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Effect of Poultry hydrolysate on growth and welfare in farmed Atlantic Salmon Smolt (*Salmo Salar*).

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Abstract

The objective of this study was to investigate the effects of different levels of poultry hydrolysate (PH) on the performance and welfare of 94g per Atlantic salmon smolts. Five experimental diets were fed to the fish for 43 days in triplicate 300L tanks, containing 0%(Control), 5%(C5), 10%(C10), 15%(C15), and 20%(C20) of poultry hydrolysate. Growth rate, feed conversion ratio, visceral fat analysis, liver, heart and faeces, whole body composition and welfare operational indicators and blood samples were examined. Results showed that the inclusion of PH 5% and 10% significantly improved growth rate and feed conversion ratio compared to the control group. Blood serum analyses revealed no adverse effects, and no mortality or cataract were observed. The control group had significantly higher score for deformity, although the level was low. Severity of scale loss was higher for the C20 group compared with the control. Scores for fin damage in active and healed phase were low, although active dorsal fin damage was highest for the C5 group, while healed pectoral fin damage was highest for the C10 group. Accumulation of fat around the viscera was lower of the C10 and C20 groups, lower fat deposits were observed on the heart surface of the C5, C10 and C15 groups compared with the control. Analyses of whole-body composition revealed decreasing dry matter content with increasing inclusion of PH. The fat content was higher in fish fed 5% PH, whereas the fat content was lowest for the fish fed 15% PH compared with the control. The ash content of the PH groups did not differ from the control group, but the condition factor was higher for the C15 group compared with the control. The slaughter yield was significantly lower for all groups fed PH (0.5-0.8% units), while inclusion of 5% PH significantly improved the faeces texture. These findings show that dietary inclusion of CH affects growth, lipid deposition pattern, fish welfare and consistency of faeces, suggesting that the inclusion of poultry hydrolysate at 5-10% in the diet for Atlantic salmon smolts can enhance production efficiency without compromising their welfare.

Acknowledgements

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1. Introduction

As the global population continues to grow, the demand for food production, including seafood, is increasing rapidly. To meet this demand, the aquaculture sector is evolving and expanding globally. In 2020, the production of fish and seafood for human consumption reached 87.5 million tonnes globally, with Salmonids accounting for 32.6% of aquaculture species. Norway is the leading producer of farmed salmon, exporting USD 11 billion worth of the fish. (FAO.,2020).

Improving the quantity and quality of salmon production requires addressing one of the main challenges: sufficient availability of sustainable feed ingredients. There are various issues with the current feed ingredients. One of the major environmental concerns is that marine sources that require a large volume of pelagic fish are disrupting the ocean ecology, leading to overfishing and a rapid decline in the availability of pelagic fish used for fish meal and fish oil. Additionally, the increasing demand for fish stocks in sea farms exacerbates the need for more feed ingredients, perpetuating the demand for captured fisheries to meet the high demand for fish meal and fish oil (Shepherd and Jackson, 2013).

Another issue is that plant-based ingredients have their own set of problems. The dependence on imported plant-based ingredients and the need for arable land is high, leading to competition for these ingredients for human consumption and other livestock. As a result, the current reliance on obtaining all these sources of ingredients is environmentally damaging and creates a broad range of issues that are volatile and unstable (Fry et al., 2016).

Feed production accounts for roughly 50% of the production costs for both in-land and net pen operations. (Torrissen et al.,2011).

In Norway, salmon feed ingredients have been developed into lowering the percentage of marine oils and protein due to the decreased availability and increasingly high cost of fishmeal and fish oil (Aas et al., 2022). The percentage of marine protein sources dropped from 65.4% in 1990 to 12.1% in 2020 as illustrated in figure 1.

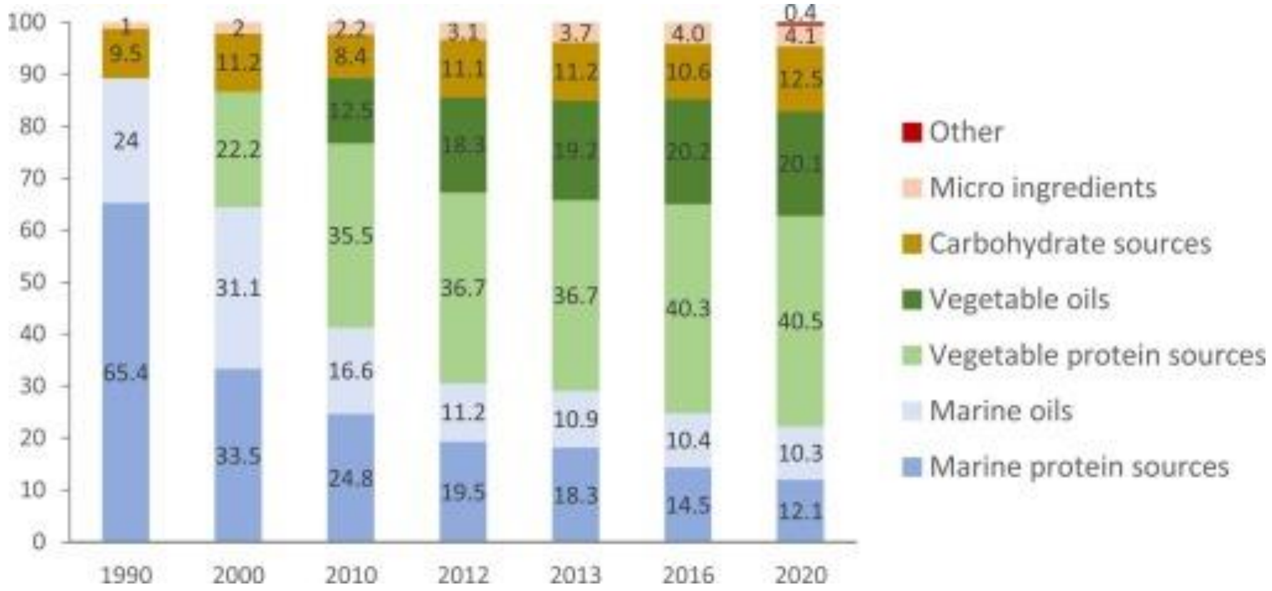

Figure 1. Percentage of feed sources in Norwegian salmon feed from 1990 to 2020 (Aas et al.,2022).

1.1 Sustainable feed ingredients

Aquaculture is a rapidly growing industry globally and has become an important source of protein for human consumption. Salmonids, such as Atlantic salmon, are the most farmed species in Norway and contribute significantly to the country's economy. The growth of this industry is, however, dependent on the availability and sustainability of high-quality feed ingredients. Fishmeal, a key ingredient in salmon feed, has become scarce and expensive in recent years. As a result, there is a need to explore alternative protein sources for use in salmon feeds.

Even though the current feed ingredients are formulated to be economically viable, there is need to search for sustainable feed ingredients. With advanced technologies, it opens opportunities for sourcing more sustainable ingredients and that provide nutrients and proteins essential for the fish.

To achieve sustainability in aquafeed, there is a Life Cycle Assessment (LCA) which is applied to feed ingredient to assess its environmental impact. This encourages good decision-making and the importance of accessing novel ingredients.

The general framework of LCA is categorised into four stages. This is usually collected data to establish the different type of levels that products should be assessed in terms of environmental impact, marketing, transportation, storage, and waste management (Hauschild et al., 2018).

1.2 Poultry Protein

Poultry protein has been widely used as a feed ingredient for various aquatic species, including salmon. Poultry protein sources such as poultry by-product meal, poultry meal, and feather meal are rich in high-quality proteins, essential amino acids, minerals, and vitamins that are important for the growth and development of fish (Dong et al., 1993).

Numerous studies have reported positive effects of including poultry protein in different species of fish diets. For example, a study found that inclusion of poultry meal in Rainbow trout (*Oncorhynchus mykiss*) diets resulted in increased growth performance and improved feed conversion (Steffens., 1994). Similarly, a recent study showed that replacing fishmeal with poultry meal in juvenile black sea bass diets did not negatively impact growth performance, feed efficiency, or nutrient digestibility (Dawson et al., 2018). However, as poultry by-product lacks some essential amino acids such as lysine and methionine (González-Rodríguez et al., 2016).

Poultry by-product meal has also been shown to be an effective protein source for salmonids. A study found that nutrient comparison between fishmeal with poultry by-product meal in Rainbow trout diets resulted in similar growth performance and nutrient utilization (Cheng and Hardy, 2002). Another study reported that fish meal substitution with poultry by-product meal in juvenile Nile tilapia (*Oreochromis niloticus*) diets resulted in similar growth performance and feed efficiency using 100% poultry by-product meal as compared to fish meal (Yones and Metwalli, 2015).

Feather meal, a by-product of poultry processing, has also been investigated as a potential protein source for salmon diets. A study found that inclusion of feather meal in Rainbow trout diets resulted in similar growth performance and nutrient utilization as diets containing fishmeal (Bureau et al.,2000). However, feather meal is known to have lower protein digestibility compared to other poultry protein sources, which may limit its inclusion in salmon diets.

In addition to its nutritional benefits, poultry protein can also be a cost-effective alternative to fishmeal, which has become increasingly expensive and limited in supply due to overfishing and environmental concerns. This makes poultry protein an attractive option for aquaculture industries seeking sustainable and cost-effective feed ingredients.

Poultry protein sources such as poultry meal, poultry by-product meal, and feather meal have been shown to be effective and sustainable feed ingredients for salmon diets, providing essential nutrients and contributing to improved growth performance and feed efficiency.

1.3 Current Poultry Protein as Feed Regulations in Norway

Norway largely adheres to the regulations set by the European Union. In 2009, a regulation was established requiring animal by-products to be free from contamination and diseases before being used in animal feed intended for human consumption (Regulation (EC) No 1069/2009). Poultry by-products can be vulnerable to contamination by several pathogenic bacteria such as Salmonella, Campylobacter, Pseudomonas, Serratia, Staphylococcus, Enterococcus, and Listeria (Rouger et al., 2017). However, these by-products can be tested and sterilized to prevent contamination. Additionally, hydrolysis may be a cost-effective method for further processing by-products and reusing them in animal feed.

1.4 Fish Welfare in Salmon Farming

Fish welfare is an essential measurement of the biological and mental state of the animal. There are several indicators to ensure good welfare for the farmed fish. In aquaculture, the standard tool to measure welfare is using Operational Welfare Indicators (OWIs).

When designing, feed experiments. It is important to look at feeding behaviours, feed intake and feeding patterns. This can also provide information for the wellbeing of the fish and its acceptability to feed consumption. For Salmonids, a common challenge would be feed competition between dominant fish and subordinate fish. This can be a stressful situation for some fish and managing the appropriate feeding times is also essential and should be included in feed trials (Campos et al., 2020).

1.5 Future of poultry hydrolysates in Salmon Farming

Several studies have suggested that replacing fish meal with poultry by-products in fish feed can be effective for different fish species (Kureshy et al., 2000, Cruz-Suárez, et al., 2007, Shapawi et al., 2007). However, concerns about salmonella in European poultry have made some consumers wary (Authority, E.F.S., 2018). Nevertheless, advances in biotechnology have made it possible to produce safer and more sustainable feed ingredients, and Norway is exploring these options (Ytrestøyl et al., 2015). To better understand the impact of poultry by-products on fish feed, it is important to investigate their effects on juvenile salmon in freshwater environments and to assess their impact on welfare.

Additionally, research is needed to determine the optimal inclusion level of poultry hydrolysate in the feed for achieving maximum growth and performance of Atlantic salmon smolts. Moreover, understanding the sustainable benefits associated with using poultry hydrolysate as a replacement for other protein sources in the feed for Atlantic salmon smolts is critical. Unfortunately, there is a lack of research in this area. Therefore, the aim of this study is to evaluate the effects of different inclusion levels of poultry hydrolysate on the performance and welfare of Atlantic salmon smolts.

2. Theoretical Background

Aquaculture feed production is heavily reliant on imported ingredients, which leads to competition for resources between human, land animal, pet food, and aquaculture consumption (Tacon and Metian, 2008). Therefore, there is a need for diversity in feed production, including locally produced and sustainable options. Poultry by-products could be a promising and sustainable alternative to traditional fish meal in fish feed for Atlantic salmon smolts. In this context, understanding the impact of poultry hydrolysate on the growth, performance, and welfare of Atlantic salmon smolts is crucial. This study aims to evaluate the effects of different inclusion levels of poultry hydrolysate on the performance and welfare of Atlantic salmon smolts in a recirculating aquaculture system (RAS).

2.1 Current Feed ingredients

2.1.1 Fish Oil and Fish Meal

Fish oil and fish meal have been widely used in the aquaculture industry as the primary ingredients in fish feed due to their high nutritional value. However, their extensive use has led to concerns regarding overfishing, sustainability, and the impact on marine ecosystems. In this section, the advantages, and disadvantages of using fish oil and fish meal as ingredients in fish feed will be discussed in table 1.

Table 1. Advantages and Disadvantages of fish oil and fish meal in fish feed ingredients.

Advantages of fish oil and fish meal	**Disadvantage of fish oil and fish meal**
High nutritional value: Fish oil and fish meal are rich in high-quality proteins, omega-3 fatty acids, and other essential nutrients that are crucial for the growth and development of fish (Turchini and Torstensen, 2009).	Overfishing: The extensive use of fish oil and fish meal in aquaculture has led to concerns regarding overfishing and the depletion of wild fish stocks (Olsen and Hasan, 2012).
Palatability: Fish oil and fish meal are highly palatable and are often preferred by fish over other types of feed (Miles and Chapman, 2006).	Sustainability: The use of fish oil and fish meal in fish feed is not sustainable in the long run, and alternative sources of protein and oil must be explored (Nordahl, 2011).
Improved growth and health: Fish oil and	Environmental impact: The use of fish oil

fish meal can significantly improve the growth and overall health of fish (Hardy,2010).	and fish meal in fish feed can have a significant impact on marine ecosystems, including the depletion of fish populations, habitat destruction, and water pollution.
Easy digestion: Fish oil and fish meal are easily digestible by fish, which helps in the efficient utilization of nutrients.	Cost: Fish oil and fish meal are expensive ingredients, and their extensive use in fish feed can significantly increase the cost of production (Tacon and Metian 2008).

