AC	3.91	1	3.91	15.99	0.0071	
BC	3.30	1	3.30	13.50	0.0104	
A^2BC	2.03	1	2.03	8.30	0.0280	
AB^2C	4.17	1	4.17	17.07	0.0061	
ABC^2	4.29	1	4.29	17.55	0.0058	
Residual	1.47	6	0.2444			
Lack of Fit	0.8000	3	0.2667	1.20	0.4422	not significant
Pure Error	0.6667	3	0.2222			
Cor Total	7.60	14				

[(1)] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A15 ANOVA for linear model from lipid in muscle analysis MU. The table provides the output summary from DX design.

ANOVA for Linear model

Response 4: % Lipid muscle MU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.5799	2	0.2900	10.95	0.0020	significant
[1]Linear Mixture	0.5799	2	0.2900	10.95	0.0020	
Residual	0.3179	12	0.0265			
Lack of Fit	0.2179	9	0.0242	0.7262	0.6888	not significant
Pure Error	0.1000	3	0.0333			
Cor Total	0.8978	14				

[1] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A16 ANOVA for linear model from water in liver analysis MU. The table provides the output summary from DX design.

ANOVA for Linear model

Response 9: % Water liver MU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	56.68	2	28.34	4.09	0.0442	significant
[1]Linear Mixture	56.68	2	28.34	4.09	0.0442	
Residual	83.14	12	6.93			
Lack of Fit	40.78	9	4.53	0.3209	0.9190	not significant
Pure Error	42.36	3	14.12			
Cor Total	139.82	14				

[1] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A17 ANOVA for linear model from minerals in liver analysis MU. The table provides the output summary from DX design.

ANOVA for Linear model

Response 10: % Minerals liver MU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0379	2	0.0190	6.08	0.0150	significant
[1]Linear Mixture	0.0379	2	0.0190	6.08	0.0150	
Residual	0.0374	12	0.0031			
Lack of Fit	0.0269	9	0.0030	0.8535	0.6266	not significant
Pure Error	0.0105	3	0.0035			
Cor Total	0.0754	14				

[1] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A18 ANOVA for linear model from protein in liver analysis MU. The table provides the output summary from DX design.

ANOVA for Linear model

Response 11: % protein liver MU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	31.81	2	15.91	17.10	0.0003	significant
[(1)]Linear Mixture	31.81	2	15.91	17.10	0.0003	
Residual	11.16	12	0.9301			
Lack of Fit	9.41	9	1.05	1.79	0.3445	not significant
Pure Error	1.75	3	0.5839			
Cor Total	42.97	14				

[(1)] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A19 ANOVA for linear model from lipids in liver analysis MU. The table provides the output summary from DX design.

ANOVA for Linear model

Response 12: % Lipid liver MU
Transform: Natural Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.2966	2	0.1483	1.46	0.2715	not significant
[1]Linear Mixture	0.2966	2	0.1483	1.46	0.2715	
Residual	1.22	12	0.1018			
Lack of Fit	0.6372	9	0.0708	0.3632	0.8957	not significant
Pure Error	0.5848	3	0.1949			
Cor Total	1.52	14				

[1] Inference for linear mixtures uses Type I sums of squares.

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A20 ANOVA for mean model from water in muscle analysis SU. The table provides the output summary from DX design.

ANOVA for Mean model

Response 5: % Water muscle SU
Transform: Natural Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0000	0				
Residual	0.0087	13	0.0007			
Lack of Fit	0.0066	10	0.0007	0.9389	0.5931	not significant
Pure Error	0.0021	3	0.0007			
Cor Total	0.0087	13				

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A21 ANOVA for mean model from minerals in muscle analysis SU.The table provides the output summary from DX design.

ANOVA for Mean model

Response 6: % Minerals muscle SU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0000	0				
Residual	0.0153	13	0.0012			
Lack of Fit	0.0148	10	0.0015	8.61	0.0514	not significant
Pure Error	0.0005	3	0.0002			
Cor Total	0.0153	13				

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A22: ANOVA for linear model from protein in muscle analysis SU. The table provides the output summary from DX design.

ANOVA for Linear model

Response 7: % Protein muscle SU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.9598	2	0.4799	1.35	0.2979	not significant
[1]Linear Mixture	0.9598	2	0.4799	1.35	0.2979	
Residual	3.90	11	0.3543			
Lack of Fit	1.90	8	0.2372	0.3557	0.8922	not significant
Pure Error	2.00	3	0.6667			
Cor Total	4.86	13				

[1] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A23 ANOVA for linear model from lipids in muscle analysis SU. The table provides the output summary from DX design.

ANOVA for Linear model

Response 8: % Lipids muscle SU
Transform: Natural Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.1600	2	0.0800	0.4976	0.6210	not significant
[(1)]Linear Mixture	0.1600	2	0.0800	0.4976	0.6210	
Residual	1.77	11	0.1607			
Lack of Fit	0.5447	8	0.0681	0.1669	0.9808	not significant
Pure Error	1.22	3	0.4078			
Cor Total	1.93	13				

[(1)] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A24 ANOVA for cubic model from water in liver analysis SU. The table provides the output summary from DX design.

