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# Assessment of Black Soldier Fly Larvae Meal as an Alternative Feed Ingredient for Atlantic Salmon (Salmo salar L.) and Rainbow Trout (Oncorhynchus mykiss): A Study on Physical, Chemical, and Sensory Quality

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Master of Science in Aquaculture

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Master thesis in aquaculture, 60 credits

by

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Zahra Yousefi Mojir

**Abstract**

The sustainability of the fish industry is crucial for ensuring food security and achieving global sustainable development goals. This has prompted the need to explore alternative and sustainable feed ingredients that can maintain or even improve the quality of fish products. This study evaluated the effect of the 4% inclusion of black soldier fly larvae (BSFL) into fish feed on the quality of farmed Atlantic salmon and rainbow trout fillets. The physicochemical and sensory properties of the fish were analysed and compared to the control group fed with a standard commercial diet.

The results showed that BSFL did not significantly affect the fillets' fat content and astaxanthin concentration. However, the Atlantic salmon fed with BSFL had firmer fillets and less gaping compared with the control group, while the rainbow trout test group tended to have a lower degree of gaping (P=0.11). In addition, the amino acid composition of the connective tissue differed significantly between the dietary groups of both species, with cysteine and glycine being significantly higher in the Atlantic salmon and proline being significantly higher in the rainbow trout. Sensory evaluation of cooked fillets by a consumer panel revealed significantly higher scores for the Atlantic salmon's firmness, juiciness, and general acceptancy and higher scores for the rainbow trout colour.

In conclusion, the findings suggest that incorporating BSFL into fish feed could be a sustainable and viable option for aquaculture without compromising the quality of the fish products.

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**Abbreviations**

AA= Amino Acid

Ala = Alanine

ALA = Alpha-Linolenic acid

ANF = Anti-nutritional Factor

Arg = Arginine

Asp = Aspartic acid

BSF = Black Soldier Fly

BSFL = Black Soldier Fly Larvae

BSFLM = Black Soldier Fly Larvae Meal

CT = Connective Tissue

Cys = Cysteine

DHA= Docosahexaenoic acid

EPA= Eicosapentaenoic acid

Glu = Glutamic acid

Gly = Glycine

His = Histidine

Ile = Isoleucine

Leu = Leucine

Lys = Lysine

Met = Methionine

NQC = Norwegian Quality Cut

Phe = Phenylalanine

Pro = Proline

Ser = Serine

Thr = Threonine

Trp = Tryptophan

Tyr = Tyrosine

Val = Valin

# 1. Introduction

The production of aquaculture has increased globally as a result of the rising demand for seafood. Fish meal and fish oil are two common ingredients of fish feed, but they are limited and the pursuit of substitute sources of protein for fish feed has therefore accelerated. Novel feed components such as insect meal have been researched recently to diversify and enhance the sustainability of fish feed in the aquaculture sector. Due to their high nutritional value, minimal environmental impact, and fast growth rates, insects have become a promising alternative to conventional protein sources. One of these insects with high potential as a component of fish feed is the black soldier fly (BSF) (*Hermetia illucens*) (Oonincx et al., 2010; Sørensen et al., 2011; Weththasinghe, 2021)

The meal made of black soldier fly (BSF) larvae is high in lipids (up to 48%) and protein content (39%), with a good amino acids profile (Weththasinghe et al., 2021). It has been demonstrated that BSF is a suitable substitute in the diet of various fish species, including Atlantic salmon and rainbow trout. The use of BSF larvae meal in fish feed can also have positive environmental impacts as it has been demonstrated. Farming of BSF can be a sustainable practice that reduces waste and greenhouse gas emissions (Oonincx et al., 2010). Additionally, incorporating alternative protein sources like black soldier fly larvae meal in fish feed can reduce pressure on wild fish populations and promote more responsible aquaculture practices.

The quality of the fish fillets is a crucial factor in determining consumer acceptance and the marketability of fish products, so it is necessary to look into the BSF potential effects on the final product's quality. Although the nutritional benefits of BSF larvae meal are promising, any modifications to the physicochemical and sensory characteristics of fillets brought on by adding feed ingredients may alter consumer preference and, in turn, impact the product's marketability. Therefore, it is crucial to investigate whether the novel feed ingredients may have an impact on the quality of the final product.

The aim of this study is to investigate the potential of using black soldier fly larvae (BSFL) (*Hermetia illucens*) as a component in fish feed and its effect on the quality of fish fillets. Specifically, this study will assess the sensory and physicochemical properties of Atlantic salmon and rainbow trout fed with diets containing 4 % of black soldier fly larvae meal. I aimed to answer the following questions:

1. How does inclusion of BSFL meal in the diet of Atlantic salmon and rainbow trout affect the physical quality of fish fillets?
2. How does inclusion of BSFL meal in the diet of Atlantic salmon and rainbow trout affect the chemical composition of fish fillets?
3. How does inclusion of BSFL meal in the diet of Atlantic salmon and rainbow trout affect the sensory quality of cooked fish fillets?

The hypothesis for both species is that the inclusion of 4% black soldier fly larvae meal in the diets would have a positive effect on the quality properties of the fillets as shown in the table below.

*Table 1. The hypotheses for the effect of 4% black soldier fly larvae meal inclusion in the diets of Atlantic salmon and rainbow trout on the physical, chemical, and sensory quality of fillets.*

| **Hypotheses** | **Atlantic salmon** | **Rainbow trout** |
|---|---|---|
| Physical quality: positive effect | ☐ | ☐ |
| Chemical composition: positive effect | ☐ | ☐ |
| Sensory quality: positive effect | ☐ | ☐ |

# 2. Theoretical background

## 2.1 Feed ingredient

More than half of the cost of fish production is allotted to feeds to optimize feed formulation and manufacturing. It's essential to focus on precise formulations and practical considerations (Hardy & Brezas, 2022). One of the most critical parts of the aquaculture industry is feed, which can significantly impact this industry. Providing feed with the best effect on fish and at a reasonable price was always the biggest concern of the fish industry. Suitable ingredients are a vital aspect of producing feed, which needs to be available on a commercial scale and be sustainable enough. Fishmeal and fish oil are one of the most suitable sources of feed for aquaculture and have been used for many decades; 90% of the ingredients in the feed for Norwegian Atlantic salmon were marine-derived in 1990 (Ytrestøyl et al., 2015) but their use in aquafeeds is now restricted because of the depletion of wild forage fish, high market prices, conflicts over the use of resources, and sustainability issues (Tacon & Metian, 2008; Weththasinghe, 2021). Over time, there has been a shift towards using more plant ingredients instead of marine ingredients, as of 2020, the average Norwegian salmon feed contained 40.5% plant protein sources (Synnøve et al., 2022). However, the presence of antinutritive factors (ANFs) (Krogdahl et al., 2010), conflicts with the consumption of human food (Ytrestøyl et al., 2015) and environmental concerns limited their application in the feed industry. An insufficient supply of sustainable protein and lipid sources for aquafeeds has become a challenge to the aquaculture industry. The feed industry has considered different novel ingredients in recent years, whereas insects have received great attention.

### 2.1.1 Black soldier fly larvae (Hermetia illucens)

Insects have been introduced as a significant source of sustainable raw materials for aquafeeds (Weththasinghe, 2021), especially as an alternative ingredient in feed for carnivorous fish (Sørensen et al., 2011). The production of insects is advantageous for the environment due to the reduction of greenhouse gas emissions (Oonincx et al., 2010) and lower usage of land and water compared to other feed ingredients, such as plant based.

The ability to convert organic waste to high-value nutrients was made insects an efficient choice as feed ingredients for aquatic animals. However, their production volume needs to be increased to compete with traditional feed sources and improve price competitiveness

(Weththasinghe, 2021). In 2017 insects as feed ingredients were approved by the European Commission (Regulation 2017/893/EC, 2017). Among the seven species approved, the black soldier fly (*Hermetia illucens*) has gained recognition as a feed source of high potential due to its significant nutritional content, ability to efficiently convert a range of organic matter, and compatibility for large-scale production (Weththasinghe, 2021).

### 2.1.2 Black soldiers fly as feed meal

The three significant fractions in the BSF are protein, lipid, and chitin (Caligiani et al., 2018). The lipid content was recorded from 8 to 48% (dry matter basis) (Weththasinghe, 2021). BSFL have a different fatty acid composition compared to other insects, such as yellow mealworms and house crickets. The quantity of fat and composition of fatty acids in BSFL depend on the substrate used for them and can be vary based on the substrate material (Ghimire, 2021; Makkar et al., 2014). The BSFL is rich in saturated fatty acids and can be used as a primary source of saturated fatty acids and a partial source of mono-unsaturated and polyunsaturated fatty acids for the aquafeed formulation, thus saving fish oil (Li et al., 2022). Compared to fish oil, the lipid fraction of BSFL has very low levels of polyunsaturated fatty acids, especially omega-3 and omega-6 fatty acids. Despite the fact that BSFL has a very low concentration of the beneficial omega-3 fatty acid, by feeding omega-3 rich diets to BSF, it is possible to enrich the diets with omega-3 fatty acids, making BSFL a suitable substitute for fishmeal and fish oil (Weththasinghe, 2021).

Earlier studies demonstrated that 50% of fish meal in the Atlantic salmon diet (Ewald et al., 2020; Lock et al., 2016) and 25% in the rainbow trout's diet (Ewald et al., 2020; Hilaire et al., 2007) could be substituted with BSFLM without any negative effects on their growth. However, such substitution reduces the content of a-linolenic acid (ALA; C18:3), eicosapentaenoic acid (EPA; C20:5), and docosahexaenoic acid (DHA; C22:6) in fish fillets, which are essential for excellent growth and reproduction in fish and shrimps and consumption benefits for humans. Thus, lower n-3 fatty acid concentrations in fish may be a problem for both producers and consumers (Ewald et al., 2020). Belghit et al. (2018), investigated the effect of Insect meal and insect oil on Atlantic salmon and concluded that BSFLM as an ingredient in the fish feed had no negative effect on feed intake or feed conversion ratio (Belghit et al., 2018).

The BSFL contains protein ranging from 31% to 59% (dry matter basis) (Weththasinghe et al., 2021). Based on the amino acid profile, BSFL has the essential and non-essential amino acids

(AA) required for Atlantic salmon and rainbow trout, except essential AAs methionine and lysine (Belghit et al., 2019). Several studies have shown that BSFL holds great potential as a source of nutrients for rainbow trout (Dumas et al., 2018; Hossain et al., 2021; Renna et al., 2017). According to Renna et al. (2017) substituting 20% and 40% of a partially defatted BSFL in rainbow trout diets had no negative effects on growth (Renna et al., 2017).

Chitin is the primary structural polysaccharide of the arthropod exoskeleton of the insect. There is much debate about the effect of chitin. According to reports, the structural form of chitin reduces the bioavailability of protein and lipids in mice and poultry by preventing nutrient absorption from the intestinal tract. Chitin makes up 5-25% of whole insects, and the exoskeletons of BSFL contain 35% of all chitins. It is still unclear whether chitin serves as an anti-nutrients factor or has prebiotic properties (Albrektsen et al., 2022). Although it is reported that the presence of chitin in BSFL meal may reduce growth, feed intake, nutrient bioavailability (Belghit et al., 2018) and decreased protein digestibility (Renna et al., 2017).

## 2.2 Sensory properties of raw fillet

### 2.2.1 Colour

Colour is one of the fundamental parameters to determine the quality of food. The evaluation of this sensory parameter is essential since it is the first visually evaluated parameter that consumers can estimate, and it can affect consumer preference. One of the characteristics that can identify Atlantic salmon from other species is the colour of the flesh, and it is the first parameter used to infer the quality of Atlantic salmon products (Alfnes et al., 2006). Atlantic salmon is known for its unique reddish-orange colour (Hardy & Lee, 2010), while fillets with pale colours will be rejected by consumers and considered a downgrade fillet in the industry. Customers have remarked that they consider Atlantic salmon's colour to be a reflection of its flavour and freshness, and research has proven that Atlantic salmon's redness greatly enhances the overall enjoyment of the fish (Alfnes et al., 2006).