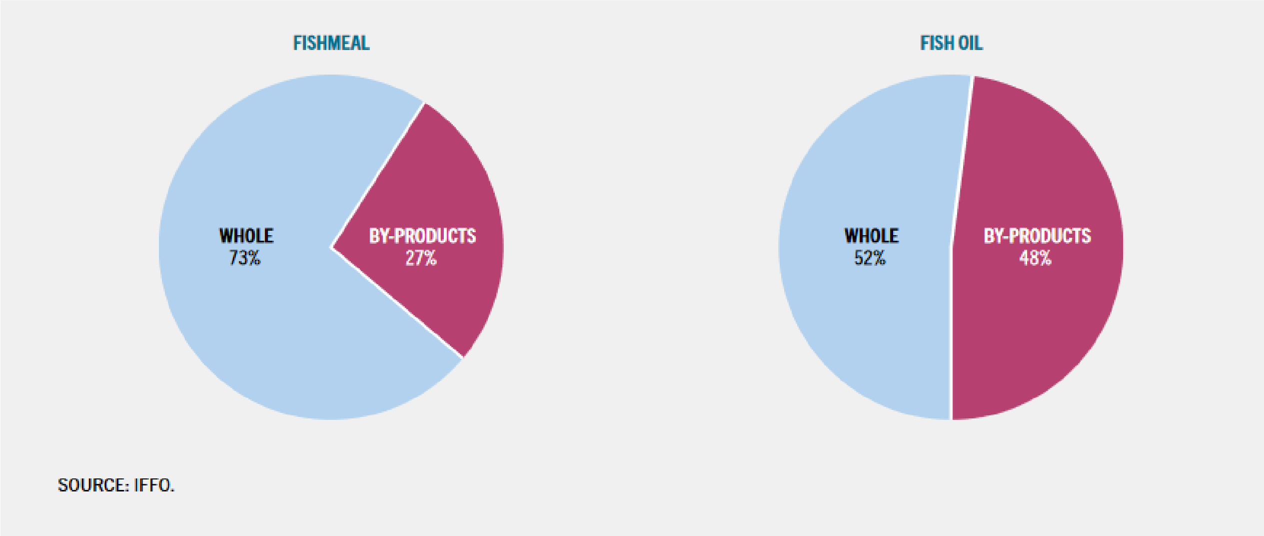

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Figure 2. Pie chart of raw material utilised for reduction into fishmeal and fish oil in 2020 (FAO 2022).

As highly nutritious fish oil and fish meal are to improve the growth and health of fish. The overreliance on these ingredients has led to overfishing, environmental degradation, and increased production costs. This highlights the need for alternative sources of protein and oil in the aquaculture industry. Additionally, as depicted in figure 2, the continued use of whole fish for fish meal and fish oil production remains at over 50%, driving prices up further. This is due to high demand and low supply. To promote sustainability in aquaculture, it is important to explore new protein sources such as insect meal, microalgae, and single-cell protein, and innovative approaches to feed processing and formulation that incorporate locally sourced, sustainable ingredients while reducing waste. Achieving these goals will require collaboration among researchers, industry professionals, and policymakers to ensure the long-term health of both the aquaculture industry and the environment.

2.1.2 Plant-based Ingredients

Fish feed has traditionally relied on animal-based ingredients, such as fishmeal and fish oil, as a source of protein and energy. However, with the increasing demand for fish feed and the limited availability of these ingredients, alternative sources of protein and energy, such as plant-based ingredients, are being explored. The advantages and disadvantages of using plant-based ingredients in fish feed are discussed in table 2.

Table 2. Advantages and disadvantages of plant ingredients in fish feed ingredients.

Advantages of plant ingredients	**Disadvantages of plant ingredients**
Cost-effectiveness: Plant-based ingredients are generally less expensive than animal-based ingredients, making them a cost-effective alternative for fish feed. Additionally, the production of plant-based ingredients is less dependent on seasonal fluctuations, making them a more reliable and sustainable source of feed(Dalsgaard et al., 2012).	Palatability: Fish can be more selective in their feeding behaviour and may not consume feed containing high levels of plant-based ingredients (Drew et al., 2007). The palatability of plant-based ingredients can also vary depending on factors such as processing, which can affect the taste and texture of the feed.
Nutrient availability: Plant-based ingredients can provide a wide range of nutrients to fish, such as carbohydrates, vitamins, and minerals. Soybean meal, for example, is a good source of protein and energy, and can replace up to 50% of fishmeal in fish feed without affecting fish growth or health (Huang et al.,2017).	Nutritional imbalances: The use of plant-based ingredients in fish feed can lead to nutritional imbalances, as these ingredients may not contain all the essential amino acids such as methionine, lysine, tryptophan, and threonine required for fish growth and health (Li et al.,2009). This can result in reduced growth rates, lower feed conversion efficiency, and an increased risk of diseases.

Sustainability: As plant-based ingredients do not require the use of marine resources, their use in fish feed can reduce the pressure on wild fish stocks (Gatlin et al., 2007). This can help to make the aquaculture industry more sustainable in the long run.	Antinutrients: Some plant-based ingredients, such as soybean meal, can contain antinutritional factors that may affect fish health and welfare (Venkata Subash et al., 2020). These antinutritional factor can cause allergic reactions, reduce feed intake, and increase the risk of diseases.

The use of plant-based ingredients in fish feed can provide a cost-effective, sustainable, and environmentally friendly alternative to traditional animal-based ingredients. However, the use of these ingredients can also pose challenges related to digestibility, palatability, nutritional imbalances, and potential allergens. Therefore, careful consideration should be given to the selection, processing, and inclusion levels of plant-based ingredients in fish feed, to ensure optimal fish growth, health, and welfare.

2.2 Alternatives to sustainable feed

Sustainable fish feed is an increasingly pressing issue in the aquaculture industry. The current reliance on marine-based feed ingredients, such as fishmeal and fish oil, is environmentally unsustainable and poses several problems, including overfishing and disruption of ocean ecology (Tacon and Metian, 2008). Additionally, plant-based alternatives have their own challenges, such as the need for arable land and competition with human consumption and other livestock.

In recent years, there has been growing interest in alternative protein sources for fish feed, including insect meal, microalgae, and single-cell protein. These sources have the potential to reduce reliance on fishmeal and fish oil, while also being more environmentally sustainable (Naylor et al.,2021). However, more research is needed to evaluate their nutritional value and cost-effectiveness, as well as their impact on fish growth and health.

Furthermore, there is a need for innovation in feed processing and formulation, with a focus on utilizing local, sustainable ingredients and minimizing waste. This may involve the use of novel feed additives, such as probiotics and prebiotics, to enhance nutrient utilization and fish health (Encarnação, 2016).

Overall, the search for sustainable alternatives to fish feed is ongoing and will require collaboration between researchers, industry, and policymakers to ensure long-term sustainability of the aquaculture industry.

2.3 Poultry Hydrolysates

Poultry by-products is an all-purpose product. For every tonne of chicken processed, 69% of the meat is processed for human consumption, while the rest consists of by-products. For feed trials, chicken by-product can be an important source of protein, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and amino acids (Emery et al., 2014).

Chicken hydrolysates have been researched as an alternative protein source in fish feed due to their high nutritional value, protein source, and digestibility. Hydrolysates are derived from the hydrolysis of proteins from various sources, including animal and plant sources. Chicken hydrolysates are obtained from the enzymatic breakdown of chicken protein, resulting in smaller peptides and amino acids that are easier for fish to digest and absorb.

Several studies have investigated the effects of chicken hydrolysates on fish growth, feed utilization, and immune response. For example, a study found that replacing fishmeal with poultry by-product meal in juvenile barramundi (*Lates calcarifer*) diets resulted in higher fatty acids intake compared to fishmeal-based diets. Although, high number of fatty acids in poultry by-product meal had an adverse effect in the immune response (Chaklader et al., 2020). Similarly, another study demonstrated that poultry hydrolysate-based diets had similar growth rates and feed utilization compared to fishmeal-based diets in humpback grouper (*Cromileptes altivelis*) (Shapawi et al., 2007).

In addition to their nutritional benefits, chicken hydrolysates have also been shown to enhance the immune response of fish. A study found that feeding common carp (*Cyprinus carpio*) with a diet containing 50% chicken hydrolysates replacing fishmeal resulted in similar growth responses and immune function compared to a 50% fish meal diet (Wu et al., 2022). In addition, a study showed that juvenile Nile tilapia (*Oreochromis niloticus*) fed with a diet containing chicken hydrolysates provided a palatability index of 12.3% and did not affect feeding behaviour (Alves et al., 2019).

Despite these benefits, there are also some potential disadvantages associated with the use of chicken hydrolysates in fish feed. One concern is the risk of transmitting diseases from poultry to fish through the feed. Another concern is the potential for the accumulation of contaminants, such as heavy metals and antibiotics, in the hydrolysates. However, these risks can be mitigated through proper processing and quality control measures.

The use of chicken hydrolysates in fish feed has several advantages, including high nutritional value, palatability, digestibility, and immune-enhancing effects. However, there are also potential disadvantages associated with their use, including the risk of disease transmission and the accumulation of contaminants.

Processing poultry by-products can result in significant greenhouse gas emissions, particularly from the use of electricity and heating. In fact, acidification and eutrophication during processing have been identified as major contributors to environmental damage (Campos et al., 2020). When compared to fish oil and fish meal, the environmental impact of the latter is even higher (table 1.). Specifically, fish oil results in 57% to 73% higher environmental damage, while fish meal causes 31% to 64% higher environmental damage.

Overall, more research is needed to fully understand the potential benefits and risks of using chicken hydrolysates in fish feed.

2.3.1 Nutrition content in poultry by-products

Poultry by-products are a valuable source of protein and nutrients that can be used in animal feed formulations. The key nutritional components of poultry by-products can be grouped into three categories: protein, amino acid and fatty acids.

Poultry by-products are a highly digestible source of protein. The protein content of poultry by-products can vary depending on the type of by-product and processing methods used. Generally, poultry by-products contain between 50-70% protein, with some by-products such as chicken meal containing up to 90% protein (dos Santos Cardoso et al, 2021). Hence poultry might be considered a good protein source as Salmonids requires around 400g/kg of protein (Hardy, 1996).

Poultry by-products are a rich source of essential amino acids, including lysine, methionine, and cysteine. These amino acids are essential for animal growth and development and must be obtained through the diet. Poultry by-products also contain non-essential amino acids such as glutamine, arginine, and glycine (Cho and Kim, 2010).

Poultry by-products are also a source of essential fatty acids, including omega-3 and omega-6 fatty acids. The fatty acid content of poultry by-products can vary depending on the fatty acids in the feed provided for the animals, the type of by-product and processing methods used. Chicken fat, for example, is generally a rich source of omega-6 fatty acids, while chicken liver is a good source of omega-3 fatty acids (Subhadra et al.,2006).

2.4 Biotechnology in protein processing

Poultry by-products are an important source of high-quality proteins and other valuable nutrients. Biotechnology plays an important role in the processing of poultry by-products, affecting their nutritional value and environmental impact.

Enzymatic hydrolysis: Enzymatic hydrolysis is a process that uses enzymes to break down proteins in poultry by-products into smaller peptides and amino acids shown in figure 3. This process can improve the digestibility and nutritional value of the proteins and produce bioactive peptides with health-promoting properties (Wubshet et al., 2018).

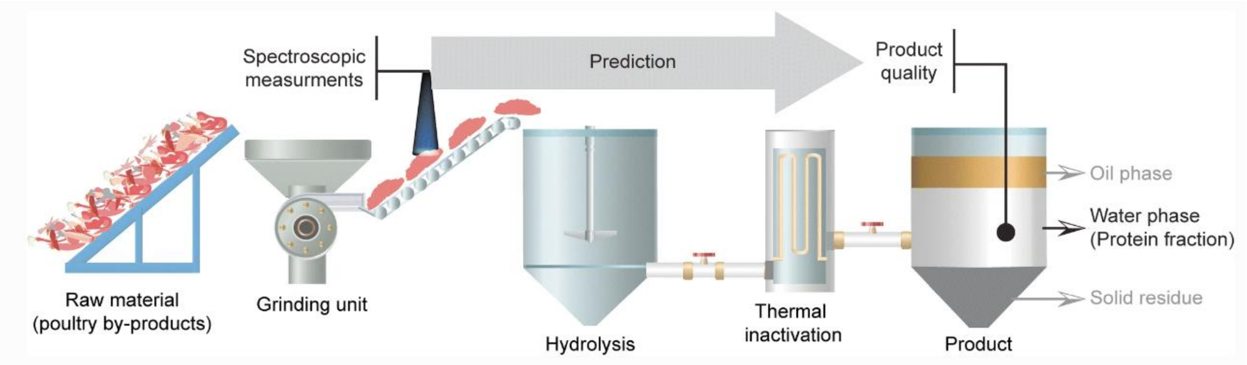

Figure 3. Processing steps in enzymatic hydrolysis of poultry by product (Wubshet et al., 2018).

Microbial fermentation: Microbial fermentation is a process that uses microorganisms to break down complex organic compounds in animal proteins into simpler compounds such as organic acids, amino acids, and vitamins. This process can improve the nutritional value of the by-products and produce value-added products such as probiotics, enzymes, and organic acids (Hou et al., 2017).

These biotechnological approaches have shown promise in improving the nutritional value and sustainability of poultry by-products. However, further research is needed to fully evaluate their potential and to address any safety and regulatory concerns.

2.5 Salmon Smolt in Recirculation Aquaculture System (RAS)

Atlantic salmon (*Salmo salar*) is an economically important species in aquaculture, and the smolts phases is a crucial step in their life cycle. Smolts are the juvenile stage of salmon that undergo a morphological transformation to adapt to seawater environments, which is essential for their survival and growth in the ocean. This process, known as smoltification, involves significant changes in the gills, enzymes, ion transporters, and ion channels of the salmon (McCormick., 1995, Nilsen., 2007).

RAS technology provides better control of water quality, which may results in better growth performance and a significant reduction in mortality (Ulgenes et al., 2008). As the farming practices vary significantly between RAS and the traditional flow through farming systems, including higher density and higher vulnerability regarding water quality. It is essential to investigate the effects of supplementing novel ingredient in feed, including inclusion of poultry hydrolysate on the performance and welfare of Atlantic salmon smolts produced in RAS.

2.6 Feed and welfare

Proper nutrition is crucial for the welfare of farmed fish, as it helps prevent diseases and improves growth rates. However, some aspects of fish feed production and use can negatively impact fish welfare. Therefore, it is important to apply the OWIs tool to monitor and evaluate the process of farming fish to ensure that the fish are raised in an ethical environment. OWIs are derived from Nofima welfare indicators (Noble et al., 2018), and a summary map is shown in figure 4. Research has shown that the type and quality of fish feed can significantly impact welfare indicators (Santurtun et al., 2018).

Several key OWIs are relevant to fish feed and welfare. These include feeding behaviour, growth rate, health, mortality rate, and water quality. Feeding behaviour involves monitoring how fish behave when feeding, and a healthy diet should promote active feeding with no aggression or competition for food. Growth rate is important to ensure healthy growth, while monitoring health and mortality rates is essential for detecting potential issues. Water quality, including temperature, pH, and oxygen levels, also affects fish welfare (Santurtun et al., 2018).