ANOVA for Cubic model

Response 13: % Water liver SU
Transform: Base 10 Log
Constant: 0

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0031	9	0.0003	2.16	0.2385	not significant
[(1)]Linear Mixture	0.0011	2	0.0006	3.46	0.1342	
AB	0.0001	1	0.0001	0.4703	0.5305	
AC	0.0001	1	0.0001	0.9009	0.3963	
BC	0.0008	1	0.0008	4.88	0.0916	
ABC	0.0006	1	0.0006	3.93	0.1184	
AB(A-B)	0.0000	1	0.0000	0.1628	0.7072	
AC(A-C)	0.0003	1	0.0003	1.63	0.2712	
BC(B-C)	1.205E-07	1	1.205E-07	0.0007	0.9795	
Residual	0.0006	4	0.0002			
Lack of Fit	0.0001	1	0.0001	0.5192	0.5233	not significant
Pure Error	0.0005	3	0.0002			
Cor Total	0.0038	13				

[(1)] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Table A25 ANOVA for cubic model from minerals in liver analysis SU. The table provides the output summary from DX design.

ANOVA for Cubic model

Response 14: % Minerals liver SU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0701	9	0.0078	30.15	0.0025	significant
[1]Linear Mixture	0.0005	2	0.0003	1.05	0.4311	
AB	0.0050	1	0.0050	19.39	0.0117	
AC	0.0000	1	0.0000	0.1392	0.7280	
BC	0.0046	1	0.0046	17.70	0.0136	
ABC	0.0094	1	0.0094	36.38	0.0038	
AB(A-B)	0.0027	1	0.0027	10.44	0.0319	
AC(A-C)	0.0005	1	0.0005	2.01	0.2289	
BC(B-C)	0.0051	1	0.0051	19.71	0.0113	
Residual	0.0010	4	0.0003			
Lack of Fit	0.0003	1	0.0003	1.32	0.3333	not significant
Pure Error	0.0007	3	0.0002			
Cor Total	0.0711	13				

[1] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A26: ANOVA for cubic model from protein in liver analysis SU. The table provides the output summary from DX design.

ANOVA for Cubic model

Response 15: % Protein liver SU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	11.04	9	1.23	33.10	0.0021	significant
[(1)]Linear Mixture	2.58	2	1.29	34.76	0.0030	
AB	1.13	1	1.13	30.48	0.0053	
AC	0.1678	1	0.1678	4.53	0.1005	
BC	3.10	1	3.10	83.67	0.0008	
ABC	4.61	1	4.61	124.42	0.0004	
AB(A-B)	0.7221	1	0.7221	19.48	0.0116	
AC(A-C)	0.2729	1	0.2729	7.36	0.0534	
BC(B-C)	0.2506	1	0.2506	6.76	0.0601	
Residual	0.1483	4	0.0371			
Lack of Fit	0.0166	1	0.0166	0.3791	0.5817	not significant
Pure Error	0.1317	3	0.0439			
Cor Total	11.19	13				

[(1)] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A27 ANOVA for linear model from lipids in liver analysis SU. The table provides the output summary from DX design.

ANOVA for Linear model

Response 16: % Lipid liver SU

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	85.47	2	42.74	4.92	0.0297	significant
[1]Linear Mixture	85.47	2	42.74	4.92	0.0297	
Residual	95.51	11	8.68			
Lack of Fit	77.41	8	9.68	1.60	0.3805	not significant
Pure Error	18.10	3	6.03			
Cor Total	180.99	13				

[1] Inference for linear mixtures uses Type I sums of squares.

These rows were ignored for this analysis:

```
10
```

Mixture Component coding is **L_Pseudo**.
Sum of squares is **Type III - Partial**

Table A28: HSI correlation. Copy of output from HSI analysis in excel

Regression Statistics	
Multiple R	0.046561431
R-Squared	0.002167967
Adjusted R-Squared	-0.080984703
Standard Error	0.333549434
Observations	14

ANOVA					
	fg	SK	GK	F	Significance-F
Regression	1	0.00290066	0.00290066	0.026072125	0.874410999
Residuals	12	1.3350627	0.111255225		
Total	13	1.33796336			

	Coefficients	*Standard error*	*t-Stat*	*P-value*	*Bottom 95%*	*Top 95%*	*Lower 95.0%*	*Upper 95.0%*
Intersection point	1.4389	0.3021	4.7631	0.0004	0.7807	2.0972	0.7807	2.0972
X-variabel 1	0.0047	0.0292	0.1614	0.8744	-0.0589	0.0683	-0.0589	0.0683

DEVIATION (OUTPUT)

Observation	Projected Y	Residuals	Standard residuals
1	1.483807514	-0.532140848	-1.660532736
2	1.468237672	0.330095661	1.030055584
3	1.484751141	-0.434751141	-1.356630496
4	1.490412902	0.059587098	0.185940109
5	1.464463165	-0.192796498	-0.601616843
6	1.517778079	0.533888588	1.665986519
7	1.481448447	0.161884886	0.5051579
8	1.505039117	-0.15837245	-0.494197428
9	1.47908938	-0.15908938	-0.496434591
10	1.496546476	-0.234879809	-0.732936804
11	1.473899433	0.2844339	0.887569156
12	1.485694768	0.004305232	0.013434374
13	1.497490103	-0.112512128	-0.351091392
14	1.469653112	0.450346888	1.405296649