The unique colour of the flesh in salmonids is due to dietary carotenoids with astaxanthin being the primary carotenoid in wild salmonids. To achieve the desired colouration of the flesh in farmed salmonids, carotenoids such as astaxanthin must be added to the feed as fish are unable to synthesize astaxanthin themselves (Hardy & Lee, 2010; Torrissen, 1989; Xiaoxiao, 2020). Target pigmentation level in Atlantic salmon fillets should be equal to or greater than 4 µg/g wet tissue to satisfy customer expectations of fillet colour. Greater pigmentation levels can be attained; however, as the human eye cannot perceive higher pigmentation levels, higher fillet carotenoid levels do not provide any additional value (Hardy & Lee, 2010).

Astaxanthin concentration varies from season to season. Atlantic salmon fillet pigment levels have decreased recently, according to the Norwegian Seafood Research Fund, and some fish farms claimed that they recorded the lowest pigment levels in Atlantic salmon in 2020 (Kraugerud, 2021).

As an antioxidant, astaxanthin is also essential for a well-functioning immune system and for reproduction. Until fish reach sexual maturity, astaxanthin slowly builds up and deposits in the flesh. Additionally, a relatively higher growth rate in the autumn would result in flesh with less pigmentation. To ensure the best quality of Atlantic salmon fillets throughout the year, it is recommended to harvest them before they reach sexual maturity, when the colour of the fillets is at its peak. (Xiaoxiao, 2020).

### 2.2.2 Gaping

When it comes to fish quality, many different characteristics come into consideration, but one of the main parameters is gaping (Jacobsen et al., 2017; Jacobsen et al., 2019). Fish fillets consist of muscle blocks or myotomes separated by thin membranes of connective tissue (CT). Gaping is a term used to define the undesirable separation of muscle blocks in a raw fillet. Gaping can be defined as the breakdown of the CT between muscle segments (Jacobsen et al., 2019). Normally it will be observed as holes or fractures in uncooked fish filets. Gaping can involve anything from a minimal separation at the cut surface to a complete slicing of the fillets into pieces (Kiessling et al., 2006). A fillet with gaping is weakened and failing to maintain the integrity of the muscle myotomes and can fall apart with ease. Moreover, gaping can cause an unpleasant appearance of fillets and cause technical problems for slicer machines, so the fillets with gaping are often downgraded and sold-out cheaper product. In addition, fish fillets with gaping have a high risk of being rejected by consumers due to their unappealing appearance (Jacobsen et al., 2019; Lu, 2017).

Different factors such as fish size, fish age, stress and protein content of the fish can affect firmness (Wang, 2016). An important cause of gaping and soft fillets is stress caused by handling before harvesting and during the process. In addition, it can cause an acidic condition in muscles, increasing the activity of Cathepsin L in the muscle, leading to degraded collagen, which can soften the fillet texture (Jacobsen et al., 2017).

### 2.2.3 Texture

Firm texture is an important criteria of fish fillets quality, which is considered a sensory characteristic for the consumer and has a critical impact on the mechanical processing of fillets. In general, customers frequently prefer salmonids with a firm texture and high juiciness instead of dry fillets. Fillets with softer textures will be downgraded (Kiessling et al., 2006; Mørkøre & Austreng, 2004), which results in as much as a 40% loss in value (Ageeva et al., 2018).

The texture of fish can be influenced by various factors, including physical factors like species, age, size, and feeding ingredients, as well as chemical composition, structural properties, and collagen content and composition (Moreno et al., 2012). These changes can occur in both myofibrillar and CT proteins, forming a supportive network throughout the fish muscle (Kiessling et al., 2006). Apart from these, the storage time and temperature of fish or secondary processes such as freezing, chilling, high-pressure processing (HPP), salting, and smoking can have a significant impact on the texture of fish fillets (Cheng et al., 2014; Moreno et al., 2012)

Fish have shorter muscle fibres and less CT compared to terrestrial animals. Fish muscles are divided into sections (myotomes) by thin layers of CT called myocommata or myosepta. These layers act like sheets that separate muscle fibres into layers (Ryu et al., 2021; Sikorski et al., 1984). The myotomes compromise muscle fibres which are surrounded by a cell wall known as the basement membrane containing thin collagen fibrils. The muscle fibres' length and diameter decrease towards the fillet's tail end. The collagen concentration in a fish fillet is higher near the tail end because of the weight ratio of the myocommata to the myotomes, which is higher near the tail than the head (Sikorski et al., 1984). The CT of fish muscles contains more collagen than any other protein, with type I collagen making up most of it. Reducible and non-reducible cross-links between adjacent collagen and elastin molecules impact the CT's mechanical strength (Moreno et al., 2016). Fish muscles are typically softer than land animals, and the main reason is a lower amount of collagen. The average amount of CT in land animals is 15% by weight (Acharya, 2012), while 3% to 10% of the protein in the CT of fish muscle consists of collagen (Cheng et al., 2014).

### 2.2.4 Collagen

The most prevalent protein in intramuscular CT is collagen. Different factors, such as fish species, the life stage of fish and diet, can affect the amount and distribution of collagen in fish muscle (Lin et al., 2022; Sikorski et al., 1984). Zhao et al. (2018) investigate how different diets affect the growth characteristics and muscle composition of juvenile grass carp (*Ctenopharyngodon idellus*). In the study, the effects of artificial feed and grass (a natural food source for grass carp) on grass carp growth and muscle quality was compared. The study demonstrated that the group fed with grass had a higher muscle protein content, a lower fat content, a higher growth rate, and a better feed conversion ratio (Zhao et al., 2018). In addition, Yu et al. (2019) found that diet enriched with Faba bean can increase the expression of type I collagen in the muscle tissue of Grass Carp (Yu et al., 2019).

Collagen is essential for both muscle development and mechanical properties (the stability and integrity of the muscle structure). Collagen plays a significant role in determining the quality of the flesh, particularly in texture characteristics like hardness, springiness, and chewiness (Lin et al., 2022). Moreno et al. in 2012 investigated the collagen characteristics between two different textures (firm and soft) of farmed Atlantic salmon. Comparing the firm texture to the soft texture showed that the firm texture was associated with higher collagen content, a higher level of collagen cross-linking, and a higher solubility of collagen in acid (Moreno et al., 2012).

Collagen is made up of different types, but the ones found in muscle are mostly type I collagen. Collagen consists of three alpha-polypeptide chains that twist together to make a strong, triple-stranded helix. The chains contain sequences of amino acids called "Gly-X-Y," with "X" and "Y" usually being proline and hydroxyproline, respectively. Glycine is an essential component of the collagen triple helix structure, providing flexibility and stability to the protein. Glycine comprises about one-third of the amino acid residues in collagen. Its small size allows it to fit well in the tight space between the collagen helices, facilitating proper collagen formation. Hydroxyproline is important for keeping the helix stable and is formed by modifying the proline after the collagen chain is built (Moreno et al., 2012). In addition to glycine, proline and hydroxyproline, cysteine and lysine are also believed to play a crucial role in the structure of collagen. Cysteine contributes to the formation of disulphide bonds between collagen fibres, enhancing collagen's strength and stability (DiChiara et al., 2018). Lysine is important for the crosslinking of collagen fibres, which further adds to collagen strength (Moreno et al., 2012).

According to Subhan et al. (2015), fish collagen has a unique composition of amino acids with a lower concentration of glycine, proline, and hydroxyproline, resulting in a lower denaturing temperature, but marine collagen contains a higher concentration of serine and threonine compared with mammalian collagen (Subhan et al., 2021).

### 2.2.5 Thermal stability of collagen

Differential scanning calorimetry (DSC) used to measure the thermal denaturation of collagen in fish fillets, as it provided information about the degree of collagen cross-linking and its thermal stability. DSC Measures heat change during thermal denaturation of biomolecule samples. DSC is used to determine the melting temperature of proteins. Protein stability increases with higher thermal transition temperature. DSC calculates the enthalpy of the melting and mesomorphic transitions (Singh & Singh, 2022). The DSC measurement curve exhibits a peak, and the area under this peak is proportional to the enthalpy associated with the process.

DSC procedure includes a sample with a specific mass being heated with an empty reference sample under a constant rise in temperature. Endothermic or exothermic processes may occur during the warm-up period, which leads to increasing or decreasing amount of heat flowing to or from the sample compared to the reference. For example, melting is an endothermic process that absorbs heat, keeping a sample at the same temperature as the reference takes more heat flow. Similarly, less heat flow is necessary since the sample emits heat during an exothermic process like polymerization (Rokvam, 2013).

### 2.2.6 Fat content

Fillet fat content affects texture, flavour, and colour. The fat content can vary depending on various factors such as species, diet, the amount of feeding and season. Atlantic salmon is considered as a fatty fish species with 17-19% lipid content on average (Mørkøre & Rørvik, 2001). Fat content varies among fish within population of farmed salmon (12-22%), fat content also can be different within fillet, being lower in the tail and the highest in the belly flap area (Mørkøre et al., 2001).

Triglycerides and phospholipids are the two main groups of lipids found in fish skeletal muscle. Triglycerides are lipids that are used to store energy in fat depots, whereas phospholipids are crucial for the structure of cell membranes (structural lipids) (Acharya, 2012). Fatty acids are carbon chains with a carboxyl group on one end and a methyl group on the other. Fatty acids can be divided into three groups based on the degree of saturation; saturated (with no double bond), monounsaturated (with one double bond) and polyunsaturated (with two or more double bonds) (Mizambwa, 2017). Fish consumption has been linked to a reduced likelihood of cardiovascular illnesses because of the presence of n-3 fatty acids (Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n3) (Méndez et al., 1996).

The lipid content and composition of the diets consumed by salmonids can affect the fatty acid composition of their tissue. There is less understanding, however, about the effects of the total amount of dietary lipids on the distribution of fat in the tissues and sensory quality. Nevertheless, Johansson et al. (1991) found that the fillet's juiciness increased with increasing fat content in rainbow trout fillets (Johansson et al., 1991).

### 2.3 Sensory assessment of cooked fillet

In sensory analysis, the quality and organoleptic properties of food products are assessed using senses of sight, taste, smell, touch, and hearing (Ruiz-Capillas & Herrero, 2021). Among fish quality attributes, colour, flavour, and odour can be modified by changing feed formulation. For example It has been reported that replacing plant protein with a fish meal in rainbow trout feed causes a less sweet taste and lower odour intensity (Francesco et al., 2004; Hardy & Lee, 2010). However, the texture is less easily modified in fish (Hardy & Lee, 2010). It has been observed that the content of collagen and lipid in muscle can affect the texture.

With the help of sensory analysis, it is possible to comprehend consumer preferences, create new products, and increase the quality of available products in the market. In order to offer objective data on the sensory qualities of food products, trained panellists or consumers are often involved in sensory analysis.

Sensory panellists are trained to assess a food product's sensory properties using standardized procedures and techniques. The panellist provides objective data on the sensory properties of food products. A trained panel evaluates products intending to identify and express the product's sensory details without assigning a personal value to the outcome. A trained panel can accurately identify and describe the sensory attributes of a food product according to an objective scale. Consumer panels assess products to provide data on their perception and emotional value. The panel typically consist of a group of consumers who represent the target market and assess consumer acceptance of or preference for food.

Hedonic testing is an example of the method often used. This method involves evaluating food products based on how much consumers like or dislike the food. Participants are asked to rate their overall liking of a product on a scale ranging from score 1 (the lowest intensity of each attribute) to score 5 (the highest intensity) (Berdos et al., 2021).