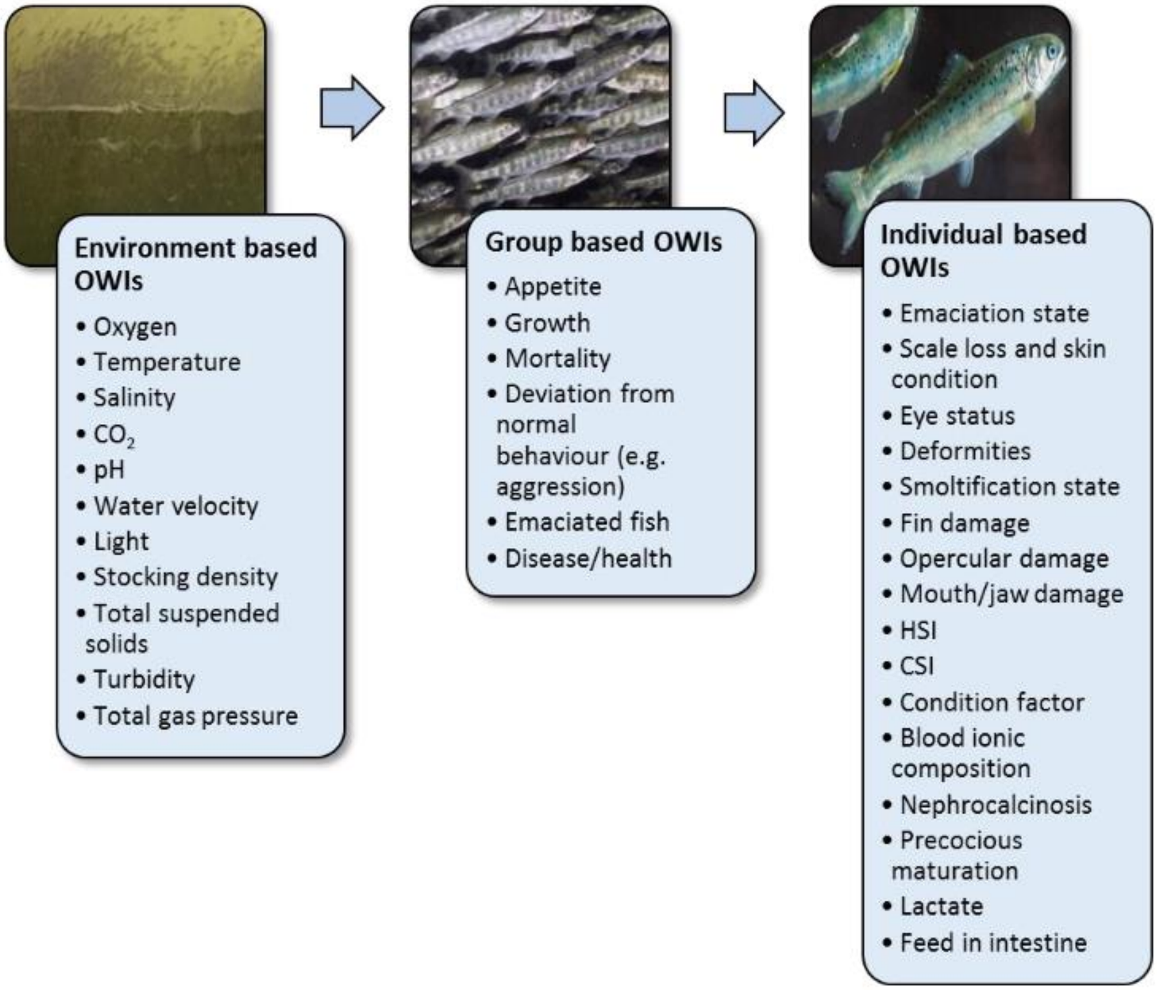

Figure 4. Operational Welfare Indicator (OWIs) presented as Environmental based, Group based and individual based OWIs (Noble et al., 2018).

3. Materials and Methods

3.1 Experimental design

The fish used for this research were farmed Atlantic salmon smolt (*Salmo salar L.*) and the feed trial was conducted at the Center for Sustainable Aquaculture, Norwegian University of Life Sciences (NMBU, Ås, Norway) using a Recirculated Aquaculture System during the period of January 6th to March 24th 2022. The fish were weight initially, after an acclimation period until February 10th and at the termination in March.

The experimental design of the study involved transferring 225 Atlantic salmon smolts to tanks at 28 weeks post hatching, with an average weight of 94.2g (range 93.7g to 94.7g) per fish. Following a 31-day acclimatization period, the fish were fed experimental diets. 15 fish were allocated per tank, and there were a total of 15 circular tanks, each approximately 300L in volume. The diets were randomly assigned to the tanks in triplicate, and the fish were fed for 24 hours every day over a period of 43 consecutive days. Any uneaten feed and faeces were collected daily on a mesh to calculate the feed conversion ratio.

3.2 The Feed and Poultry hydrolysates coating procedures

The feed pellets, formulated by Aller Aqua A/S (Christiansfeld, Denmark) Thalassa Ex 2mm, were coated with chicken hydrolysates purchased from GePro Geflügel-Protein (Diepholz, Germany), the formulated list is shown in table 4. The minerals and amino acids found in the chicken hydrolysates are listed in Table 3 and Appendix 1.

Table 3. A summary of minerals and amino acids found in the Chicken hydrolysates, GePro Geflügel-Protein,AquaTrac Sol SD.

Trace Elements	
Copper (Cu)	10.3 mg/kg
Zinc (Zn)	34.4 mg/kg
Iron (Fe)	102.9 mg/kg
Manganese (Mn)	8.3 mg/kg
Amino Acids	
Alanine	3.4%
Arginine	4.1%
Aspartic Acid	4.9%
Cystine	0.6%
Glutamine acid	9.3%
Glycine	8.9%
Histidine	1.0%
Isoleucine	1.8%
Leucine	3.4%
Lysine	3.5%
Methionine	1.0%
Phenylalanine	1.8%
Proline	5.1%
Serine	2.6%
Taurine	1.1%
Threonine	2.1%
Tyrosine	0.9%
Valine	2.2%

Table 4. Ingredients list formulated by Aller Aqua (Aller Thalassa Ex,2mm).

Raw Material	**Feed Composition (%)**
Fish Meal	24.00
Maize Gluten	17.50
Soya Meal	14.68
Wheat	14.31
Sunflower Protein Concentrate	8.00
Rapeseed Oil	4.54
Fish oil	4.50
Pea Protein	4.00
Shrimp Meal	3.00
Soya Protein Concentrate	3.00
MonoAmmonium Phosphate	0.66
Vitamin A & D3	0.50
Minerals (Mn,Calsium,Zn,Cu)	0.15
Propyl Gallate (E310)	1.00

3.2.1 Preparation of Chicken Hydrolysate Mixture

AquaTrac Sol SD, a commercial chicken hydrolysate powder, was mixed with water in a ratio of 3:2 (60% chicken hydrolysate powder to 40% water. The mixture was then heated and kept warm in a bath at 40°C to prevent clumping.

3.2.2 Preparation of Poultry Hydrolysate-Enriched Pellets

To prepare the feed for the fish trial, the Aller Thalassa Ex 2mm pellets were air-fried with intermittent mixing until their temperature reached 30-40°C. Afterward, one kilogram of Aller Aqua pellets was mixed with varying percentages (0%, 5%, 10%, 15%, and 20%) of a chicken hydrolysate mixture that had been previously prepared. These different feed mixtures were labeled as Control diet (no poultry inclusion), C5 diet (5% poultry hydrolysate inclusion), C10 diet (10% poultry hydrolysate inclusion), C15 diet (15% poultry hydrolysate inclusion), and C20 diet (20% poultry hydrolysate inclusion). To homogenize the mixture, a Kenwood mixer was used at low speed for a few seconds, while maintaining the temperature at 40°C. Any excess fluids were removed, and the pellets were manually mixed to ensure uniformity.

3.2.3 Drying and Storage

The chicken hydrolysate-enriched pellets were spread on a tray and left to dry. After drying, they were packed in an airtight container and stored at 9°C in a dry storage facility.

3.3 Water Quality

During the feed experiment, the water temperature in the system was maintained at 14°C throughout the experimental period, and the temperature was monitored regularly to ensure consistency. However, there were some fluctuations in temperature over a period of three days, when it temporarily dropped to 10.2°C on the 11th of March 2022, but subsequently rose back to 14.1°C within two days (12th March: 12.7°C and 13th March: 13.6°C). The pH of the water in the outlets was also monitored, with readings of 7.9 on the 13th of January 2022, 7.8 on the 1st of February 2022, and 7.8 on the 22nd of March 2022.

The concentration of NH4-N mg/L in the inlet was 0.09, while the outlet consistently recorded a value of <0.05 mg/L. The concentration of NO2-N mg/L in the outlet was approximately 0.03. To ensure optimal conditions for the aquatic organisms, the water was constantly oxygenated to maintain levels above 85%. The water flow was also maintained at a rate of 8.0 to 10.0 L/min.

3.4 Sampling procedures

In this study, individual numbering and weighing of all fish were carried out on the last feeding day until the end of the trial. On the 35th day of the feeding trial, one fish per tank was removed for a smoltification test (Appendix 2), leaving 14 fish per tank for sampling. Prior to sampling, fish were then individually collected and anesthetized with Finquel vet (Trikainmesilat, MSD Animal Health Norge AS). A blow was administered to ensure death, after which the fish were transported to a nearby lab section for examination. Each individual fish was photographed under standardized light conditions, using an iPhone camera to assess fin damage, cataracts, scale loss, and vertebral deformities.

The first 10 fish from each tank were then gutted to determine gender by inspecting the gonads, and visceral fat was visually scored on a scale of 1 (clearly visible) to 5 (not visible)

(Figure 5). Hearts were visually scored on a scale of 0-3, where 0 indicated no visible fat on the ventricle, and 3 indicated visible fat on the ventricle (Figure 6). Fecal scoring was done on a scale of 1 (solid excrement) to 3 (loose excrement similar to diarrhea). The livers and hearts of the fish were dissected, weighed, and pooled per tank to calculate the hepatosomatic index (HSI) and the cardiosomatic index (CSI). Additionally, fecal samples were collected and pooled per tank for further analysis.

Finally, the last four fish from each tank were frozen at -18 degrees Celsius to analyze the whole-body composition. The fish were not starved and were provided with feed for 24 hours, except for the fish used for analysing the whole-body composition.

Figure 5. . Scale of assessment of visual liver colour (a) and visceral fat according to visibility of pyloric cacea (b) of Atlantic Salmon (Salmo Salar L.) (Mørkøre et al., 2020).

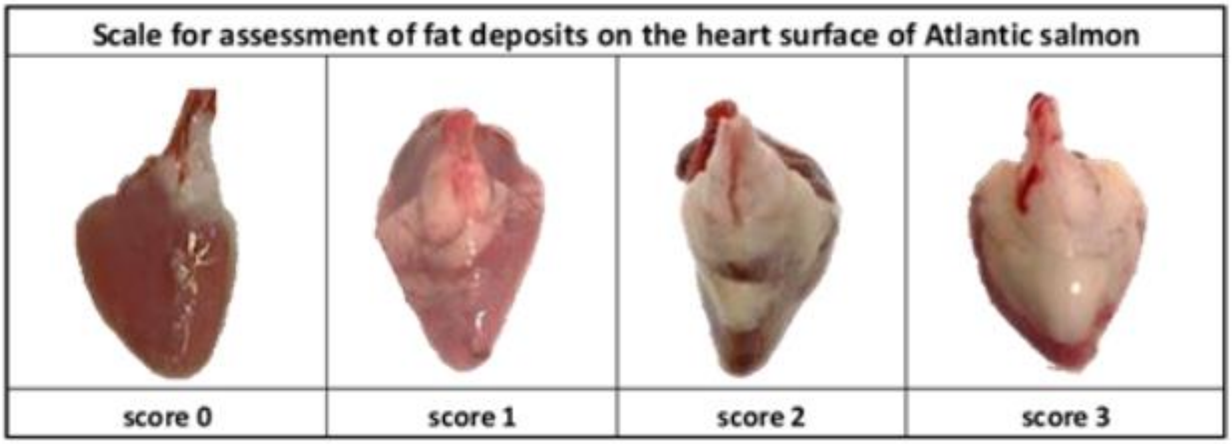

Figure 6. Scoring assessment with the visible fat on the heart in Atlantic Salmon (Salmo Salar L.) (Formanowicz, 2022).

3.5 Welfare Assessment

The welfare of the experimental fish was evaluated through visual scoring of OWIs using photographs taken with an iPhone camera of ten slaughtered fish per tank. The reliability and non-invasive nature of this method have been previously established. The OWIs are detailed in Appendix 3(Noble et al., 2018).

Assessment of fin damage was done on four regions: Dorsal, Pectoral, Caudal and Anal. The severity of the damage was classified as active or healed, with scores ranging from 0 (no damage) to 3 (severe damage). Scale loss was assessed using a score of 0 (no loss) to 3 (large area of loss ≥10% of the fish). Vertebral deformity was scored from 0-3, with 0 indicating no deformity and 3 indicating extreme deformity.

Skin hemorrhages were evaluated on a scale of 0 (no haemorrhaging) to 3 (significant bleeding), along with severe scale loss and skin blisters. Cataracts were assessed using a visual score illustrated in figure 7.

0. No cataract

1. Cataract covers less than 10% of lens diameter

2. Cataract covers between 10 and 50% of lens diameter

3. Cataract covers 50 to 75% of lens diameter

4. Cataract covers over 75% of lens diameter

Figure 7. Diagnosing and classifying eye cataracts. (Noble et al., 2018).

3.6 Blood plasma

Blood samples were obtained from the ductus cuvieri of fish, with each specimen yielding 1.00-1.50 ml of blood. To prevent clotting, an anticoagulant, haperinnatrium 100 IU/ml (see Appendix 4), was added to the samples. The samples were then subjected to centrifugation at 4000g for 10 minutes to separate the plasma and serum components. Only the plasma fraction was collected and stored at -25°C, with each blood sample yielding 0.5-0.75 ml of plasma. Pooled sampled per tank were subjected to analysis at NMBU Sentrallaboratoriet (S-lab) in Ås, Norway, following the method described by Tietz (1995).

3.7 Calculations

Feed Intake was calculated as,

$$Feed\ Intake\ (grams\ in\ DM\ per\ fish) = \frac{Total\ Feed\ Intake\ per\ Tank}{Number\ of\ fish\ per\ tank}$$

The Feed Conversion Ratio (FCR) was calculated as,

$$FCR\left(\frac{gram}{gram}\right) = \frac{Total\ Feed\ Intake}{Final\ Body\ Weight\ (Wet) - Initial\ Body\ Weight\ (Wet)}$$

Specific Growth Rate (SGR) was calculated as,

$$SGR\left(\frac{\%Body\ Weight}{Day}\right) = \frac{[(\ln final\ Body\ Weight - \ln Initial\ Body\ Weight) \times 100]}{Total\ Experiment\ Days}$$

Where, In = Natural Logarithm

Condition Factor (CF) was calculated as,

$$CF = \frac{Body\ weight(g)}{(Body\ Length(cm))^3}$$

Slaughter yield (%) was calculated as,

$$SY = \frac{Gutted\ weight\ (g)}{Body\ weight\ (g)} \times 100$$

Hepatosomatic Index (HSI) was calculated as,

$$HSI = \frac{Liver\ weight\ (g)}{Body\ weight\ (g)} x\ 100\%$$

Cardiosomatic Index (CSI) was calculated as,

$$CSI = \frac{Heart\ weight\ (g)}{Body\ weight\ (g)} \times 100\%$$

3.8 Statistical Analysis

In this study, the performed statistical analysis using the Statistical Analyses ANOVA to determine significant differences among the dietary groups. The software used for the statistical analysis was SAS software programming (SAS Institute, Cary, NC, USA; version 9.4). The P-values were calculated and a value below 0.05 was considered statistically significant.

4. Results

4.1 Weight gain

Weight gain was observed in all treatment groups as shown in figure 8, with the highest weight gain observed for fish fed the C5 diet, which had a weight increase of 103.94±6.6 g. The C5 diet also resulted in a significantly higher body weight ($P \leq 0.0055$) compared to the Control, C10, C15, and C20 diets. However, there was no significant difference in weight gain observed among the other diets except for C5. C10, C15, and C20 had slightly lower weight gain compared to the Control diet.

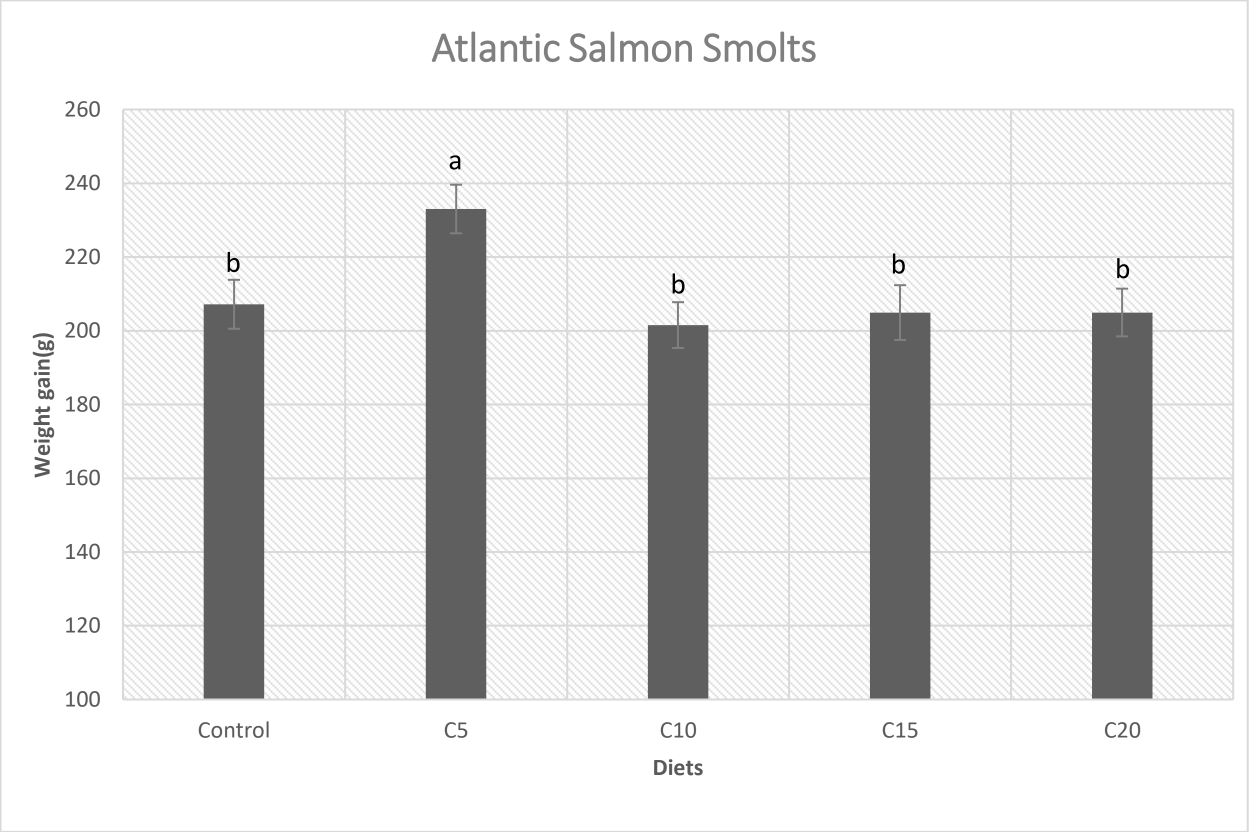

Figure 8. Weight gain in grams for the different diets. The superscripts above the error bars indicate significant differences between dietary treatments ($P \leq 0.05$).

4.2 Fish Growth Performance

The growth performance of Atlantic smolt fed on poultry-by product inclusion is presented in table 5. Although C10, C15, and C20 diets resulted in slightly lower weight gain compared to Control, the differences were not statistically significant (P≤0.247) for Specific Growth Rate (SGR). There was no mortality observed in any of the diets during the trial period. The initial weight of fish in all diets was similar, ranging from 109.67±0.29 grams to 128.09±0.35 grams. The final weight of fish fed on different diets ranged from 202.33±1.26 grams to 232.03±6.25 grams. The highest weight gain was observed in fish fed C5 diet 103.94±6.55 grams, while the lowest was in fish fed C20 diet 80.87±4.84 grams.

Table 5. Body weight after a four-week acclimation period, final body weight, weight increase (grams) and specific growth coefficient (%) (mean ± standard error).

Indicators	Control	C5	C10	C15	C20
Initial weight	120±2.27	128.09±0.35	109.67±0.29	115.33±3.83	123±0.11
Final weight	206.6±10.4	232.0±6.3	202.3±1.3	205.9±13.5	203.9±4.7
Weight gain	86.6±12.7	103.9±6.6	92.7±1.6	90.6±17.3	80.9±4.8
SGR%/day	1.12±0.06	1.28±0.06	1.09±0.06	1.11±0.06	1.1±0.06

4.3 Whole Body Composition

4.3.1 Dry Matter

The whole-body analysis dry matter content varied significantly between the dietary treatment (figure 9). The highest dry matter content was observed in salmon fed the control diet 30.1 ± 0.3, which was significantly greater than the dry matter content of the C20 diet 29.03 ± 0.3 with a p-value of 0.0278 and of 29.07 ± 0.3 C15 diet with p-value of 0.0319. Additionally, a regression line was plotted from the control diet with the highest dry matter content to the C20 diet with the lowest dry matter content, indicating a gradual decrease in dry matter content with increasing levels of C20 in the diet.

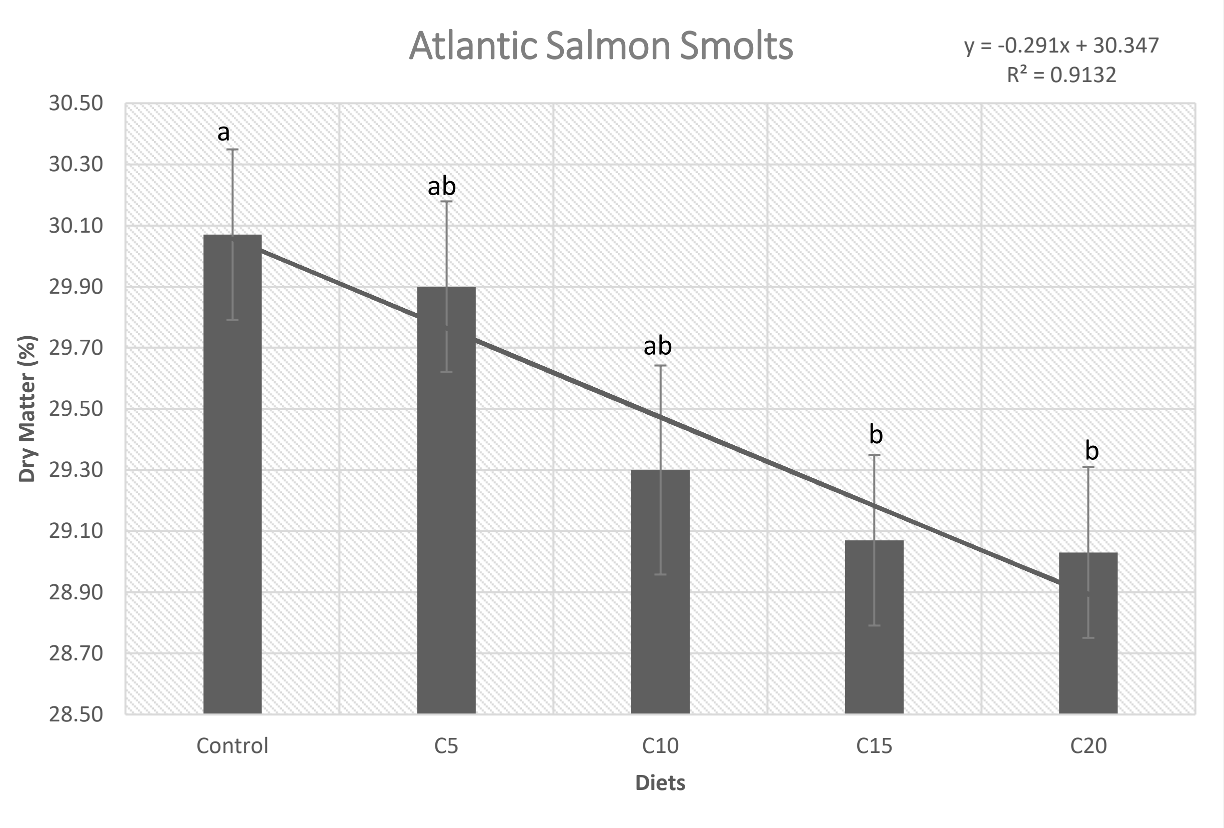

Figure 9. Dry matter in percentage of whole body of salmon smolts fed increasing inclusion of poultry hydrolysate from 0% (Control), 5% (C5), 10% (C10), 15% (C15) to 20% (C20). Different letters above the error bars indicate significant differences between dietary treatments (P<0.05).

4.3.2 Ash

The whole body of the salmon smolts fed the C15 diet 2.6 ± 0.08 had significantly higher ash content as compared to the C5 diet 2.33 ± 0.08 at a p-value of 0.0455. The lowest ash content was observed for the salmon fed in the C20 diet 2.30 ± 0.08 with a p-value of 0.0283(figure 10).

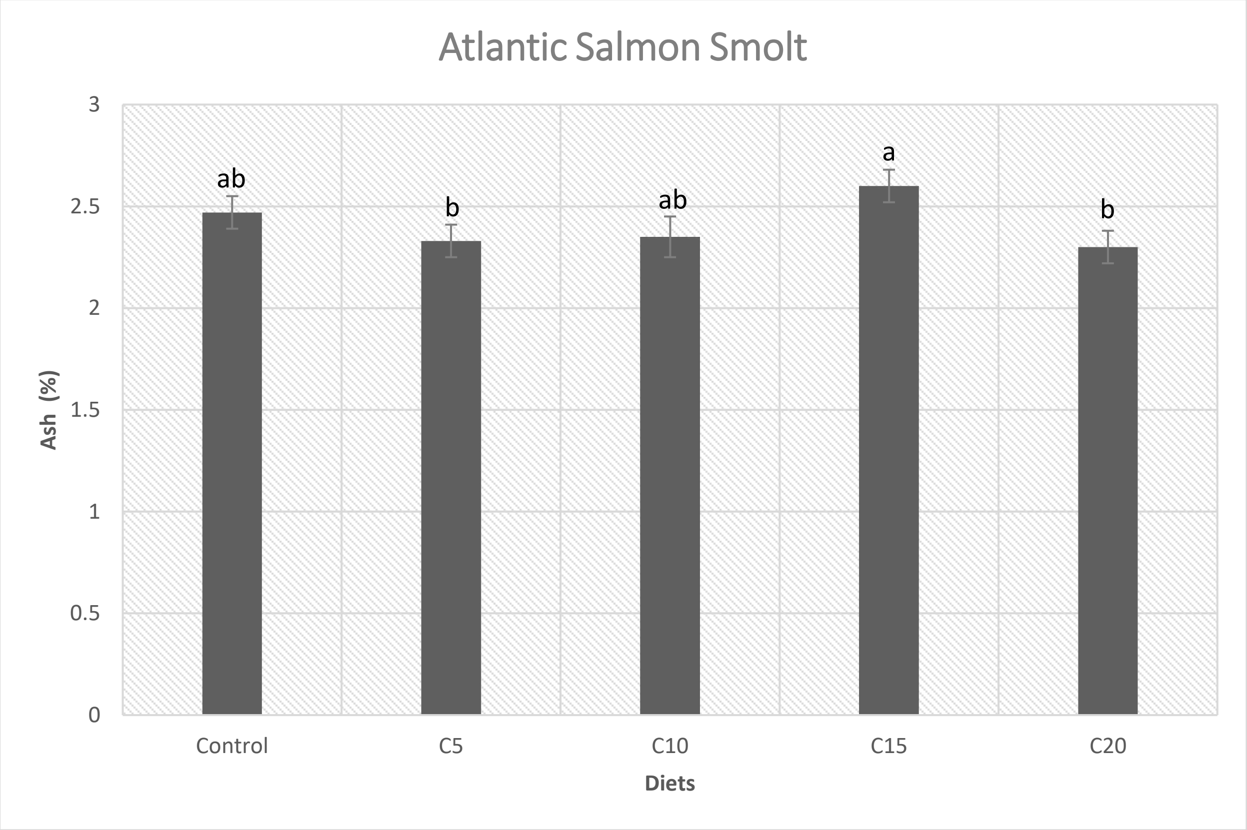

Figure 10. Ash in percentage of whole body of salmon smolts fed increasing inclusion of poultry hydrolysate from 0% (Control), 5% (C5), 10% (C10), 15% (C15) to 20% (C20). Different letters above the error bars indicate significant differences between dietary treatments (P<0.05).

4.3.3 Crude Fat

The percentage of fat content among the experimental diets is shown in figure 11. Salmon fed the C5 diet had the highest fat content compared to the other diets with a p-value of 0.0086. Conversely, the C15 diet had the lowest fat content 6.90 ± 0.168 with a p-value of 0.0010 between Diet C5 8.03 ± 0.168.