# 3. Material and methods

## 3.1 Biometric traits

In October 2022, the Atlantic salmon and rainbow trout biometric traits were meticulously measured during a single sampling event. Comprehensive information regarding the measurements of their body weight, length, gutted weight, and fillet weight were recorded. Platform scale was used to determine fish's body weight, gutted and fillet weight. Additionally, based on the collected data, slaughter yield, fillet yield, and condition factor were calculated using the following formulas.

$$Slaughter\ Yield\ (\%) = \frac{Gutted\ weight\ (g)}{Body\ weight\ (g)} \times 100$$

$$Fillet\ Yield\ (\%) = \frac{Fillet\ weight\ (g)}{Body\ weight\ (g)} \times 100$$

$$Condition\ factor\ (\%) = \frac{Round\ body\ weight\ (g)}{Body\ length\ (cm)^3} \times 100$$

## 3.2 Fish feed

In this experiment, fish were fed two different diets, commercial standard feed, which is indicated as the control diet, and the same commercial standard feed with 4% inclusion of black soldier fly (*Hermetia illucens*) larvae meal, indicated as test diet. During the experiment, the fish were fed with control diets or test diets for the entire production cycle in seawater. The feeds used in the experiment were produced by Aller Aqua. The specific control feeds used for Atlantic salmon and rainbow trout were named 'Atlantic' and 'Nordic proof ', respectively. The test feed was prepared using black soldier fly larvae (BSFL) at a rate of 4% by weight. The BSFL were defatted and ground into meal by the raw material producer.

The feeds were formulated based on the nutritional need of the fish. The feed for Atlantic salmon had a protein-to-fat ratio of 37:34 (9 mm pellet), while the feed for rainbow trout had a protein-to-fat ratio of 38:32 (9 mm pellet). Table 2 and 3 provide the composition of the feed used for the Atlantic salmon and the rainbow trout, respectively.

*Table 2. Composition of commercial feed and commercial feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens) fed to Atlantic salmon.*

| **Diet** | **Control (Atlantic)** | **Test (Atlantic BSFL)** |
|---|---|---|
| Dry matter % | 93.4 | 93.9 |
| Ash % | 7.6 | 8.0 |
| Starch % | 9.6 | 9.5 |
| Energy MJ/kg | 25.6 | 24.5 |
| Astaxanthin mg/kg | 40 | 38 |
| Protein % | 36.2 | 37.1 |
| Fat content % | 33.8 | 32.6 |

*Table 3. Composition of commercial feed and commercial feed with 4% inclusion black soldier fly larvae meal (Hermetia illucens) fed to rainbow trout.*

| **Diet** | **Control (Nordic proof)** | **Test (Nordic proof BSFL)** |
|---|---|---|
| Dry matter % | 92.8 | 92.8 |
| Ash % | 8.6 | 8.1 |
| Starch % | 10.5 | 9.7 |
| Energy MJ/kg | 23.8 | 24.3 |
| Astaxanthin mg/kg | 40 | 30 |
| Protein % | 37.7 | 36.8 |
| Fat content % | 30.9 | 31.7 |

## 3.3 Fish materials

The experiment consisted of two salmonid species, including Atlantic salmon (*Salmo salar L.*) and rainbow trout (*Oncorhynchus mykiss*). Atlantic salmon was farmed at Klammerholmen by Austevoll Melaks, and rainbow trout was farmed at Leiholmane by Firda Seafood (figure 1&2).

*Figure 1.Map of Norway. The pin mark is the location of Austevoll Melaks at Klammerholmen in the municipality Austevoll*

*Figure 2. Map of Norway. The pin mark is the location of Firda Seafood at Leiholmane in the municipality of Gulen.*

The study involved a total of six net pens of Atlantic salmon, where three net pens were fed with the test diet and three net pens were fed with the control diet. Five net pens of rainbow trout were used, where of three net pens were fed with the test diet and two with the control diet. In total, 48 Atlantic salmon and 40 rainbow trout were sampled including eight fish from each net pen. Fish were harvested, bled, gutted, and packed on ice in styrofoam boxes before being sent to NMBU in Ås, Norway.

## 3.4 Fillet quality analyses

In this study, various physical quality parameters of fish fillet such as colour, texture, and gaping, were analysed.

### 3.4.1 Colour

Visual colour measuring was used to evaluate the colour of fish fillets. The SalmoFan™ colour measurement scale by DSM was used for comparing the fillet; this scale is designed for salmonid fillets (figure 3).

The visual colour was evaluated by the fillets under standard lighting in a box within a controlled environment to reduce the effect of varying light conditions and reflective surfaces. The colour measurement was performed above the midline of the Norwegian quality cut (NQC), which is the cutlet between the posterior part of the dorsal fin and the gut. The SalmoFan scores from 20 to 34, which 20 referring to the palest colour and the most intense red colour scoring 34.


*Figure 3.SalmoFan by DSM*

### 3.4.2 Gaping

Degree of fillet gaping was assessed by considering the number and size of slits in the fillet were used to determine the gaping (figure 4). This method involved sliding a flat palm under the fillet to look for splits and holes after filleting. The number and size of the holes in the fillet are raging on a scale of 0 to 5 based (Andersen et al., 1994). Gaping was scored using a scale where fillets with no slits or holes receive a score of 0, fillets with fewer than 5 small slits (< 2 cm) receive a score of 1, fillets with less than 10 small slits receive a score of 2, fillets with more than 10 small slits or some large slits (>2 cm) receive a score of 3, while fillets with severe gaping and fillets that fall apart or have extreme gaping receive a score of 4 or 5, respectively (table 4).

*Table 4.Scale used to classify fillet gaping (Andersen et al., 1994)*

| Score | Description |
|---|---|
| **0** | No gaping |
| **1** | Few small slits < 5 |
| **2** | Some small slits < 10 |
| **3** | Many slits >10 or a few large slits(>2cm) |
| **4** | Severe gaping (Many large slits) |
| **5** | Extreme gaping (the fillet falls apart) |

Small slits < 2 cm
Large slits > 2 cm


*Figure 4. Gaping in fillets of rainbow trout*

### 3.4.3 Texture analyses

In this experiment, raw fillet texture was analysed with objective mechanical method based on Mørkøre & Einen 2006. In this method, a texture analyser containing an arm moved down to penetrate or compressed the product, and the fillet's resistance force or break point was measured. The raw fillet analyses were conducted using a TA-XT2; (stable Micro Systems Ltd., Surrey, England) equipped with a flat-ended cylinder probe (12.5 mm diameter, type P/0.5) pressed into the fillets at a constant rate of 1 mm/s. The trigger force for the texture analyser was 0.2 N, and it had a 5 kg load cell (Mørkøre & Einen, 2006). A computer recorded the breaking force and the total area (N*S) under the force-time graphs served as a measure of the firmness of the fillet. The region of fillet used for the analysis, was the fillet area below the dorsal fin, above the lateral line (figure 5).

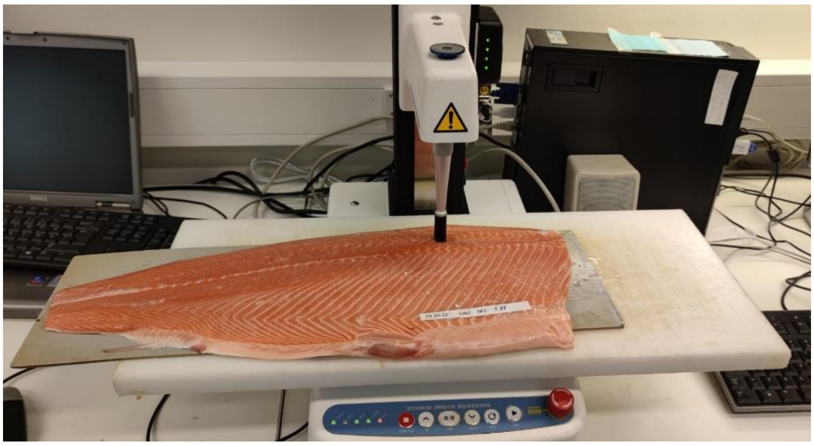


*Figure 5.Texture analyser*

## 3.5 Chemical analyses

In this study, various chemical quality parameters of fish fillet such as fat content, astaxanthin content, amino acids profile of CT, and thermal behaviour of CT were analysed.

### 3.5.1 Fat content

Fat content analysis was carried out at LabTek (Analysis lab for livestock and aquaculture) at NMBU based on Commission Regulation (EC) No 152/2009. The analysis was conducted by a fully automated system, Soxtec™ 8000. Fat was extracted using petroleum ether as a solvent of the Soxhlet method. The sample was weight into cellulose thimbles (33x80 mm) and submerged in the solvent (40-60 °C). After that, the extract was transferred from the cellulose thimbles to aluminium cups and put in a drying cabinet at 103 °C for 30 minutes to let the solvent evaporate. After drying, the resulting fat residue was quantified gravimetrically.

### 3.5.2 Astaxanthin

Astaxanthin analysis was carried out at LabTek (Analysis lab for livestock and aquaculture) at NMBU using the "CEN/TS 16233-1:2011 (E) - HPLC method for the determination of xanthophylls in fish flesh. Part 1: Determination of astaxanthin and canthaxanthin ". The analysis began with weighing approximately 1.5 mg of homogenized fish flesh and 1 g of BHT (2,6-Di-tert-butyl-p-cresol) into a volumetric flask, 5 mL of tetrahydrofuran was added, and the flask was filled to the mark with tetrahydrofuran. 10 mL of this solution was transferred to a 100 mL volumetric flask, and 85 mL of heptane was added. The analysis was performed using an Ultimate 3000 UHPLC system with a UV detector (Thermo Scientific), which measured the concentration of astaxanthin.

### 3.5.3 Amino acid analysis of connective tissue

Connective tissue from fish muscles was isolated according to the method described by Moreno et al. (2012), with slight modifications. The first step included homogenization. The samples were homogenized for 15 seconds using the Robot 600W blender by Taurus with NaCl 8‰ (8 per 1000) nearby. The same blender with the same speed was used for all the samples. In order to keep the temperature as low as possible (0-5 °C), all the steps were done in the vicinity of ice. The resulting solution was filtrated with a strainer, and the mixture was washed with consecutive rinses of cold tap water. This process was repeated several times until whitish CT was obtained. In the next step, in order to remove as much fat as possible, the solution of 1:10 butanol: water (v:v) was added to the CT for two hours. The resulting CT was rewashed with distilled water and then manually dried by pressing the paper filter. Finally, the CT was stabilized at -80 °C (Moreno et al., 2012) (Appendix 8.1).

The next step was to analyse the amino acid profile using HPLC. In order to perform the analysis, the samples were carefully weighed and hydrolysed using constant boiling 6 N HCl that contained 0.1% phenol. Norleucine (Sigma-Aldrich, Inc.) was used as an internal standard to ensure accuracy. After hydrolysis, the samples were vacuum-dried and then dissolved in an application buffer before being injected into a Biochrom 30+ Amino Acid Analyser (Biochrom, Kaysville, USA).

The selection of amino acids for this study was made with a specific focus on those that are known to play a critical role in the structure of collagen, as collagen is a key component of fish fillet texture under investigation in this master thesis.

### 3.5.4 Differential Scanning Calorimeter

A differential scanning calorimeter (DSC Q1000, TA Instruments, New Castle, USA) was used to determine the CT's thermal behaviour according to the method described by Moreno et al., (2012). An electronic balance determined the sample's weight. Samples were placed in aluminium pans, which were sealed hermetically. A drop of 8‰-NaCl solution was added to samples to prevent the collagen from melting due to a lack of water. Under a dry nitrogen purge at a 50 mL/min rate, the samples were scanned in two replicates at a rate of 10 °C/min from 5 to 90 °C. After cooling to 5 °C with a rate of 30 °C/min, second scans were taken to look for any residual/new effects. To normalize thermal data to dry matter content, the water content of each individually encapsulated sample was calculated using desiccation at 105 °C. Temperature, Tpeak (°C), and enthalpy of transition DH (J/gdm) data were recorded (Moreno et al., 2012).

### 3.6 Sensory analysis of cooked fillet

A consumer evaluation of the Atlantic salmon and rainbow trout samples was conducted by untrained participants, 50 participants evaluated the Atlantic salmon samples, while 30 participants evaluated the rainbow trout samples. All consumer panel members were volunteer students and staff at the Norwegian University of Life Sciences (NMBU).