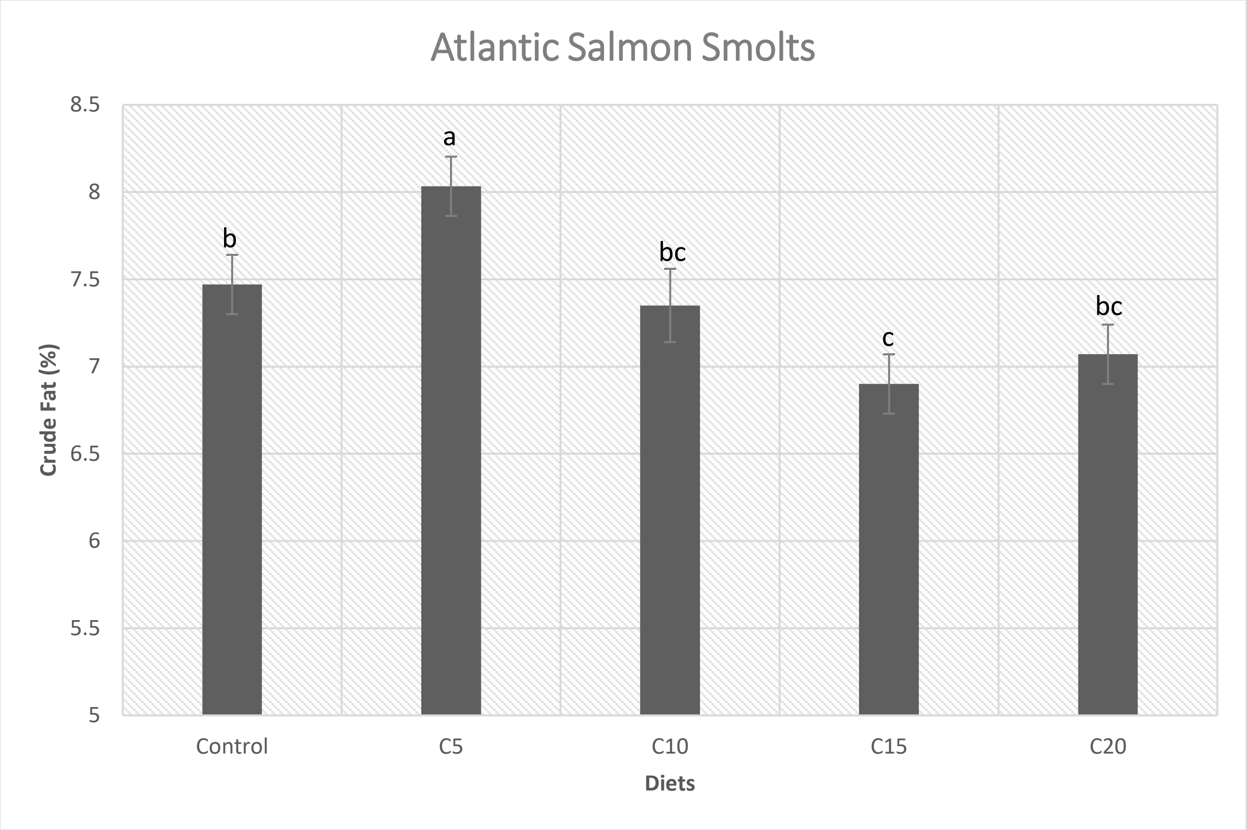

Figure 11. Fat in percentage of whole body of salmon smolts fed increasing inclusion of poultry hydrolysate from 0% (Control), 5% (C5), 10% (C10), 15% (C15) to 20% (C20). Different letters above the error bars indicate significant differences between dietary treatments (P<0.05).

4.4 Condition Factor

The condition factor was highest for salmon fed the C15 diet 1.31±0.038 and lowest for the salmon fed the Control diet 1.25±0.042, with a significant difference (P=0.0406) observed in figure 12. No significant difference was observed between the salmon fed C5, C10 and C20 diets.

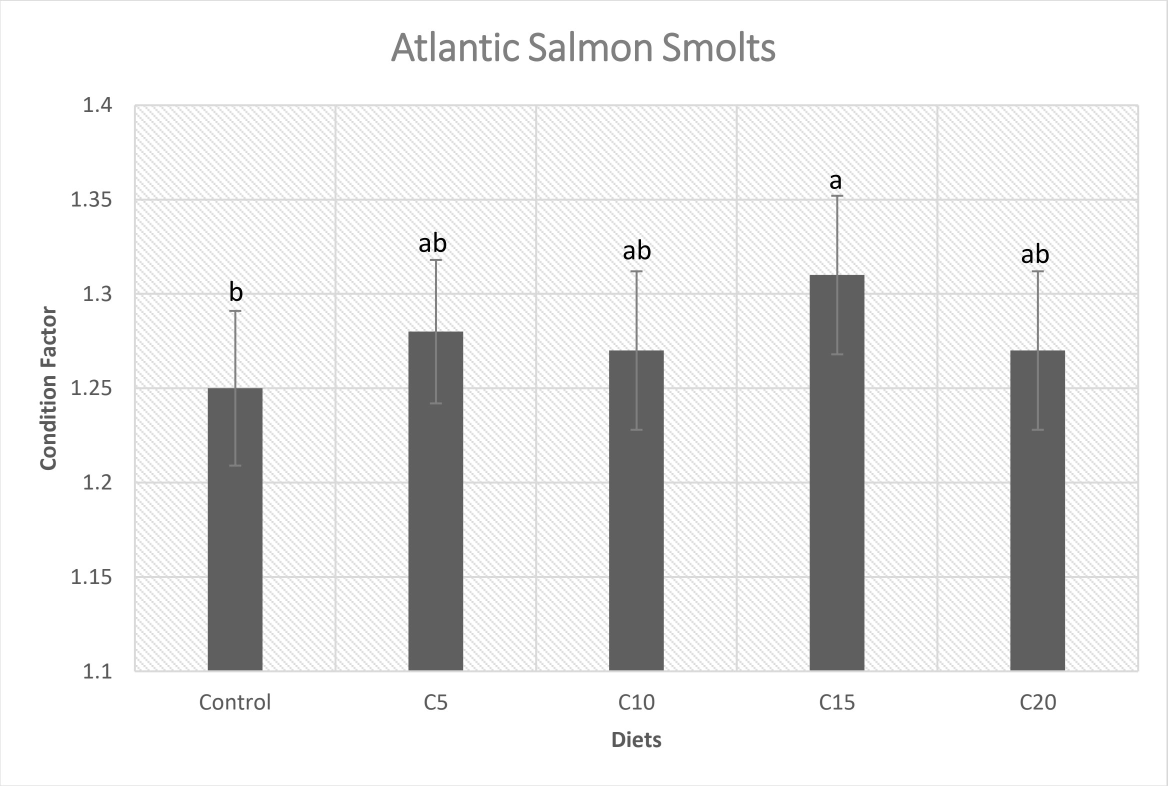

Figure 12. The condition factor of salmon smolts fed increasing inclusion of poultry hydrolysate from 0% (Control), 5% (C5), 10% (C10), 15% (C15) to 20% (C20). Different letters above the error bars indicate significant differences between dietary treatments (P<0.05).

4.5 Feed Conversion Ratio (FCR)

The feed conversion ratio (FCR) was the highest for the salmon fed the control diet 0.803 ± 0.0477 and between the C20 diet 0.786 ± 0.0477, that was significantly lower compared with the salmon fed the C10 diet 0.6197 ± 0.0477 (P=0.03) as described in figure 13.

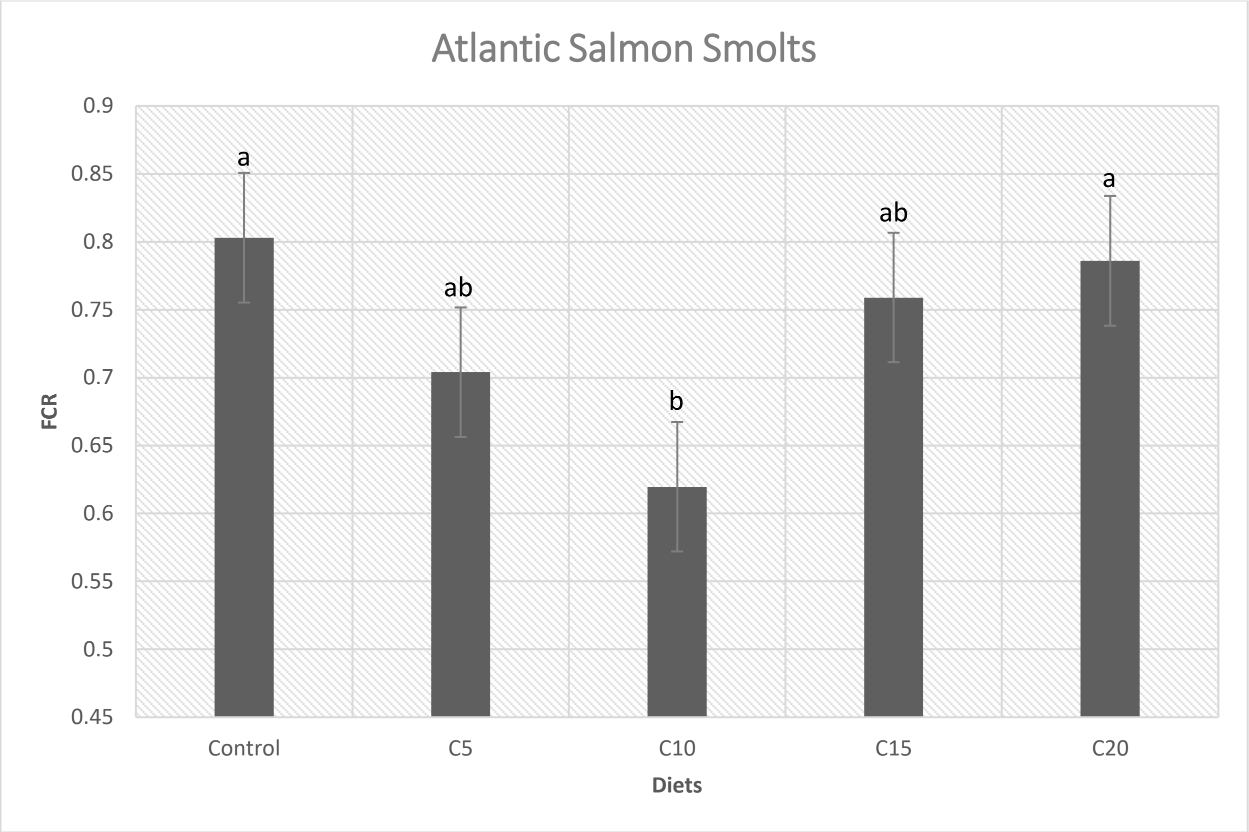

Figure 13. Feed Conversion Ratio (FCR) of salmon smolts fed increasing inclusion of poultry hydrolysate from 0% (Control), 5% (C5), 10% (C10), 15% (C15) to 20% (C20). Different letters above the error bars indicate significant differences between dietary treatments (P<0.05).

4.6 Gutted weight and Slaughter yield

Gutted weight was highest for C5 diet and significantly different from dietary groups C10, C15, and C20. The Control diet had a significantly different gutted weight compared to the C10 diet, which had the lowest gutted weight. Slaughter yield was significantly different among diets, with the Control diet having the highest yield, while C5, C10, C15, and C20 had the lowest yields (Table 6).

Table 6. Gutted weight and slaughter yield of Atlantic smolt salmon fed with by-poultry inclusions from 0%, 5%, 10%, 15% and 20%. Results are shown mean ± standard error and different letters indicate significant differences between dietary treatments (P<0.05).

Diets	Control	C5	C10	C15	C20	P-Value
Gutted Weight(g)	188.7±13.6ab	204.1±13.5a	170.63±13.7c	184.7±12.5bc	184.6±13.6bc	0.043
Slaughter yield (%)	89.1±0.5a	88.3±0.5b	88.40±0.5b	88.6±0.2ab	88.34±0.5b	0.09

4.7 Blood serum

The results of the blood analysis showed no significant differences for several parameters including amylase, AP (Alkaline Phosphate), albumin, AST(Aminotransferase), FFA (Free Fatty Acids), globulins, glucose, chloride, TP (Total Protein), CK (Creatine Kinase) or bile salts among the different dietary treatments (Table 7). However, phosphorus levels were significantly different (P=0.1454), with the highest levels observed in the C10 diet 5.04±0.26 and the lowest in the C15 diet 4.14±0.26 with a P-value of 0.0510. Potassium levels also showed a significant difference (P-Value = 0.0455), with the highest levels observed in the C10 diet and the lowest in the C15 diet. Sodium levels were also significantly different, with the C10 diet having the highest levels compared to the C5 diet having the lowest levels, with a P-value of 0.053.

Table 7. Different blood enzymes, fatty acids, minerals are presented among the different diets. Significant differences are marked as alphabets, SEM represents Standard Error Mean.

Blood Analysis	**Treatment Types**						
	C0	**C5**	**C10**	**C15**	**C20**	**SEM**	**P-value**
Amylase	1284	1247	1359	1261	1214	80.18	0.7629
AP	203.67	204	216.67	207	214.67	21.25	0.9616
Albumin	19	18.67	19	18.33	18.67	0.93	0.9836
AST	359.33	345.33	373.67	336.67	397.33	54.29	0.9349
CK	5335.33	4846.67	10181	5934.33	8035	2345.74	0.5032
Phosphorus	4.27ab	4.24ab	5.04a	4.14b	4.17b	0.26	0.1454
FFA	0.27	0.2	0.33	0.23	0.23	0.064	0.6679
Bile Salts	24.67	14.67	41.33	13.33	10.33	12.58	0.5049
Globulins	14	13.67	14	13.67	14.67	0.68	0.8335
Glucose	5.47	5.3	5.83	5.43	5.37	0.29	0.7288
Chloride	132.67	132.67	132.67	132	132	0.76	0.8409
Calcium	2.77	2.73	2.97	2.77	2.80	0.10	0.5017
Sodium	159.67ab	158.67b	162.67a	159.00ab	159.00ab	1.29	0.2440
Potassium	5.07ab	5.4ab	5.87a	4.93b	5.27ab	0.29	0.2472
Na:K Ratio	31.77	31.07	27.8	32.27	30.4	1.82	0.4875
Cholesterol	9.17	8.8	9.1	8.67	9.2	0.40	0.8327
Creatine	13.67	20.33	27	22.33	29.33	5.75	0.3948
Lipase	14.67	12.67	13.67	13.67	14	0.91	0.6490
Amyloid	10	9.33	10.33	9.67	9.67	0.39	0.4853
TP	33.33	32.33	33	32.33	33.33	1.42	0.9695
Triglyceride	2.33	2.70	2.87	2.30	3.20	0.401	0.5052

4.8 Fish Welfare

No mortality or cataracts were observed during the feed trial. From table 8, the viscera fat score was found to be significantly different between the control group and the groups fed with C10 and C20 diets, with the control group having the highest fat score and C20 having the lowest. The highest HSI (%) value was observed for the group fed with C10, while the lowest was observed for the control group. Heart score was highest for the control group, while it was lowest for the C15 group. Liver weight was found to be highest in the C5 group and lowest in the control group. Faeces scoring showed the highest value for the control group and lowest in the C5 group.

Further analysis of the data revealed that there was no significant difference in liver score and CSI (%) among the groups. Heart weight was also found to be similar among all groups. The P-values for all the parameters are reported in table 8.

Table 8. Visual scoring of visceral fat, fat accumulation on the heart surface and liver colour and faeces, and the liver and heart weight (g) and % of heart and liver relative to the body weight (HSI and CSI,%). Results are shown as mean ± standard error for Atlantic Salmon smolts fed diets with increasing levels of poultry hydrolysates 0% (Control), 5% (C5), 10% (C10) 15%(C15) and 20%(C20).

Diets	Control	C5	C10	C15	C20	P-value
Viseral fat score	2.49±0.20a	2.40±0.20ab	2.22±0.20bc	2.34±0.18ab	2.10±0.20bc	0.058
Liver colour score	3.71±0.18	3.55±0.18	3.52±0.18	3.67±0.16	3.66±0.18	0.416
HSI, (%)	1.07±0.07c	1.11±0.07ac	1.20±0.07a	1.10±0.06b	1.16±0.07ab	0.094
CSI, (%)	0.12±0.01	0.13±0.01	0.13±0.01	0.13±0.01	0.12±0.01	0.454
Heart score	1.06±0.22a	0.68±0.21b	0.68±0.22b	0.61±0.20b	0.82±0.22ab	0.061
Heart weight(g)	0.26±0.02ab	0.29±0.02a	0.25±0.02b	0.28±0.02ab	0.26±0.02b	0.213
Liver weight(g)	2.27±0.21b	2.55±0.21a	2.35±0.21ab	2.32±0.20ab	2.41±0.21ab	0.536
Faeces score	1.93±0.19a	1.57±0.19b	1.80±0.19ab	1.70±0.18ab	1.86±0.19a	0.113

4.8.1 Fin Damage

Operational Welfare Indicators were also used to assess fin damage with visual scoring ranging from 0 to 3, 0 indicating a whole fin present and 3 indicating very little fin remaining described in table 9. The highest active fin damage score was observed in the dorsal fin, with a score of 0.37±0.10 of the C5 diet group. In the pectoral fin, the highest active fin damage score was 0.30±0.09 in the C5 diet group.

Table 9. Damage of dorsal, pectoral and caudal fin in active phase. Results are presented as mean ± standard error for Atlantic salmon smolts fed diets with poultry hydrolysates ranging from 0% (Control), 5% (C5), 10% (C10) 15%(C15) and 20%(C20).

Fin Damage (Active)	**Control**	**C5**	**C10**	**C15**	**C20**	**P-Value**
Dorsal	0.34±0.088	0.37±0.102	0.20±0.075	0.20±0.075	0.33±0.088	0.459
Pectoral	0.10±0.057	0.30±0.086	0.13±0.064	0.03±0.033	0.17±0.069	0.057
Caudal	0.90±0.057	0.73±0.082	0.67±0.088	0.77±0.079	0.87±0.064	0.177
Anal	0.07±0.047	0.07±0.046	0.13±0.064	0.03±0.033	0.03±0.033	0.529

Another welfare indicator considered was the healed fin damage (table 10). For the dorsal fin, the highest healed fin damage score was observed in the C10 0.67±0.01 diet group. In the pectoral fin, the C10 diet group was the highest while the other diets showed no signs of fin damage (healed). In the pectoral fin, C19 and C15 shows similar fin damage, while the other diets showed no signs of fin damage (healed). Lastly, in the anal area, C5 and C10 showed minor fin damage 0.03±0.033 while the other diets have no signs of fin damage (healed).

Table 10. Damage of dorsal, pectoral, and caudal fin in healed phase. Results are presented as mean ± standard error for Atlantic salmon smolts fed diets with poultry hydrolysates ranging from 0% (Control), 5% (C5), 10% (C10) 15%(C15) and 20%(C20).

Fin Damage (Healed)	**Control**	**C5**	**C10**	**C15**	**C20**	**P-Value**
Dorsal	0.38±0.089	0.47±0.104	0.67±0.100	0.53±0.093	0.47±0.093	0.459
Pectoral	0.00±0.00	0.00±0.00	0.03±0.033	0.00±0.00	0.00±0.00	0.057
Caudal	0.00±0.00	0.00±0.00	0.03±0.033	0.03±0.033	0.00±0.00	0.177
Anal	0.00±0.00	0.03±0.033	0.03±0.033	0.00±0.00	00.00±0.00	0.529

4.8.2 Scale loss

Figure 14 represents the scale loss scores obtained during the study. The results indicate that the highest scale loss score was observed in the C20 diet group with a score of 2.50±0.104. In contrast, the control group had the lowest score of 1.90±0.113. The statistical analysis revealed a p-value of 0.007, indicating a highly significant difference between the two groups.

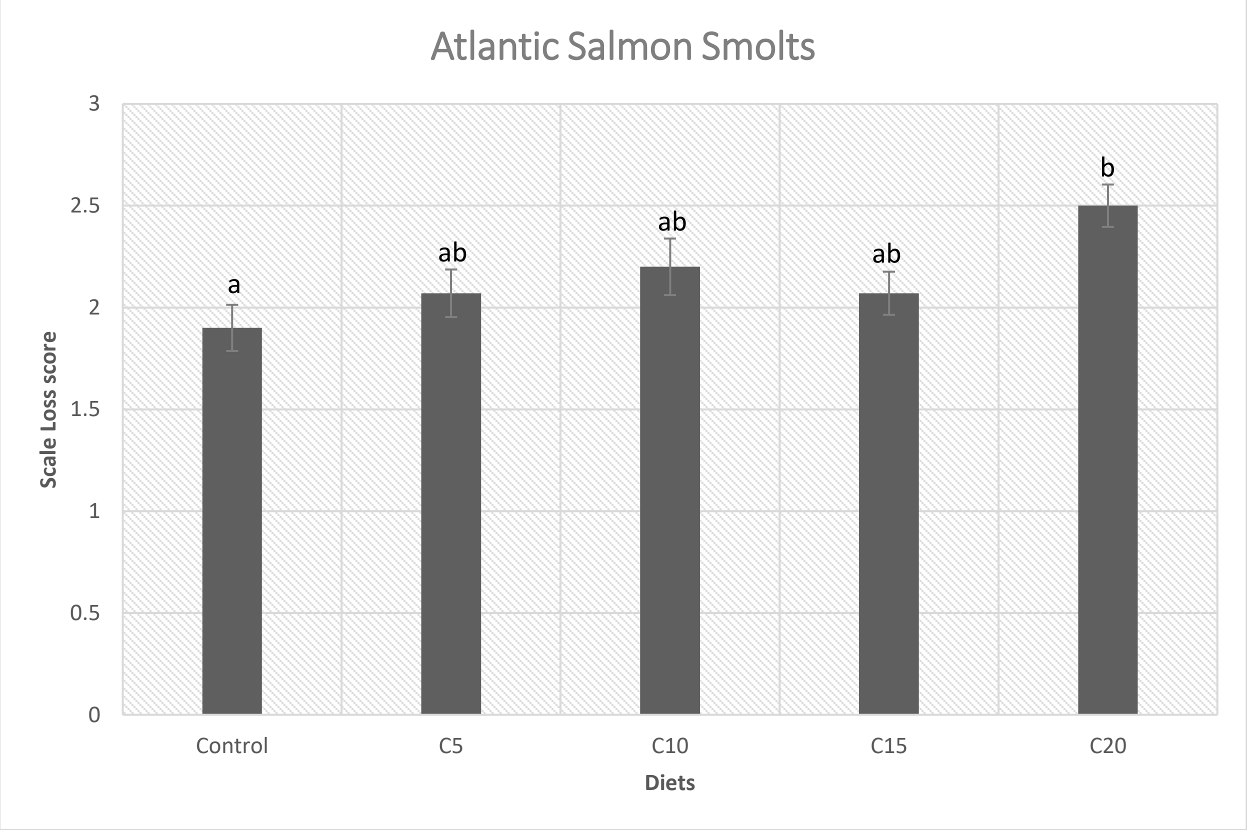

Figure 14. Scale loss of salmon smolts fed increasing inclusion of poultry hydrolysate from 0% (Control), 5% (C5), 10% (C10), 15% (C15) to 20% (C20). Different letters above the error bars indicate significant differences between dietary treatments (P<0.05).

4.8.3 Vertebrate deformities

Minor vertebrate deformities were observed in the control group (0.10 ± 0.057), whereas the inclusion of poultry hydrolysates from C5 to C20 diets did not show any deformities. The p-value of 0.012 indicates that minor vertebrate deformities were detected in the control diet.

5. Discussion

5.1 Weight gain

The results showed that weight gain was observed in all treatment groups, with the highest weight gain seen in fish fed the C5 diet, which had a weight increase of 103.94±6.55 g. This suggests that the C5 diet may be more effective in promoting weight gain in fish compared to the other diets.

Further analysis showed that the C5 diet also resulted in a significantly higher body weight ($P \leq 0.0055$) compared to the Control, C10, C15, and C20 diets. This finding indicates that the C5 diet may be more suitable for fish growth and development, at least in the context of this study.

Even though there was no significant difference in weight gain observed among the other diets except for C5 diet. C10, C15, and C20 diets had slightly lower weight gain compared to the Control diet. This suggests that while these diets may not be as effective as the C5 diet in promoting weight gain, the different diets still show weight gain throughout the feed trial.

In addition, a previous study has reported that higher substitutions of poultry by-product meal, ranging from 50% to 75%, can cause a significant decrease in growth performance in gilthead seabream (*Sparus aurata L.*) compared to fish meal (Nengas et al., 1999). It is possible that higher inclusion of poultry by-product in fish feed may lead to issues with palatability or digestion. However, the specific effects of higher inclusion levels of poultry by-product on fish growth and development are not well understood and require further investigation.

5.2 Fish Growth Performance

From the results, C5 diet showed the highest weight gain, the differences in weight gain among the other diets were not statistically significant, indicating that these diets did not have a negative impact on fish health. The absence of mortality in any of the diets during the trial period further supports this claim.

These results are consistent with previous studies that suggest poultry by-product meal can serve as an alternative protein source in fish diets. Studies have shown that poultry by-product meal is a good source of protein for fish and replacing fishmeal with poultry by-product did not significantly affect growth performance in Nile Tilapia (*Oreochromis niloticus*) (Soltan, 2009; Yang et al., 2004). Thus, these findings support the notion that the inclusion of poultry by-products in fish diets can lead to improved growth performance.

5.3 Whole Body Composition

The present study investigated the impact of including poultry hydrolysate at different levels in the diets of Atlantic salmon smolts on their dry matter, ash, and crude fat. The results revealed significant differences in dry matter content among the experimental diets, with the control diet exhibiting the highest dry matter content. Furthermore, the ash content of the fish varied among diets, with the C15 diet displaying a significantly higher ash content than the C5 diet, while the C20 diet had the lowest ash content. Interestingly, the C5 diet had the highest fat content among all the diets, while the C15 diet had the lowest fat content. These findings demonstrate that including poultry hydrolysate in fish diets can have a significant impact on the composition of the fish, and therefore should be considered when formulating fish feeds.

5.4 Condition factor

From the results, the effect of different diets on the condition factor of salmon smolts. The results showed that the C15 diet had the highest condition factor and was significantly different from the control diet. This finding indicates that the inclusion of poultry by-products can enhance the overall condition and health of salmon smolts. These results are consistent with a previous study that investigated the replacement of fishmeal with poultry by-product in juvenile hybrid grouper fish (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) diets (Zhou et al., 2019). The study found that the grouper fish with 10% to 20% poultry by-product replacement had a higher condition factor than those with higher percentages of replacement. These findings suggest that there may be an optimal level of poultry by-product inclusion that can lead to improved condition and health in fish.

5.5 Feed Conversion Ratio

The results showed that the control group had the highest FCR, while the group fed with the C10 diet had the lowest FCR. These findings suggest that a 10% inclusion of poultry hydrolysate in the diet may have a positive impact on FCR.

However, as the inclusion level of poultry hydrolysate increased to 15% and 20%, the FCR became similar to that of the control diet. This finding is consistent with a previous study conducted on catfish (*Clarias geriepinus*) that found replacing 20% of fishmeal with poultry by-products resulted in a lower FCR compared to the control group (Abdel-Warith et al., 2001). However, as the inclusion level of poultry by-products increased beyond 20%, the FCR ratio increased with the percentage increment of poultry by-products.

5.6 Gutted Weight and Slaughter yield

The results of the experiment revealed that the C5 diet had the highest gutted weight of 204.07g, which could be attributed to the higher weight gain observed in this group during the experiment. However, the slaughter yield was lower in the C5 diet at 88.30% as compared to the control diet with the highest yield of 89.13%. Although the difference in yield was not statistically significant, it may be noteworthy from a commercial perspective.

Interestingly, the study also revealed that there was a significant difference in gutted weight between the C5 diet (204.07±13.54g) and the C10 diet (170.63±13.65g). This finding suggests that the inclusion level of poultry hydrolysate in the diet has a significant impact on the growth of Atlantic salmon smolts. Additionally, the results for slaughter yield were significantly different between the control diet (89.1±0.5) and the C5 diet (88.3±0.5). These results suggest that the inclusion of poultry hydrolysate in the diet may have an impact on the processing yield of Atlantic salmon smolts.

5.7 Blood serum

The blood analysis revealed that the tested diets did not significantly impact most of the parameters such as amylase, AP (Alkaline Phosphate), albumin, AST(Aminotransferase), FFA (Free Fatty Acids), globulins, glucose, chloride, TP (Total Protein), CK (Creatine Kinase) or bile salts. However, significant differences were found in phosphorus, potassium, and sodium levels among the diets. The C10 diet had the highest levels of phosphorus and potassium, while sodium levels were slightly higher in the C10 diet but consistent in the other diets. The observed differences in electrolyte levels could be attributed to differences in diet composition.

These findings are consistent with previous research, which has suggested that poultry hydrolysate in salmon feed can improve the availability of phosphorus (Skonberg et al, 1997). Further research is needed to understand the exact mechanisms behind these differences and their potential health outcomes. Additionally, the study found that the higher levels of sodium and potassium in the C10 diet could have positive effects on the overall performance and welfare of smolts, as these electrolytes are essential for physiological changes in smolts, including osmoregulation (Philip et al, 2022).

Overall, the results suggest that while the different diets may not have a significant impact on most blood parameters, there may be subtle differences in electrolyte levels.