The consumer sensory assessment was conducted using the selected part of fillet shown in figure 6. The fillet pieces were cut into 2 cm × 2 cm at the filleting time, placed in vacuum plastic bags, and stored at -20 °C until sensory analysis. The fillet pieces were thawed at room temperature for four hours. The thawed fish pieces were soaked in a 5% salt solution for 15 minutes. Fish were cooked in a preheated oven with a fan for 12 minutes at 175 °C. Since there were two dietary treatments, a triangle test was decided to perform for each fish species. Participants received three fish fillet samples on a clean plastic plate, consisting of either two pieces of the control group or one piece of the test group, or vice versa (figure 7).

Each plate was marked with specific 3-digit codes, and each fish piece was marked with letters A to C to distinguish between the samples. It was offered to have some sips of water and a small bite of cracker between samples to cleanse the palate in order to ensure the smell and taste of one sample don't affect the next one.

Each participant completes a questionnaire regarding colour, odour, tastiness, firmness, juiciness, and general acceptability of the samples based on a categorical scale from 1- 5, shown in table 5. Details such as age, gender, nationality, occupation, and frequency of fish consumption were asked in the questionary (Appendix 8.3). During tasting and completing the questionnaire, communication between assessors was avoided.


*Figure 6. The selected portion of the fillet (rainbow trout) for sensory evaluation of quality attribute*

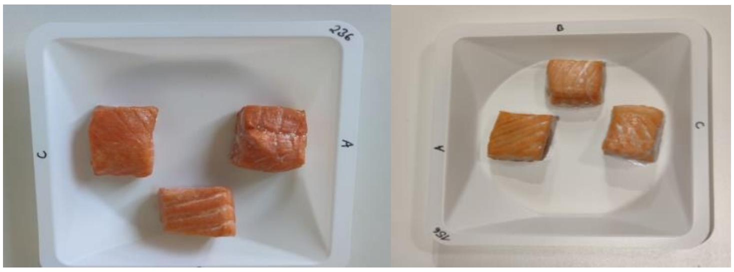


*Figure 7. Visual representation of the fillet samples used in sensory evaluation, Rainbow trout (left) and Atlantic salmon (right).*

*Table 5.Evaluation criteria employed by the consumer panel for assessing quality attributes of cooked fish fillets of Atlantic salmon and rainbow trout.*

| **Parameters** | | | **Scores** | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| **Colour** | Dislike | Acceptable | Likes slightly | Good | Excellent |
| **Odour** | Dislike | Acceptable | Likes slightly | Good | Excellent |
| **Tastiness** | Dislike | Acceptable | Likes slightly | Good | Excellent |
| **Firmness** | Soft | Acceptable | Medium | Good | Excellent |
| **Juiciness** | Dry | Acceptable | Medium | Good | Excellent |
| **General Acceptability** | Dislike | Acceptable | Likes slightly | Good | Excellent |

## 3.7 Statistical analyses

Statistical analyses were performed using ANOVA with the SAS software package (SAS Institute, Cary, NC, USA; version 9.4). The test aimed to investigate the effect of dietary treatment for Atlantic salmon, and rainbow trout. The quality parameters were adjusted for body weight and gender in the statistical analyses when deemed significant. For the significant differences among means (LSMeans) of dietary treatments, pdiff and Duncan's multiple range test were used for ranking, with a level of significance set to 5% ($P \leq 0.05$).

# 4. Results

## 4.1 Biometric traits

The biometric traits of the Atlantic salmon and the rainbow trout fed dietary inclusion of 4 % BSFL meal (test group), or a control diets (control group) were measured and recorded to determine the physical characteristics and yields of the fish. The collected data are presented in table 6.

The biometric traits of the Atlantic salmon, including whole body weight, length, fillet weight, fillet yield, and condition factor, were numerically higher of the test group compared to the control group, although the differences were not statistically significant. However, the gutted weight and the slaughter yield of the test group were significantly higher (P<0.05) compared with the control group.

Regarding the rainbow trout, the biometric traits including body weight, length, gutted weight, and fillet weight were found to be significantly higher of the control group compared to the test group (P<0.05).

*Table 6. whole body weight, gutted weight, fillet weight, body length, condition factor, slaughter yield, and fillet yield of Atlantic salmon and rainbow trout fed commercial feed or commercial feed with 4% inclusion of defatted black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences between the groups (P≤0.05).*

| Biometric traits | Atlantic salmon | | Rainbow trout | |
|---|---|---|---|---|
| | **Control** | **Test** | **Control** | **Test** |
| **Fish length (cm)** | $69.8 \pm 0.8^{a}$ | $71.7 \pm 0.8^{a}$ | $61.7 \pm 1.2^{b}$ | $58.1 \pm 0.8^{c}$ |
| **Body weight (g)** | $4260 \pm 153^{a}$ | $4669 \pm 210^{a}$ | $4193 \pm 253^{a}$ | $3634 \pm 146^{b}$ |
| **Gutted weight (g)** | $3758 \pm 138^{b}$ | $4226 \pm 188^{a}$ | $3618 \pm 210^{b}$ | $3070 \pm 117^{c}$ |
| **Fillet weight (g)** | $1290 \pm 49^{a}$ | $1438 \pm 72^{a}$ | $1302 \pm 76^{a}$ | $1099 \pm 47^{b}$ |
| **Calculated traits** | | | | |
| **Slaugther yield (%)** | $88.3 \pm 0.8^{b}$ | $90.6 \pm 0.4^{a}$ | $86.5 \pm 0.3^{c}$ | $85.0 \pm 0.6^{c}$ |
| **Fillet yield (%)** | $61.0 \pm 0.7^{a}$ | $61.6 \pm 0.5^{a}$ | $61.5 \pm 0.5^{a}$ | $60.7 \pm 0.5^{a}$ |
| **Condtiton factore (%)** | $1.24 \pm 0.02^{b}$ | $1.25 \pm 0.03^{b}$ | $1.76 \pm 0.03^{a}$ | $1.84 \pm 0.04^{a}$ |

## 4.2 Fillet quality analyses

### 4.2.1 Colour

The fillet colour of Atlantic salmon and rainbow trout fed with test diet and control diet was analysed using SalmoFan (figure 8).

The Atlantic salmon fed with the control diet had a mean colour score of 25.0±0.2, whereas those fed with test diet had a numerically higher mean value of 25.5±0.2 (P=0.09). Similarly, the rainbow trout control group had a numerically higher colour score of 27.7±0.2 compared to the test group which had mean colour score of 27.9±0.2 (P=0.58).

The rainbow trout had a significantly higher colour score compared to the Atlantic salmon (overall colour score 27.8 and 25.2 for the rainbow trout and the Atlantic salmon, respectively) ($P \leq 0.05$).

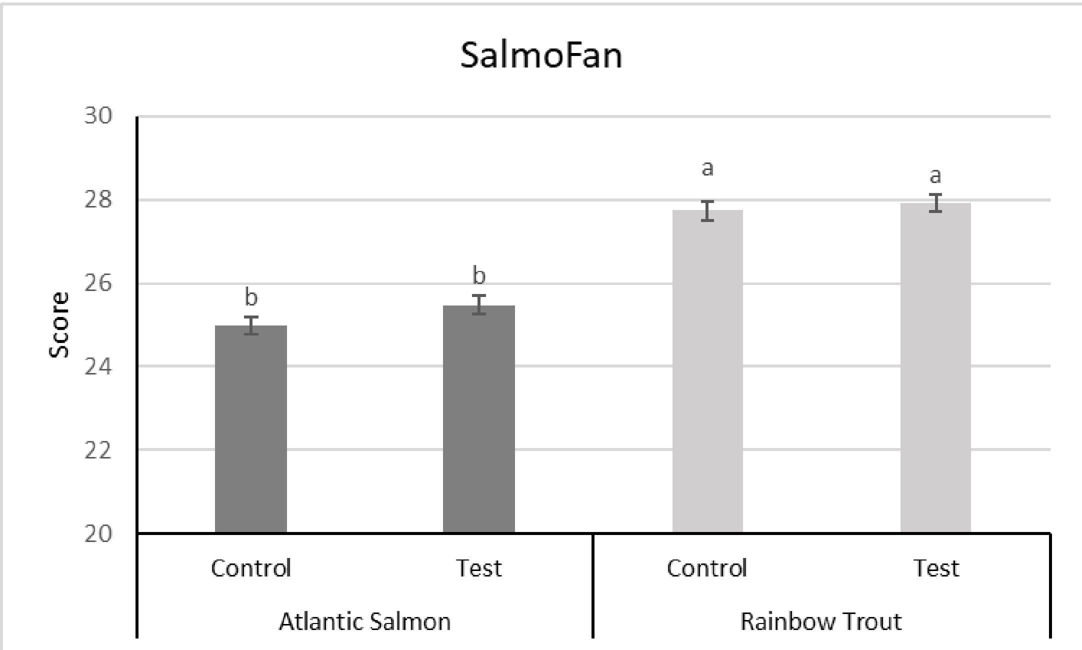



*Figure 8. The average fillet colour (SalmoFan) of Atlantic salmon and rainbow trout fed commercial feed or commercial feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences between the groups ($P \leq 0.05$).*

### 4.2.2 Gaping

The occurrence of gaping in fillets of Atlantic salmon and rainbow trout fed with test diet, and control diet was evaluated and shown in figure 9.

Fillet gaping differed significantly between the control and test groups for the Atlantic salmon. The control group had a mean value of 1.5±0.2, while the test group had a significantly lower mean value of 0.9±0.2 (P=0.02).

No significant differences were observed between the control and test groups for the rainbow trout. The control group had a mean value of 1.8±0.3, while the test group had a mean value of 1.3±0.19, (P=0.11).

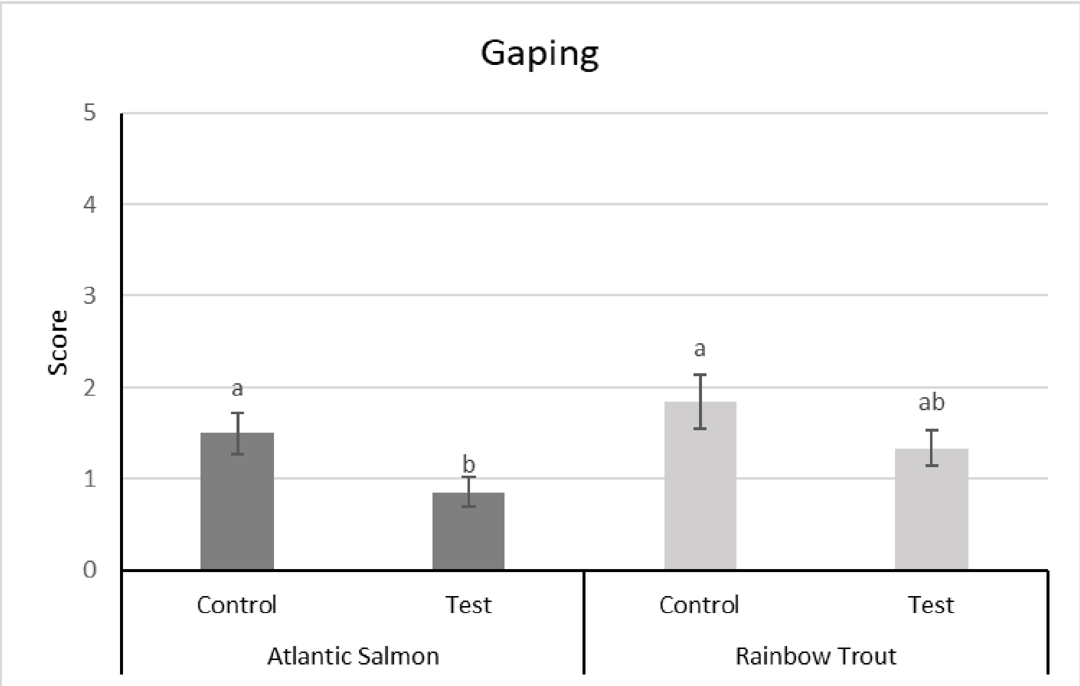



*Figure 9. The average gaping score of fillets of Atlantic salmon and rainbow trout fed commercial feed or commercial feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences between the groups (P≤0.05).*

### 4.2.3 Texture

The firmness (total area, N*s) of Atlantic salmon and rainbow trout fed with test diet and control diet was analysed and shown in figure 10.