5.8 Fish welfare

The present study investigated the effects of different inclusion levels of poultry hydrolysate on the welfare of Atlantic salmon smolts by measuring the visceral fat score, heart score, and liver score, as well as the HSI and CSI across the various diets. The results suggest that the diets were nutritionally balanced and met the requirements of the fish, as indicated by the absence of mortality or cataracts in all groups. Furthermore, the inclusion of poultry hydrolysate had a positive effect on reducing fat accumulation in the fish, as evidenced by the significant differences observed in fat score and HSI values between the control group and the groups fed with C10 and C20 diets. The study also showed that a high-lipid diet can lead to frequent cataract formation, highlighting the positive effects of including poultry hydrolysates on preventing cataracts (Waagbø et al., 2003).

The liver weight was highest in the C5 group, which may indicate that this group had a higher metabolic rate and was utilizing nutrients more efficiently. However, the faeces scoring showed the highest value in the control group, which suggests that the control group had a less efficient digestion and absorption of nutrients. The absence of significant differences in liver score and CSI (%) among the groups suggests that the different inclusion levels of poultry hydrolysate did not have a significant effect on liver health or protein utilization efficiency.

5.8.1 Fin damage

The present study utilized Operational Welfare Indicators to assess the extent of fin damage in Atlantic salmon smolts. The results showed that the highest active fin damage score was observed in the dorsal region of the fish, with the C5 diet group having a score of 0.37±0.102. Similarly, in the pectoral region, the highest active fin damage score was 0.30±0.086 in the C5 diet group. Although the scores indicate minor damage, it is important to note the potential welfare implications for the fish.

Another welfare indicator considered in this study was healed fin damage. The healed fin damage scores obtained for the various diets are presented in Table 10. In the dorsal region, the C10 diet group had the highest score for healed fin damage. In the caudal area, the control group had the highest score for healed fin damage, with a score of 0.90±0.057. For the anal area, the highest healed fin damage score was observed in the C10 diet group, with a score of 0.13±0.064. The C10 and C15 diets had the highest scores for healed fin damage in the caudal region, while the C5 and C10 diets had the highest scores in the anal region.

Overall, the results suggest that the inclusion of different diets had an impact on the extent of fin damage in Atlantic salmon smolts. However, it is not proven if this is due to diet or external factors.

5.8.2 Scale loss

Scale loss is another important welfare concern in fish, and the results of this study suggest that diet plays a crucial role in scale loss and fish welfare. The higher scale loss score observed in the C20 diet group may be due to the diet's composition or nutrient deficiencies. Further research is needed to investigate the specific dietary factors that impact scale loss in fish and to develop dietary interventions to promote fish welfare.

5.8.3 Vertebral deformities

The study found that the control group had minor vertebral deformities, whereas the inclusion of poultry hydrolysates in diets from C5 to C20 did not result in any deformities. This suggests that poultry hydrolysates could be a promising solution to prevent vertebral deformities in fish. The vertebrae play a crucial role in the biomechanical function of fish, allowing for muscle anchoring, propulsion, and flexibility during locomotion (Webb., 1984), as well as in maintaining calcium and phosphorus homeostasis (Graff et al., 2002). Although the analysis of blood serum revealed that the C10 diet had the highest levels of phosphorus, while the C15 diet had the lowest levels. This observation may be attributable to the higher vitamin D content present in poultry by-products, as previous research has demonstrated that vitamin D significantly influences the roles of calcium and phosphorus in Atlantic salmon (Lock et al., 2007).

6. Conclusion

To summarize, this research provides insights into the use of poultry by-products and hydrolysate as dietary ingredients in fish feed. While these ingredients can benefit fish growth and nutrient availability, it's important to carefully consider the optimal inclusion level to avoid negative impacts on processing yield and fish welfare. More research is needed to determine the long-term effects of different diets on fish health and development, and their interactions with factors like smoltification and fin healing.

Based on this study, an optimal range of poultry inclusion is between 5% to 10%, with C5 (5% inclusion) diet showing the highest growth performance, good scores in liver, heart and faeces analysis, low scale loss and no fin damages, and C10 (10% inclusion) diet showing the highest condition factor, lowest FCR, and lowest fin damage. Further research with dietary factors in this range may lead to promising results for sustainable and cost-effective aquaculture diets.

7. Appendix

7.1 Appendix 1.

Information of Protein Hydrolysate from GePro, Aquatrac Sol.

AQUATRAC® SOL
SPRAY DRIED

AquaTrac®

Art.-No.
5508

Current revision number: 023, December 2020
Last revision number: 022, July 2020

DEFINITION

The natural attractant AquaTrac® sol SD is a spray-dried and processed animal protein, consisting of highly soluble protein, peptide and amino acid compounds. AquaTrac® sol SD has been developed to significantly enhance the acceptance of all kinds of aquatic feed (finfish and crustaceans). AquaTrac® sol SD also has strong binder properties.
Recommended inclusion in aquatic feed: 3-10%

SPECIFICATION

Nutrients		
Moisture	< 6.0 %	VDLUFA III 3.1
Crude Protein	> 70.0 %	VDLUFA III 4.1.1
Crude Fat	> 9.0 %	VDLUFA III 5.1.1
Crude Ash	< 10.0 %	VDLUFA III 8.1
In Vitro Digestibility, Pepsin-conc. 0.02 %	> 96.0 %	VDLUFA III 4.2.1
In Vivo Digestibility (shrimps)	94.8 %	

Volume elements		
Calcium (Ca)	0.2 %	VDLUFA III 10.3.2
Phosphorus (P)	0.9 %	VDLUFA III 10.6.5
Ca:P ratio	0.2	
Magnesium (Mg)	0.1 %	DIN CEN/TS 15621
Potassium (K)	2.2 %	VDLUFA III 10.2.1
Sodium (Na)	1.4 %	VDLUFA III 10.1.1

Trace elements		
Copper (Cu)	10.3 mg/kg	DIN15763-SOP#41474
Zinc (Zn)	34.4 mg/kg	DIN15763-SOP#41474
Iron (Fe)	102.9 mg/kg	DIN CEN/TS 15621
Manganese (Mn)	0.3 mg/kg	DIN CEN/TS 15621

Amino acids		
Alanine	3.4 %	VDLUFA III 4.11.1
Arginine	4.1 %	VDLUFA III 4.11.1
Aspartic acid	4.9 %	VDLUFA III 4.11.1
Cystine	0.6 %	VDLUFA III 4.11.1
Glutamine acid	9.3 %	VDLUFA III 4.11.1
Glycine	8.9 %	VDLUFA III 4.11.1
Histidine	1.0 %	VDLUFA III 4.11.1
Isoleucine	1.8 %	VDLUFA III 4.11.1
Leucine	3.4 %	VDLUFA III 4.11.1
Lysine	3.5 %	VDLUFA III 4.11.1
Methionine	1.0 %	VDLUFA III 4.11.1
Phenylalanine	1.8 %	VDLUFA III 4.11.1
Proline	5.1 %	VDLUFA III 4.11.1
Serine	2.6 %	VDLUFA III 4.11.1
Taurine	1.1 %	VDLUFA III 4.11.1
Threonine	2.1 %	VDLUFA III 4.11.1
Tyrosine	0.9 %	VDLUFA III 4.11.1
Valine	2.2 %	VDLUFA III 4.11.1

The data in the specification, which are not specified specifically (">" or "<") represent mean values of the organic substance. These data should be considered as typical values, without any commitment.

Microbiology		
Salmonella	negative in 25 g	LFGB L00.00-98
Enterobacteria	<10 CFU /g	LFGB L05.00-5

Colour	light brown powder
Flavour	fresh
Contamination	technically free from pesticides, lumps, insects and other foreign bodies

LEGISLATION / HEALTH CERTIFICATE

AquaTrac® sol SD has been manufactured out of low risk poultry material only, which has passed the slaughtering process. It is classified to be "category 3 material" (Regulation (EC) No. 1069/2009; 999/2001; 142/2011 and amendments).
It is declared as processed animal protein.

HALAL CERTIFIED (BY HQC)
This product is in compliance with the Halal requirements stipulated by Halal Quality Control (HQC).

USAGE
AquaTrac® sol SD is used as a raw material in diets for aquatic feed.

STABILITY / STORAGE

AquaTrac® sol SD has a shelf life of 6 months in well closed bags in a cool and dry place.
This may vary in case of other conditions.
Opened packages should be processed as soon as possible.

GEPRO Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG
Im Moore 1 - 49356 Diepholz - Germany
Tel.: +49 5441 5925-0 · info@ge-pro.de · www.ge-pro.de

Veterinary-Control-No. & EU Vet. Category III No.: DE-03 251 0061 34

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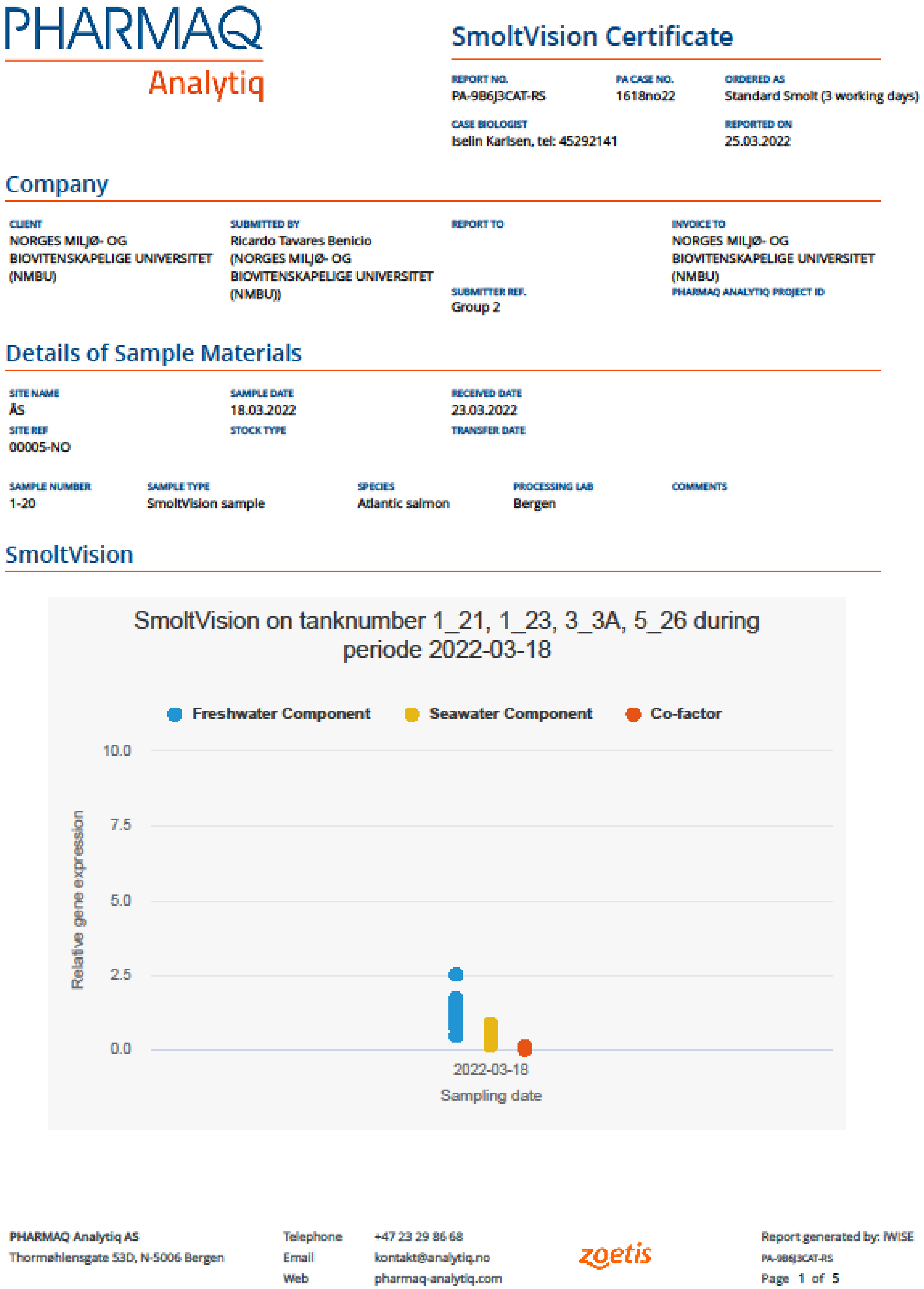
GEPRO We go beyond.

7.2 Appendix 2.

Smoltification Results for Sentral laboratory.



PHARMAQ
Analytiq

SmoltVision Certificate

REPORT NO.	PA CASE NO.	ORDERED AS
PA-9B6J3CAT-RS	1618no22	Standard Smolt (3 working days)

CASE BIOLOGIST	REPORTED ON
Iselin Karlsen, tel: 45292141	25.03.2022

Company

CLIENT	SUBMITTED BY	REPORT TO	INVOICE TO
NORGES MILJØ- OG BIOVITENSKAPELIGE UNIVERSITET (NMBU)	Ricardo Tavares Benicio (NORGES MILJØ- OG BIOVITENSKAPELIGE UNIVERSITET (NMBU))		NORGES MILJØ- OG BIOVITENSKAPELIGE UNIVERSITET (NMBU)
		SUBMITTER REF.	PHARMAQ ANALYTIQ PROJECT ID
		Group 2	

Details of Sample Materials

SITE NAME	SAMPLE DATE	RECEIVED DATE
Ås	18.03.2022	23.03.2022
SITE REF	STOCK TYPE	TRANSFER DATE
00005-NO		

SAMPLE NUMBER	SAMPLE TYPE	SPECIES	PROCESSING LAB	COMMENTS
1-20	SmoltVision sample	Atlantic salmon	Bergen	

SmoltVision

PHARMAQ Analytiq AS
Thormøhlensgate 53D, N-5006 Bergen

Telephone	+47 23 29 86 68
Email	kontakt@analytiq.no
Web	pharmaq-analytiq.com

zoetis

Report generated by: IWISE
PA-9B6J3CAT-RS
Page 1 of 5

Individual data

DATE	REPORT NR	FRESHWATER COMPONENT	SEAWATER COMPONENT	CO-FACTOR	SMOLT INDEX	K-FACTOR	WEIGHT
2022-03-18	[illegible]	1,14	0,42	0,05	2,8	1,22	195,7

Temperature / Light regime

DATE	TEMPERATURE	SALINITY
2022-03-18	14.60 °C	-

LIGHT REGIME	DESCRIPTION OF DIFFERENT LIGHT REGIME
Other light regime	24:0

Salt feed

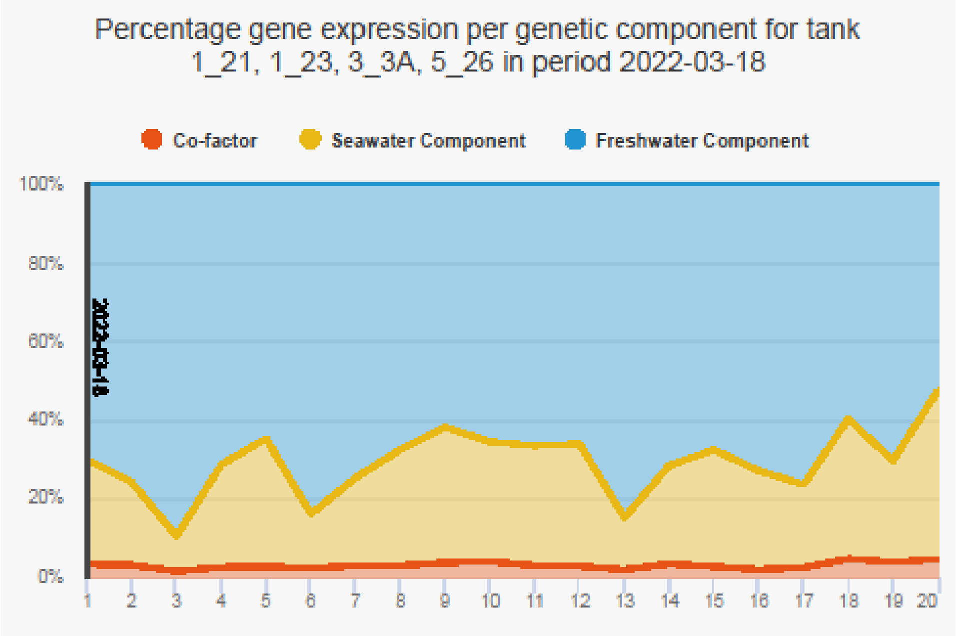
FEED SUPPLIER	SALT FEED START DATE	SALT FEED END DATE
-	-	-

Conclusions/Comments

[illegible]

SmoltVision is showing in average a higher expression of freshwater ATPase (1,14) than seawater ATPase (0,42) and a co-factor of 0,05. The co-factor is within the normal range (makes up ±20% of the total gene expression, as seen in the graph below), indicating that there are no disturbances of the gills or the smoltification process being picked up at this sampling point. The group is showing some variation in the gene expression. 20 out of 20 individuals have a dominating production of freshwater ATPase at this sampling point, which is not compatible with seawater tolerance. 3 individuals (fish no. 3, 6, and 13) stand out from the rest of the group with a very strong freshwater expression. The group have a large and robust size, which may compensate to some degree for the lack of seawater ATPase at seawater transfer. Based on the size of the fish it's likely that the fish have been through the smoltification process at least once at an earlier stage, and we cannot exclude the possibility that the group is now desmoltifying. Smolt index is low at 2,8, while condition factor is good at 1,22. The fish are on a 24:0 hour light regime. The estimated date of transfer is not stated. Based on the results the fish have a gene expression that is not compatible with seawater tolerance at this sampling point. The large and robust size may compensate for this to some degree. We recommend a new sampling point prior to seawater transfer, to follow the development and smolt status of the fish. Iselin Karlsen

Extended report



Additional information

TRANSFER DATE	AVERAGE WEIGHT	WATER TYPE	WATER TEMP.	VACCINE	DIAGNOSIS/SYMPTOMS
		Saltwater	14,6 °C		

Received samples - detailed list

INDIVIDUAL	SAMPLE TYPE	ID	BARCODE	CAGE	BATCH	TISSUE	SAMPLING DATE
1	SmoltVision sample	16, LV2004197030	LV2004197030	5_26		Gill	2022-03-18
2	SmoltVision sample	17, LV2004197139	LV2004197139	5_26		Gill	2022-03-18
3	SmoltVision sample	18, LV2004197140	LV2004197140	5_26		Gill	2022-03-18
4	SmoltVision sample	19, LV2004197141	LV2004197141	5_26		Gill	2022-03-18
5	SmoltVision sample	20, LV2004197142	LV2004197142	5_26		Gill	2022-03-18
6	SmoltVision sample	21, LV2004197143	LV2004197143	3_34		Gill	2022-03-18
7	SmoltVision sample	22, LV2004197144	LV2004197144	3_34		Gill	2022-03-18
8	SmoltVision sample	23, LV2004197271	LV2004197271	3_34		Gill	2022-03-18
9	SmoltVision sample	24, LV2004197272	LV2004197272	3_34		Gill	2022-03-18
10	SmoltVision sample	25, LV2004197274	LV2004197274	3_34		Gill	2022-03-18
11	SmoltVision sample	26, LV2004197275	LV2004197275	1_21		Gill	2022-03-18
12	SmoltVision sample	27, LV2004197277	LV2004197277	1_21		Gill	2022-03-18
13	SmoltVision sample	28, LV2004197281	LV2004197281	1_21		Gill	2022-03-18
14	SmoltVision sample	29, LV2004197487	LV2004197487	1_21		Gill	2022-03-18
15	SmoltVision sample	30, LV2004197488	LV2004197488	1_21		Gill	2022-03-18
16	SmoltVision sample	31, LV2004197489	LV2004197489	1_23		Gill	2022-03-18
17	SmoltVision sample	32, LV2004197500	LV2004197500	1_23		Gill	2022-03-18
18	SmoltVision sample	33, LV2004197501	LV2004197501	1_23		Gill	2022-03-18
19	SmoltVision sample	34, LV2004197504	LV2004197504	1_23		Gill	2022-03-18
20	SmoltVision sample	35, LV2004197511	LV2004197511	1_23		Gill	2022-03-18

Information to smolt certificate

DATE SAMPLED	BATCH	TEMPERATURE	SALINITY	TANK NUMBER
2022-03-18		14,60 °C		1_21, 1_23, 3_34, 5_26

INDIVIDUAL ID	LABEL	BARCODE	LENGTH	WEIGHT	PARR MARKS	SILVER COLOURING	FIN MARGINS	COMMENTS	K-FACTOR	SMOLT INDEX	FRESHWATER COMPONENT	SEAWATER COMPONENT	CD-FACTOR
1	16, LV2004-197030	LV2004197030	251,0	182,1	4,0	3,0	3,0	Treatment 5	1,14	3,0	1,41	0,62	0,57
2	17, LV2004-197139	LV2004197139	253,0	199,1	4,0	3,0	2,6	Treatment 5	1,24	3,0	1,04	0,07	0,86
3	18, LV2004-197140	LV2004197140	255,0	216,1	4,0	2,6	2,6	Treatment 5	1,30	2,7	1,15	0,13	0,82
4	19, LV2004-197141	LV2004197141	258,0	227,4	4,0	2,6	2,6	Treatment 5	1,32	2,7	1,50	0,47	0,86

INDIVIDUAL ID	LABEL	BARCODE	LENGTH	WEIGHT	[illegible]	[illegible]	FIN [illegible]	COMMENTS	K-FACTOR	SMOLT INDEX	FRESHWATER COMPONENT	SEAWATER COMPONENT	[illegible]
5	20, [illegible]	[illegible]	[illegible]	[illegible]	4,0	[illegible]	[illegible]	Treatment [illegible]	1,28	2,7	[illegible]	[illegible]	[illegible]
6	21, [illegible]	[illegible]	[illegible]	[illegible]	4,0	[illegible]	[illegible]	Treatment L	1,12	3,0	0,90	[illegible]	[illegible]
7	22, [illegible]	[illegible]	[illegible]	[illegible]	4,0	[illegible]	4,0	Treatment L	1,10	3,7	0,77	[illegible]	[illegible]
8	23, [illegible]	[illegible]	281,0	214,0	4,0	[illegible]	[illegible]	Treatment L	0,95	[illegible]	[illegible]	[illegible]	[illegible]
9	24, [illegible]	[illegible]	[illegible]	214,2	4,0	[illegible]	[illegible]	Treatment L	1,15	3,0	1,10	[illegible]	[illegible]
10	25, [illegible]	[illegible]	287,0	[illegible]	4,0	[illegible]	[illegible]	Treatment L	1,18	3,0	[illegible]	[illegible]	[illegible]
11	26, [illegible]	[illegible]	[illegible]	165,1	4,0	2,0	1,0	Treatment C	1,32	[illegible]	0,74	0,04	[illegible]
12	27, [illegible]	[illegible]	240,0	[illegible]	4,0	2,0	2,0	Treatment C	1,18	3,7	[illegible]	[illegible]	[illegible]
13	28, [illegible]	[illegible]	226,0	[illegible]	[illegible]	2,0	1,0	Treatment C	1,20	3,0	[illegible]	[illegible]	[illegible]
14	29, [illegible]	[illegible]	[illegible]	145,7	[illegible]	2,0	2,0	Treatment C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
15	30, [illegible]	[illegible]	267,0	[illegible]	4,0	3,0	2,0	Treatment C	1,31	2,7	[illegible]	[illegible]	[illegible]
16	31, [illegible]	[illegible]	242,0	[illegible]	[illegible]	3,0	2,0	Treatment T	1,30	2,5	[illegible]	[illegible]	[illegible]
17	32, [illegible]	[illegible]	231,0	151,3	4,0	3,0	2,0	Treatment T	1,23	2,7	1,70	[illegible]	[illegible]
18	33, [illegible]	[illegible]	[illegible]	218,3	4,0	3,0	3,0	Treatment T	1,22	2,7	[illegible]	[illegible]	[illegible]
19	34, [illegible]	[illegible]	[illegible]	[illegible]	4,0	3,0	1,0	Treatment T	1,22	2,5	[illegible]	[illegible]	[illegible]
20	35, [illegible]	[illegible]	[illegible]	[illegible]	4,0	3,0	3,0	Treatment T	1,28	2,7	[illegible]	[illegible]	[illegible]
Min			[illegible]	[illegible]	[illegible]	[illegible]	1,0	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Max			[illegible]	[illegible]	4,0	[illegible]	4,0	-	[illegible]	3,7	[illegible]	[illegible]	[illegible]
Average			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	1,14	[illegible]	[illegible]
Median			[illegible]	214,1	[illegible]	[illegible]	[illegible]	-	[illegible]	3,7	[illegible]	[illegible]	[illegible]
Std. Dev.			[illegible]	[illegible]	[illegible]	[illegible]	0,7	-	[illegible]	0,4	[illegible]	0,17	[illegible]

-END OF REPORT-

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PA Diagnostics Terms of Business.

7.3 Appendix 3.

Operational Welfare Indicator

Nofima · INSTITUTE OF MARINE RESEARCH · Veterinærinstituttet Norwegian Veterinary Institute · NORD universitet · UNIVERSITY of STIRLING

FISHWELL Morphological Operational Welfare Indicators (OWI's) for farmed Atlantic salmon v1.1

Level 0: Little or no evidence of this OWI, i.e. normal (not illustrated).
Level 1, minor to Level 3, clear evidence of the OWI.

	Eye haemorrhaging	Exophthalmia	Opercular damage	Snout damage	Upper jaw deformity	Lower jaw deformity	Emaciation
1	Minor haemorrhages	Eye protruding a little	Operculum only partly covering gills	Minor wound on snout (either jaw)	Suspected malformation	Suspected malformation	Potentially emaciated
2	Larger haemorrhages, or traumatic injury	Moderate eye protrusion	Operculum absent on one of the gills (gill exposed)	Moderate wound and broken skin on snout	Distinct malformation	Distinct malformation	Emaciated
3	Large haemorrhages / traumatic injury. Eye may be ruptured	Major eye protrusion	Both opercula absent (both gills exposed)	Large deep and extensive wound. Can cover the whole head	Major malformation, jaw pointing backwards	Major malformation, jaw pointing backwards	Extremely emaciated

	Vertebral deformity	Skin haemorrhages	Lesions / wounds [a,b]	Scale loss	Sea lice infection	Healed fin damage	Active fin damage [c]
1	Signs of deformed spine	Minor haemorrhaging, often on the belly of the fish	One small wound (< 10 pence piece)[a], subcutaneous tissue intact (no muscle visible)	Loss of individual scales	Light infection	Most of the fin remaining	Most of the fin remaining
2	Clearly visible spinal deformity (e.g. short tail)	Large area of haemorrhaging, often coupled with scale loss	Several small wounds	Small areas of scale loss (< 10% of the fish)	0.05 - 0.08 pre-adult or adult lice cm² of fish skin	Half of the fin remaining	Half of the fin remaining
3	Extreme deformity	Significant bleeding, often with severe scale loss, wounds and skin edema	Large, severe wounds, muscle often exposed (≥ 10 pence piece)	Large areas of scale loss (≥ 10% of the fish)	≥ 0.08 pre-adult or adult lice cm² of fish skin	Very little of the fin remaining	Very little of the fin remaining

Figure: C. Noble, D. Izquierdo-Gomez, L. H. Stien, J. F. Turnbull, K. Gismervik, J. Nilsson. Photo: K. Gismervik, L. H. Stien, J. Nilsson, C. Noble, J. F. Turnbull, P. A. Sæther, I. K. Nerbøvik, I. Simon, B. Tørud, B. Kløvagg, R. Andersen, C. Karlsen, K. J. Merok, F. Gregersen

[a] For pre-smolts "one small wound" should be < 1 cm

[b] NB! Wounds that penetrate the abdominal cavity should be scored as a 3) irrespective of size

[c] Splitting and/or haemorrhaging

Welfare indicators for farmed Atlantic salmon: tools for assessing fish welfare

The FISHWELL scoring scheme for morphological OWI's for farmed Atlantic salmon is reproduced from the manual "Welfare indicators for farmed Atlantic salmon: tools for assessing fish welfare" written by researchers from Nofima, Institute of Marine Research, Norwegian Veterinary Institute, Nord University (all Norway) and the University of Stirling (UK).

This handbook suggests a unified scoring system that is primarily aimed at farmers to help them assess welfare and rapidly detect potential welfare problems out on the farm.

It is an amalgamation of the injury scoring schemes used in the Salmon Welfare Index Model (SWIM) (Stien et al., 2013), the injury scoring scheme developed by the Norwegian Veterinary Institute (NVI) (Grøntvedt et al., 2015, Gismervik et al., 2016) and also from other schemes developed by J. F. Turnbull (University of Stirling) and J. Kolarevic and C. Noble (Nofima).

The handbook standardises scoring for 14 different indicators to a 0-3 scoring system.

Pictures used in the system represent examples of each scoring category. We suggest dorsal, caudal and pectoral fins as the primary fins to monitor for fin damage. As a comprehensive system for the classification of vertebral deformities, similar to that in human medicine, has not yet been developed for Atlantic salmon, we suggest a simplified scoring system similar to that used in the RSPCA welfare standards for farmed Atlantic salmon (RSPCA, 2018).

For the full references for the four citations listed above, please refer to the FISHWELL handbook.

The PDFs of the manual and scoring scheme can be downloaded from www.nofima.no/fishwell/english.

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7.4 Appendix 4.

Blood Serum Heparin LEO used for centrifugation.

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