Firmness of the test group of Atlantic salmon exhibited a significantly higher mean value of 261.9±7.6 N*s compared to the control group, which had a mean firmness value of 236.9±7.1 N*s (P=0.006). For the rainbow trout, the control group showed a significantly higher firmness value of 225.9±5.4 N*s, while the test group showed a lower firmness value of 200.5±4.6 N*s (P=0.01).

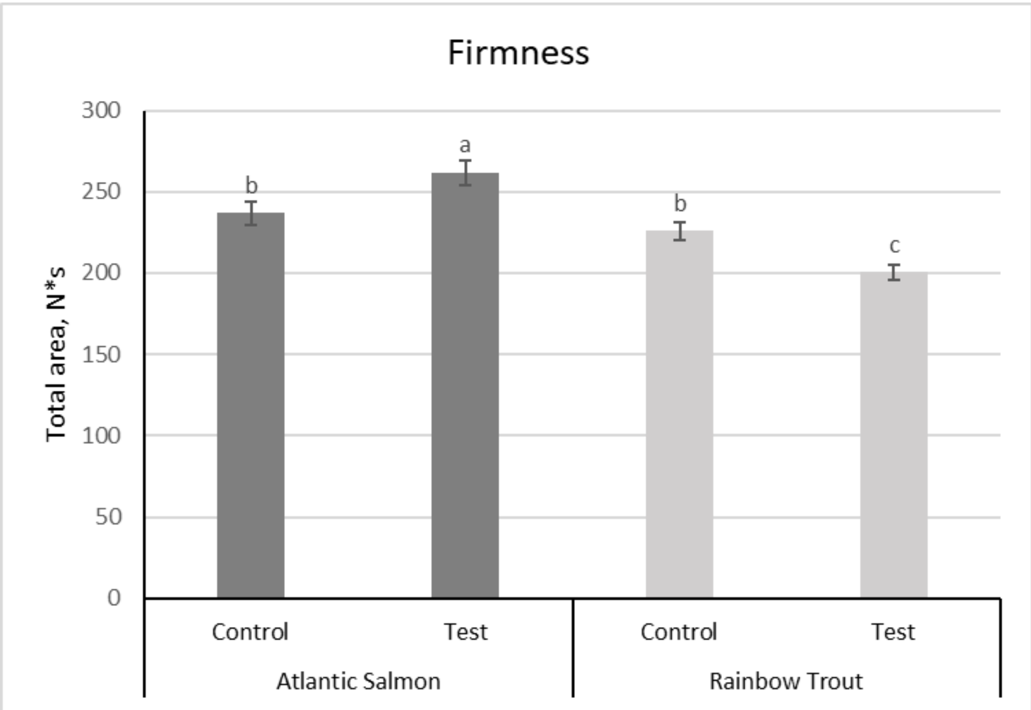



*Figure 10. Fillet firmness (total area, N*s) of Atlantic salmon and rainbow trout fed commercial feed or commercial feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences between the groups (P≤0.05).*

### 4.3 Chemical analyses

#### 4.3.1 Fat Content

The fillet fat contents of Atlantic salmon and rainbow trout fed with test diet and control diet was analysed and shown in figure 11.

The Atlantic salmon fed control diet had an average fat content of 12.5%±0.6, while the Atlantic salmon fed test diet had an average fat content of 13.30%±1, which was numerically higher (P=0.43).

The average fat content in the rainbow trout fed the control diet was 12.3%±0.2, which was numerically higher than the fat content in the rainbow trout test group with an average of 11.2%±0.66 (P=0.39).

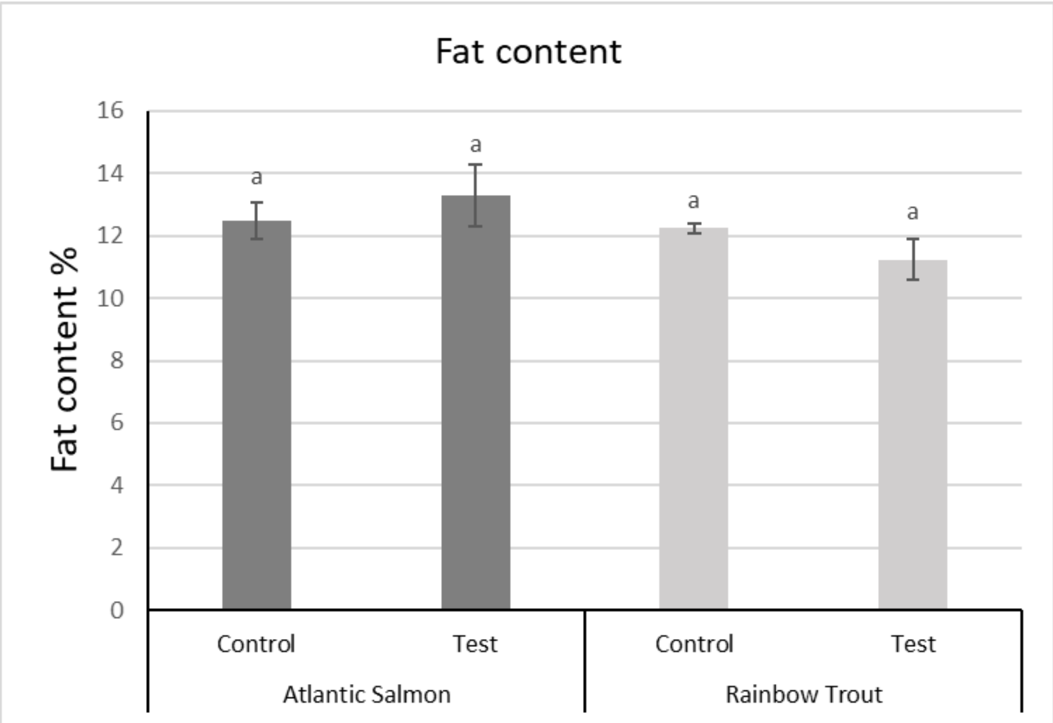



*Figure 11. The average fillet fat content (%) (Norwegian quality cut, NQC) of Atlantic salmon (Salmo salar L.) and rainbow trout (Oncorhynchus mykiss) fed commercial standard feed or commercial standard feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences between the groups (P≤0.05).*

### 4.3.2 Astaxanthin

The fillet astaxanthin contents of Atlantic salmon and rainbow trout fed with test diet and control diets was analysed and shown in figure12.

Astaxanthin content of the Atlantic salmon fillets ranged from 7.8±0.3 mg/kg in the control group to 8.1± 0.8 mg/kg in the Atlantic salmon fed by the test diet, (P=0.78). Likewise, the results showed no significant differences in the astaxanthin content in the rainbow trout, where both dietary groups had an average content of 11.3±1.3 mg/kg (P=1.0).

A significant difference (P≤0.05) in astaxanthin content between the Atlantic salmon and the rainbow trout was observed, where the rainbow trout fillets had a higher average astaxanthin content than the Atlantic salmon fillets. (Overall astaxanthin content of 7.98 mg/kg and 11.30 mg/kg for the Atlantic salmon and the rainbow trout, respectively).

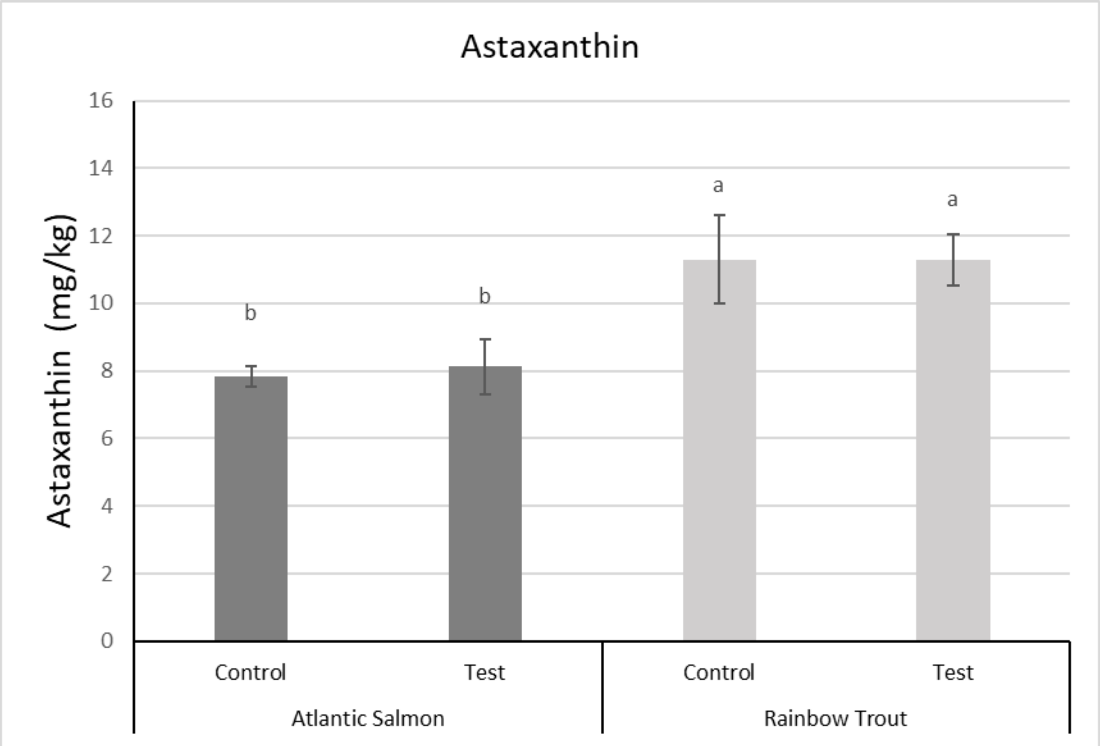



*Figure 12. The average fillet astaxanthin content (mg/kg) (Norwegian quality cut, NQC) of Atlantic salmon (Salmo salar L.) and rainbow trout (Oncorhynchus mykiss) fed commercial standard feed or commercial standard feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences between the groups (P≤0.05).*

### 4.3.3 Amino acid composition of connective tissue

The amino acid composition of CT, extracted from fillet of Atlantic salmon and rainbow trout fed with test diet and control diet was analysed and shown in table 7.

The major component of the amino acids of the Atlantic salmon and the rainbow trout muscle was glycine. Analyses revealed that the Atlantic salmon test group had significantly higher glycine concentration than the control group (P=0.004). No significant differences in glycine concentration were observed between the rainbow trout groups (P=0.30).

Proline was numerically higher of the Atlantic salmon test group (P=0.29) and significantly higher of the Rainbow trout test group compared with the control group (P=0.02).

Hydroxyproline of the test group of both species showed a numerically higher value. However, no significant difference was observed between the Atlantic salmon (P=0.59) and rainbow trout (P=0.16) groups.

Lysin in Atlantic salmon fed by test diet showed significantly lower values (P=0.003) compared to the control group, while no significant differences were observed between the groups of rainbow trout (P=0.65).

Cysteine concentration in the Atlantic salmon test group increased significantly (P=0.02) compared to the control group, but there were no significant differences between the rainbow trout groups (P=0.54).

*Table 7. Amino acid composition (number of residues/1000 amino acid residues) of connective tissue samples in fillets obtained from Norwegian quality cut (right NQC) of Atlantic salmon and rainbow trout fillets fed commercial feed or commercial feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences (P≤0.05) between the groups (P≤0.05).*

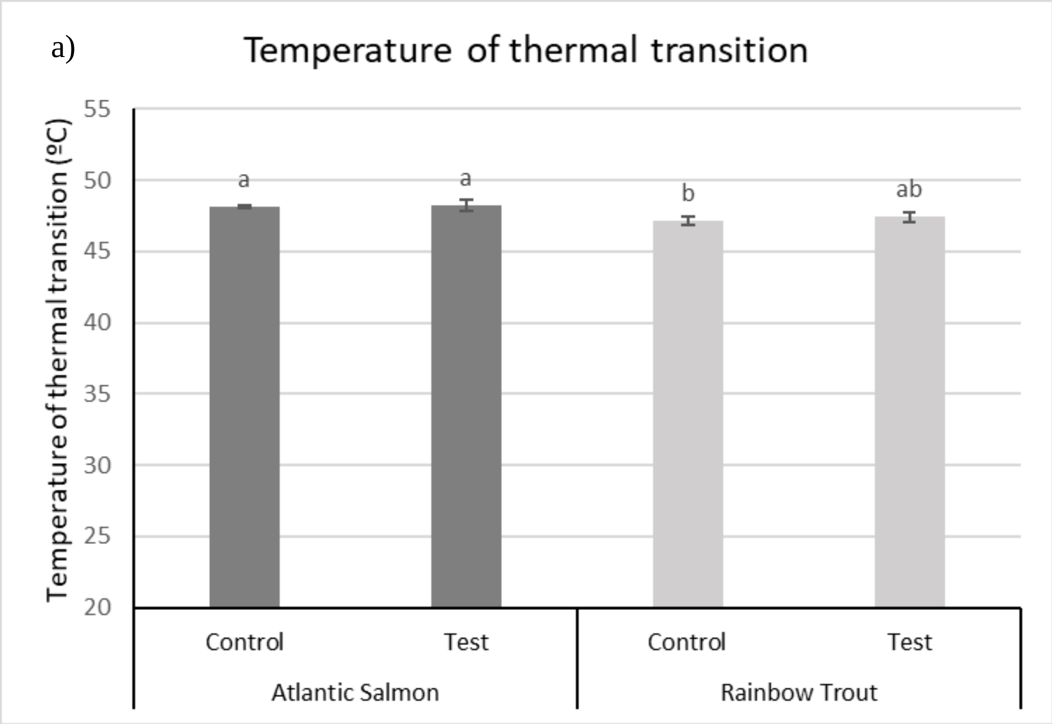
| Amino Acids | Atlantic salmon Control | Atlantic salmon Test | Rainbow rout Control | Rainbow trout Test |
|---|---|---|---|---|
| **Aspartic acid** | 64.99 ± 0.89 $^{c}$ | 63.55 ± 0.39 $^{c}$ | 69.00 ±0.54 $^{a}$ | 67.02 ± 0.31 $^{b}$ |
| **Threonine** | 32.04 ± 0.53 $^{a}$ | 29.57 ± 0.81 $^{b}$ | 26.70 ± 0.82 $^{c}$ | 26.82 ± 0.28 $^{c}$ |
| **Serine** | 49.31 ± 0.26$^{b}$ | 48.42 ± 0.2 $^{b}$ | 52.00 ± 0.76 $^{a}$ | 50.22 ± 1.03 $^{ab}$ |
| **Glutamic acid** | 84.36 ± 0.99 $^{ab}$ | 80.74 ± 1.42 $^{bc}$ | 86.19 ± 3.11 $^{a}$ | 78.77 ± 1.39 $^{c}$ |
| **Glycine** | 276.54 ± 4.38 $^{c}$ | 296.28 ± 6.19 $^{b}$ | 305.14 ± 1.18 $^{ab}$ | 312.30 ± 2.58 $^{a}$ |
| **Alanine** | 106.31 ±0.68 $^{ab}$ | 106.90 ± 0.79 $^{a}$ | 105.57 ± 1.09 $^{ab}$ | 104.39 ± 0.52 $^{b}$ |
| **Cysteine** | 4.42 ±0.2$^{c}$ | 5.17 ± 0.27 $^{b}$ | 6.82 ± 0.24 $^{a}$ | 7.02 ± 0.15 $^{a}$ |
| **Valine** | 27.53 ± 0.7 $^{a}$ | 23.80 ± 1.51 $^{b}$ | 19.80 ± 0.48 $^{c}$ | 20.21 ± 0.46 $^{c}$ |
| **Methionine** | 19.42 ± 0.31 $^{b}$ | 19.72 ± 0.61 $^{b}$ | 21.70 ± 0.5 $^{a}$ | 21.68 ± 0.43 $^{a}$ |
| **Isoleucine** | 18.69 ± 0.58 $^{a}$ | 15.93 ± 0.86 $^{b}$ | 13.93 ± 0.26 $^{c}$ | 13.88 ± 0.44 $^{c}$ |
| **Leucine** | 35.42 ± 0.98 $^{a}$ | 32.21 ± 0.84 $^{b}$ | 30.83 ± 1.37 $^{b}$ | 31.16 ± 0.64 $^{b}$ |
| **Tyrosine** | 10.62 ± 0.4 $^{b}$ | 10.28 ± 0.36 $^{b}$ | 13.77 ± 0.1 $^{a}$ | 12.82 ± 0.26 $^{a}$ |
| **Phenylalanine** | 20.49 ± 0.43 $^{c}$ | 20.50 ± 0.77 $^{c}$ | 26.77 ± 1.00 $^{a}$ | 24.20 ± 0.55 $^{b}$ |
| **Hydroxylysine** | 8.51 ± 0.11 $^{a}$ | 8.61 ± 0.09 $^{a}$ | 8.03 ± 0.19 $^{b}$ | 8.11 ± 0.09 $^{b}$ |
| **Histidine** | 11.57 ± 0.32 $^{a}$ | 10.29 ± 0.34 $^{b}$ | 9.30 ± 0.28 $^{c}$ | 9.43 ± 0.15 $^{c}$ |
| **Lysine** | 37.07 ± 0.94 $^{a}$ | 33.73 ± 0.81 $^{b}$ | 33.74 ± 0.49 $^{b}$ | 33.23 ± 0.36 $^{b}$ |
| **Arginine** | 50.68 ± 0.13 $^{a}$ | 49.58 ± 0.61 $^{a}$ | 45.77 ± 0.56 $^{b}$ | 46.63 ± 0.53 $^{b}$ |
| **Hydroxyproline** | 51.85 ± 0.94 $^{a}$ | 52.53 ± 1.16 $^{a}$ | 44.30 ± 0.23 $^{b}$ | 46.30 ± 0.66 $^{b}$ |
| **Proline** | 90.17 ± 1.01 $^{a}$ | 92.20 ± 1.23 $^{a}$ | 80.64 ± 2.45 $^{c}$ | 85.81 ± 1.11 $^{b}$ |

### 4.3.4 Differential scanning calorimetry

The temperature of thermal transition (ºC) and the Enthalpy of transition (J/g) of CT extracted from fillet of Atlantic salmon and rainbow trout fed with test diet and control diet was analysed and shown in figure 13.

The results of thermal transition temperature showed an average value of 48.2±0.1 ºC for control group and 48.2±0.4 ºC for the test group of the Atlantic salmon (P=0.90). No significant difference was observed in the temperature of thermal transition between the rainbow trout control and test group (P=0.61).

The Enthalpy of transition results revealed an average value of 1.1±0.2 J/g for the Atlantic salmon control group and showed numerically higher average of 1.9±0.4 J/g for the test group (P=0.06). No significant differences were observed in the two groups of the rainbow trout regarding Enthalpy of transition (P=0.86).



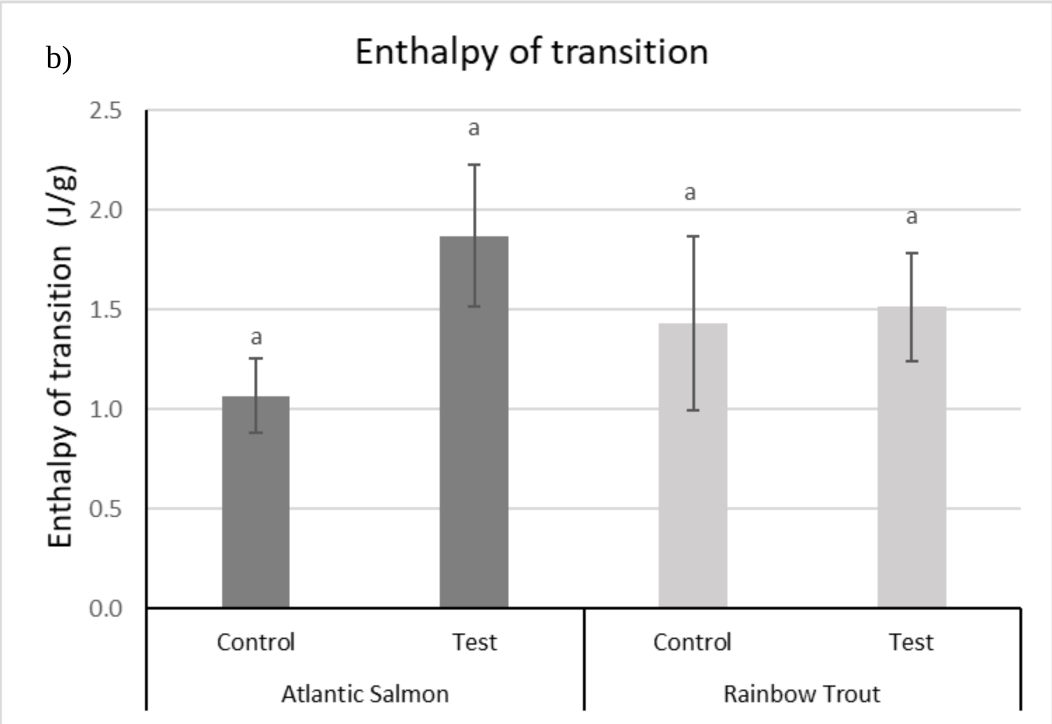




*Figure 13. The temperature of thermal transition (ºC)(a) and the Enthalpy of transition (J/g) (b) of connective tissue extracted from fillets (Norwegian quality cut, NQC) of Atlantic salmon and rainbow trout fed commercial feed or commercial feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences between the groups (P≤0.05).*

### 4.4 Sensory analyses of cooked fillets

The sensory analyses of cooked fillets of Atlantic salmon and rainbow trout fed with test diet and control diet was analysed and shown in figure 14. The graphs show colour, odour, tastiness, juiciness, firmness, and general acceptability.

The sensory assessment of the Atlantic salmon and the rainbow trout was done using a scale of 1 to 5 (table 5.). According to the results, both species were given an average score above 3, indicating that the sensory panel had an overall positive impression of both species.

The colour of the rainbow trout fed with the test diet displayed significantly higher scores (4.7±0.2) compared to the control group (4.2±0.1) (P=0.03). For the Atlantic salmon no significant difference was observed between the dietary groups, test group showed an average score of 3.9±0.09 and the control group had a score of 3.9±0.1 (P=0.93).

The odour scores of the Atlantic salmon control and test groups were 3.8±0.9 and 4±0.09, respectively (P=0.31). For the rainbow trout, the control and test groups scored 4.1±0.1 and 3.9±0.1, respectively (P=0.25).

The tastiness scores for the control and test groups of Atlantic salmon were 3.8±0.9 and 4±0.09, respectively (P=0.13). Meanwhile, for the rainbow trout, the control and test groups received scores of 4.1±0.1 and 3.9±0.1, respectively (P=0.52).

The juiciness of the Atlantic salmon fed the test diet was significantly higher, with an average score of 3.9±0.1, compared to the control group with an average score of 3.3±0.1 (P=0.001). No significant difference in juiciness was observed between the control and test groups of the rainbow trout, which had average scores of 4.0±0.1 and 3.7±0.2, respectively (P=0.21).

The firmness scores for the Atlantic salmon test group were significantly higher, with an average of 4.1±0.09, compared to the control group, with an average of 3.7±0.1(P=0.02). There was no significant difference in firmness between the control and test groups of the rainbow trout, which had average values of 3.9±0.1 and 3.7±0.1, respectively (P=0.53).

The general acceptability scores of the Atlantic salmon test group (4.1±0.7) were significantly higher than the control group (3.81±0.09) (P=0.02). There was no significant difference between the control and test groups of the rainbow trout, with scores of 4.07±0.1 and 4.0±0.1, respectively (P=0.67).

The sensory panel rated the Atlantic salmon fed test diet a higher score than the other groups; 64% of participants admitted that the Atlantic salmon fed test diet was the best choice, indicating a preference for this treatment (Appendix 8.4).

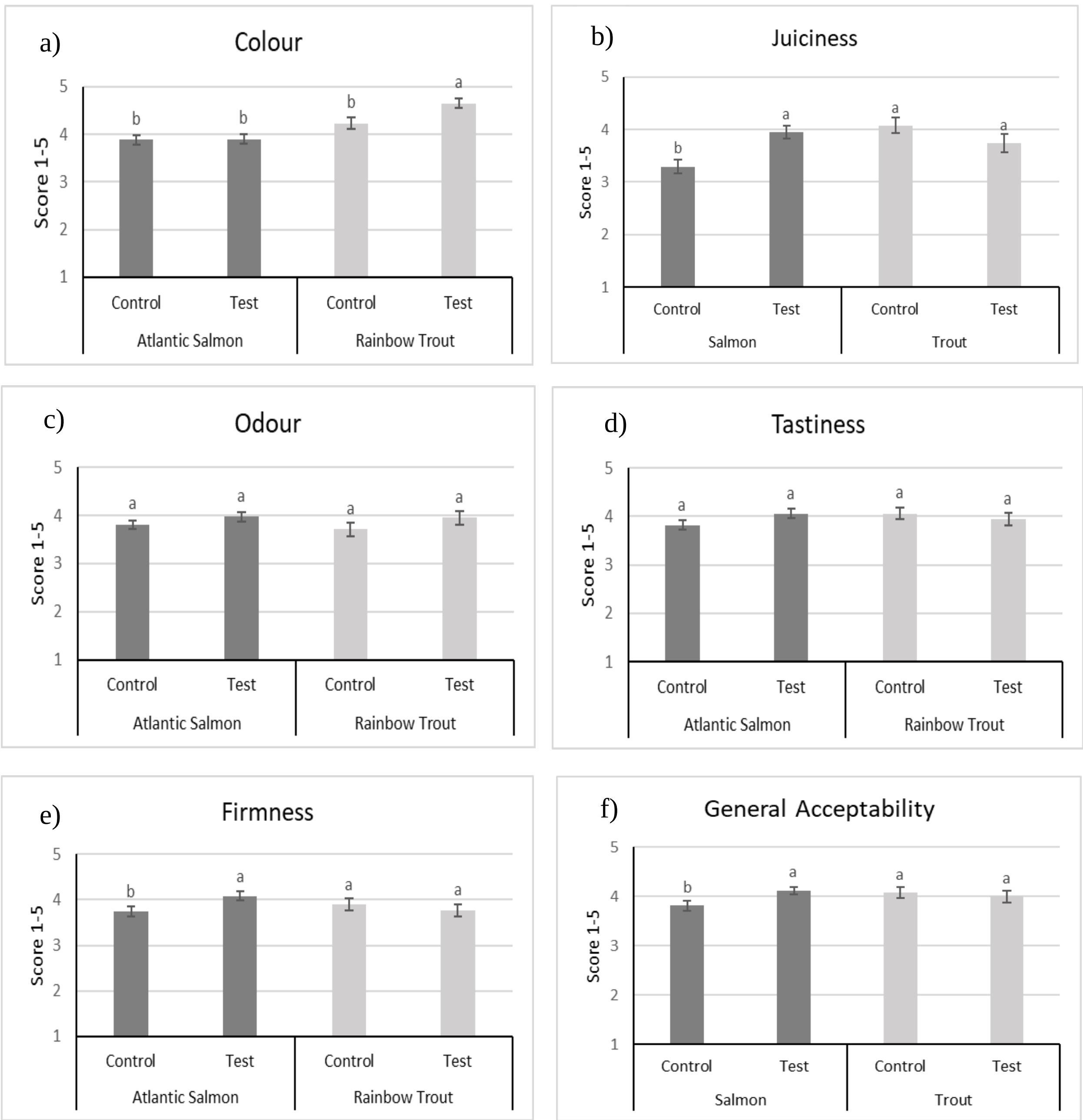



*Figure 14. Sensory assessment of colour (a), juiciness (b), odour (c), tastiness (d), firmness(e) and general acceptability (f) of cooked fillet of Atlantic salmon and rainbow trout fed commercial feed or commercial feed with 4% inclusion of black soldier fly larvae meal (Hermetia illucens). Values are expressed as means ± standard errors (SE). Different superscripts indicate significant differences between the groups ($P \leq 0.05$).*

# 5 Discussion

## 5.1 Fillet quality analyses

### 5.1.1 Colour

The present study investigated the effect of incorporating BSFL into Atlantic salmon and rainbow trout diets on the colour of raw fillets.

The results of this study suggest that feeding BSFL to Atlantic salmon and rainbow trout did not have a significant effect on the colour of the raw fillets. Although the test group of both species showed numerically higher mean colour score, the difference was not significant. This is aligned with some previous studies reporting that 40% of the inclusion level of BSFL did not influence the colour parameters (Renna et al., 2017). However, the lack of significant difference in this study could be attributed to the low level of BSFL inclusion in the diets used in the current study.

Another possible explanation for the lack of significant difference could be related to the method used to measure the colour in this study. Although SalmoFan is widely used to measure the colour of fish, it has some limitations, such as the inability to distinguish between different pigment types or to account for changes in the background colour. Consequently, other techniques, like spectrophotometry or ChromaMeter, may offer more precise information on the colour of fish.

It is important to note that the two species' colours differ significantly, with the rainbow trout having a higher mean value of colour compared to the Atlantic salmon. This may be due to variations in these species' metabolism and pigment deposition. In addition, the diets and lifestyles of rainbow trout and Atlantic salmon may differ, which may also have an impact on their pigmentation.

In conclusion, this study provides some insight into the effect of feeding BSFL on the colour of fish, but more research is needed to fully understand this phenomenon. Future studies could explore different levels of black soldier fly larvae inclusion in diets and use other colour measurement methods to provide a more comprehensive understanding of fish colouration.

### 5.1.2 Gaping

The present study investigated the effect of incorporating BSFL into Atlantic salmon and rainbow trout diets on the occurrence of gaping in raw fillets.

The results of this study suggest that the inclusion of BSFL in the diets of Atlantic salmon and rainbow trout may have differing effects on the occurrence of gaping. The significantly lower occurrence of gaping observed in the test group of Atlantic salmon fed with BSFL suggests that including this ingredient may positively impact flesh quality by reducing the occurrence of this undesirable trait. A similar trend was observed in the rainbow trout group, with a numerically lower gaping score in the test group compared to the control group. However, the lack of significant differences in the occurrence of gaping between the control and test groups of rainbow trout suggests that the inclusion of BSFL in the diet may not significantly impact this trait's occurrence in this species.

To date, no research has explored the potential impact of BSFL into fish feed on the gaping susceptibility of fish fillets. One possible explanation for the significant reduction in gaping occurrence in Atlantic salmon fed with BSFL could be due to the amino acid composition of CT. Atlantic salmon fed by BSFL showed higher levels of glycine, alanine, and proline, which are important for collagen synthesis (Moreno et al., 2016). As collagen is a key structural protein in fish muscle, an increase in its synthesis may lead to stronger muscle fibres and reduced gaping in fillets.

Possible explanations for the observed differences between the Atlantic salmon and the rainbow trout could be related to their differences in physiology and dietary requirements. Further research could investigate the underlying mechanisms that lead to these differences to better understand the effects of BSFL on fish flesh quality.

In conclusion, the results of this study suggest that the inclusion of BSFL in the diet of Atlantic salmon may reduce the occurrence of gaping, while its effects on the occurrence of this trait in rainbow trout are less clear. These findings could have implications for the use of BSFL as a sustainable alternative ingredient in fish feed formulations.

### 5.1.3 Texture

The present study investigated the effect of incorporating BSFL into Atlantic salmon and rainbow trout diets on the firmness (total area N*s) of the raw fillets.

In this study, the test group of Atlantic salmon exhibited a significantly higher firmness value compared to the control group, while the test group of rainbow trout showed a significantly lower firmness value compared to the control group. These results suggest that including BSFL meal in the diets of Atlantic salmon can increase the firmness of the fillet, but it may have a negative impact on rainbow trout. This is in contrast with the findings of Bruni et al. (2019), who reported no significant effect of BSFL on the texture of farmed Atlantic salmon. The difference in results could be due to differences in the experimental design, fish species, or even the origin and composition of the BSFL used in the studies.

The improved firmness of the Atlantic salmon fillet observed in this study could potentially be attributed to the effect of BSFL on protein content of fish fillet. It is possible that BSFL may facilitate the deposition of amino acids, which can promote muscle development and improve the texture of fish fillets. This deposition of amino acids may lead to the formation of stronger muscle fibres, resulting in a firmer texture of the fillets. This hypothesis is supported by this study's amino acid composition analysis results, which showed a significantly higher amount of glycine in the Atlantic salmon test group. Glycine is a key amino acid in collagen structure and a major component of CT in fish muscle (Moreno et al., 2012). Therefore, the higher amount of glycine in the test group may have contributed to the improved firmness observed in the Atlantic salmon fillet. However, the opposite effect was observed in the rainbow trout, with a slightly lower firmness score in the test group compared to the control group, suggesting that the impact of BSFL on fish raw fillets texture may be species-specific.

Overall, the present study suggests that the inclusion of BSFL in the diets of salmon and trout may have a limited effect on their firmness. However, further research is needed to fully understand the mechanisms underlying the effects of BSFL on fish texture and to identify the optimal inclusion level of BSFL in the diets of different fish species.

## 5.2 Chemical analyses

### 5.2.1 Fat content

The present study investigated the effect of incorporating BSFL into Atlantic salmon and rainbow trout diets on the fat content of the raw fillets.

The results from the fat content analysis indicate no statistically significant differences between the dietary groups for Atlantic salmon and rainbow trout. Interestingly the Atlantic salmon fed test diet had a numerically higher average fat content compared to the control group. At the same time, the result showed lower fat content in rainbow trout fed by test diet compared to the control group.

These findings suggest that using BSFL as a feed ingredient does not significantly affect the fat content of fish fillets. The results of earlier studies by Belghit et al. (2019) and Belghit et al. (2018), which also found no effect of the BSFL diet on the whole-body lipids of Atlantic salmon, support the current finding. In addition, Bruni et al. (2019) reported no effect of complete dietary substitution of fishmeal with black solider fly larvae meal on the total lipid content of Atlantic salmon fillet.

According to the present study, it was concluded that adding 4 % BSFL meal to the Atlantic salmon and rainbow trout diet did not significantly affect the fat content of fillets. However, it needs to be considered that the BSFL meal used in this trial was defatted; therefore, it was expected that no significant effect on the fat content of the fish fillet would be observed. However, further studies with larger sample sizes may be necessary to confirm these findings.

Despite that, it should be considered that the composition of fatty acids in fish fillets plays an important role in consumers' health. For example, the essential fatty acids omega-3 and 6 in fish fillets are crucial for maintaining cardiovascular health (Méndez et al., 1996). For this reason, if the use of BSFL in the diet reduces the essential fatty acids in fish fillets, it may harm the marketability of farmed Atlantic salmon and rainbow trout as health-promoting food for consumers. Therefore, more research is needed to investigate the effect of a BSFL-based diet in fish on the composition of fatty acids in fish fillets.

### 5.2.2 Astaxanthin content

The present study investigated the effect of incorporating BSFL into Atlantic salmon and rainbow trout diets on the astaxanthin content of the raw fillets.

Based on the result of the present study, it has been concluded that astaxanthin content in the Atlantic salmon was not significantly affected by the diet. In addition, there were no significant differences in the astaxanthin content of rainbow trout samples, with both groups showing the same average value.

However, the present study showed that rainbow trout had a higher average astaxanthin content than Atlantic salmon, which is consistent with the result from the colour measurement (SalmoFan) of the fillet in the present study, where rainbow trout gained a higher score compared to the Atlantic salmon groups. To our knowledge, no study with a similar objective was found to investigate the impact of BSFL on the Astaxanthin content in the fish muscle.

Since BSFL is not a source of carotenoid pigments, it was expected to achieve no significant difference between the treatment of each species. Nevertheless, further studies with larger sample sizes are needed to confirm these results and to explore the potential effects of BSFL on the content of pigments in fish fillets.

### 5.2.3 Amino acid composition of connective tissue

The current study investigated the effect of BSFL on the amino acid composition of CT in the Atlantic salmon and rainbow trout muscle.

According to the study's findings, the major amino acid present in the CT of both species was glycine. In general terms, glycine accounted for approximately one-third of the amino acid residues of the collagen as also reported by Moreno et al. (2012). Additionally, the results showed that the salmon test group had significantly higher concentration of glycine compared to the control group. However, while there were no significant differences observed in the rainbow trout groups, the glycine content was found to be higher in rainbow trout test group.

These findings would support the hypothesis that dietary inclusion of BSFL meal would increase glycine concentration in both species' CT. Moreover, this higher amount of glycine in the CT would result into more stabilized collagen by the formation of hydrogen bonds in which glycine is involved (Montero & Borderías, 1990).

Similarly, the test group of both the Atlantic salmon and rainbow trout showed higher values of proline compared to the control group, with rainbow trout test group showing significantly higher values compared to the control group. These results also support the hypothesis that BSFL meal would increase the proline concentration in both species' CT. Interestingly, the Atlantic salmon treatments had significantly higher hydroxyproline values than the rainbow trout treatments, indicating that the effect of dietary inclusion of BSFL meal may vary among different fish species. In contrast, Dumas et al. (2018) reported that hydroxyproline was the sole nitrogenous compound analysed that increased in rainbow trout fed BSFL meal diet. Since insect collagen contains no hydroxyproline, the elevated levels observed in rainbow trout fed by BSFL products indicated that this amino acid was more digestible or may have been spared or synthesized by rainbow trout. Therefore, BSFL meal may contain nutrients that promote prolidase activity, which is the specific enzyme responsible for the hydrolysis and absorption of proline and hydroxyproline in the small intestine (Dumas et al., 2018). Hydroxyproline is believed to play an important role in the stabilization of the triple-stranded collagen helix due to its hydrogen bonding ability through its hydroxyl group (Moreno et al., 2012) so it could be suggested that dietary BSFL contributed to a more stabilized collagen structure.

At the same time, no significant difference was observed in the lysine concentration between the control and test groups of rainbow trout treatments, while Atlantic salmon fed by BSFL showed significantly lower values compared to the control group. Lysine is present in the helical and non-helical regions of the collagen molecule. Lysine is essential for collagen synthesis, as it is involved in cross-linking collagen molecules, giving collagen strength and stability (Moreno et al., 2016).

The cysteine concentration in the test group of Atlantic salmon showed a significantly higher value, while no significant differences were observed in rainbow trout dietary groups. The positive effect of BSFL in the diet of the Atlantic salmon might be explained by the composition of the diet, which might provide a higher concentration of cysteine, or by the possibility that BSFL might promote the accumulation of amino acids in fish fillets. On the other hand, the study's results indicated that rainbow trout are less responsive to changes in dietary cysteine. However, it is important to note that other factors, such as genetics, could also influence the response of rainbow trout to dietary cysteine.

However, it is difficult to completely attribute changes in the amino acid composition of fish fillets to dietary variations due to the lack of an amino acid profile of the fish feed used in this experiment. For future research, it is recommended to incorporate the feed amino acid profile to better comprehend the influence of dietary composition changes on the amino acid composition of fish fillets.

Nonetheless, these findings highlight the need for further research to better understand how the BSFL diet could influence the collagen content and its properties in the CT of these species.

### 5.2.4 Differential scanning calorimetry

The present study investigated the effect of BSFL on the thermal properties of Atlantic salmon and rainbow trout CT extracted from muscle. Differential Scanning Calorimetry (DSC) was performed to determine the thermal transition temperature and enthalpy of transition for the four groups.

The results showed no significant differences in thermal transition temperature between the Atlantic test and control groups, indicating that the incorporation of BSFL meal did not affect the thermal properties of Atlantic salmon CT. Similar observations were made for the rainbow trout, where no significant difference was observed in the thermal transition temperature between the test and control group. However, the rainbow trout control group had a significantly lower thermal transition temperature compared to the Atlantic salmon.

The enthalpy of transition results showed that the Atlantic salmon test group had a numerically higher value than the control group. However, no significant differences were observed between the two dietary group. Interestingly, rainbow trout test group exhibited lower enthalpy values compared to Atlantic salmon test group. Again, no significant differences were observed between the dietary treatments.

Some authors have indicated a higher thermal transition temperature and enthalpy of conformational transition data for the treatments with the greater presence of several amino acids in collagen (Moreno et al., 2016). However, in the present study, there are different findings. A possible explanation for the lack of differences in thermal transition temperature and enthalpy in the four groups of the Atlantic salmon and the rainbow trout would be related to the differences in collagen composition in the CT. Glycine, proline, and hydroxyproline are the main amino acid involved in collagen stabilization, and although the presence of them is slightly higher in the Atlantic salmon and the rainbow trout test groups, but no significant differences were observed in most of the cases (table 7), which would result in a lack of effect in the thermal transition temperature and enthalpy.

Based on the results, it appears that the incorporation of BSFL meal into the diets of Atlantic salmon and rainbow trout did not have a significant impact on the thermal properties of the CT extracted from muscle.

#### 5.2.5 Sensory analyses of cooked filet

The results of this study suggest that the inclusion of BSFL in the Atlantic salmon and rainbow trout diet had significant effects on some sensory characteristics of the cooked fish fillets. Overall, both species were rated positively regarding colour, odour, tastiness, juiciness, firmness and general acceptability, with an average score above 3 on a scale of 1 to 5.

Regarding colour, the Atlantic salmon showed no statistically significant difference between the dietary groups. This contrasts with the finding from Belghit et al. (2019), who reported lower colour intensity of cooked salmon in the group fed by insect meal. On the other hand, the rainbow trout test group displayed a significantly higher value than the other groups. This contrasts with the finding from Sealey et al. (2011) who reported no effect of BSFL on the colour of rainbow trout fillets. However, in the present study, there were no significant differences in colour measurement results of raw fillet (SalmoFan) between the control and test groups for either species. One possible solution can be related to the sensory evaluation; it may have been affected by individual variations in the panellists' perceptions and preferences or lighting conditions and the background in which the samples were evaluated, which could have led to inconsistencies in the results. Selecting panellists with greater sensory expertise could help improve the reliability of the results.

In terms of juiciness, Atlantic salmon test group had a significantly higher value than the control group, suggesting that including BSFL in the diet could enhance the juiciness of the Atlantic salmon. However, no significant difference in juiciness was observed between the control and test groups for the rainbow trout. This could be due to differences in the muscle structure and composition between the two species. A study by Bruni et al. in 2019 reported lower scores for juiciness in the group of Atlantic salmon, which had 66% replacement of fish meal with BSFL compared to the other groups, which contrasts with the current study's findings.

The Atlantic salmon test group showed significantly higher firmness than the control group. At the same time, there was no significant effect on the rainbow trout groups. The result of this study is consistent with the findings of Belghit et al. (2019), who reported that higher inclusion levels of BSFL resulted in firmer cooked salmon fillets, However, Sealey et al. (2011) reported no effect of diet enriched with black soldier fly prepupae on the sensory evaluation of rainbow trout fillets. This could be due to the amino acid composition of BSFL, which may have led to an increase in collagen crosslinking, resulting in a firmer texture as the result of the amino acid profile showed higher values of Hyp and Pro in the Atlantic salmon test group compared to the Atlantic salmon control group, although the difference wasn't significant.

In terms of odour and tastiness, no significant difference was observed between the control and test groups in both species. This suggests that the inclusion of BSFL in the diet did not have a negative impact on the sensory quality of the cooked fish fillets. These findings are consistent with the studies conducted by Sealey et al. (2011) and Belghit et al. (2019), who reported no significant differences in the odour and taste of rainbow trout and Atlantic salmon fed with lower amounts of BSFL meal. However, according to a study conducted by Borgogno et al. (2017), the inclusion of black soldier fly larvae meal in rainbow trout feed resulted in a dominant metallic flavour (Borgogno et al., 2017). In addition, Bruni et al. (2019) reported that the sensory evaluation scores of odours and flavour were lower for Atlantic salmon group with 66% replacement of fish meal with BSFL compared to the other groups.

The sensory panel rated the Atlantic salmon test group, with a higher value than the other groups, with 64% of participants admitting that it was the best choice, indicating a preference for this treatment. This could be due to the improved juiciness and firmness of The Atlantic salmon fed the test diet.

Overall, the results of this study suggest that the inclusion of BSFL meal in the diet of Atlantic salmon and rainbow trout could have some positive effects on the sensory characteristics of the cooked fish fillets, particularly in terms of juiciness and firmness for Atlantic salmon and colour for rainbow trout. However, further research is needed to evaluate a sensory test with a trained panel that can help to minimize variability in sensory evaluations. It is essential to ensure that all panellists use the same sensory evaluation methods and are consistently trained to recognize and identify sensory attributes makes the resulting data more consistent and reliable.

# 6 Conclusion

This study provides valuable insights into the potential effects of feeding BSFL on physical parameters of raw fillets including colour, gaping, and texture of Atlantic salmon and rainbow trout. The results suggest that the inclusion of BSFL in the diets of these species may have limited effects on the colour of raw fillets, but it can positively impact the firmness of Atlantic salmon fillets and reduce the occurrence of gaping. However, the differences observed between the two species highlight the need for further research to fully understand the effects of BSFL on fish flesh quality.

The results indicated that using BSFL as a feed ingredient did not significantly affect the fat content and astaxanthin concentration of fish fillets in Atlantic salmon and rainbow trout. However, the dietary inclusion of BSFL meal increased the glycine, proline, hydroxyproline and cysteine concentrations in both species' CT, which supports the hypothesis that the BSFL meal could increase the quality of the fish's CT. The inclusion of BSFL meal in the diets did not significantly impact the thermal transition temperature and enthalpy of transition of the CT.

The study suggests that incorporating BSFL in the diet of Atlantic salmon and rainbow trout can positively affect the sensory characteristics of the cooked fish fillets. Specifically, rainbow trout fed the test diet had a higher colour rating, while Atlantic salmon fed the test diet had higher ratings for juiciness, firmness, and general acceptability. However, further research is necessary to investigate other potential mechanisms underlying the effects of BSFL on the quality parameters of fish fillets.

In conclusion, based on the results obtained in this study, it can be concluded that the 4% inclusion of black soldier fly larvae meal as a component of fish diets can hold promising potential to enhance the physical and sensory quality of fish fillets of Atlantic salmon and could be a promising and sustainable alternative to traditional feed ingredients sources (table 8).

*Table 8. Effect of 4% black soldier fly larvae meal on physical, chemical, and sensory quality of Atlantic salmon and rainbow trout fillets: Hypotheses and Results*

| Hypotheses | Atlantic salmon | Rainbow trout |
|---|---|---|
| Physical quality: positive effect | ☑ | ☐ |
| Chemical composition: positive effect | ☑ | ☑ |
| Sensory quality: positive effect | ☑ | ☑ |

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# 8 Appendix

## 8.1 Procedure of connective tissue extraction from fish muscle

1- Homogenization of muscles in NaCl 0.8% (8 per 1000)

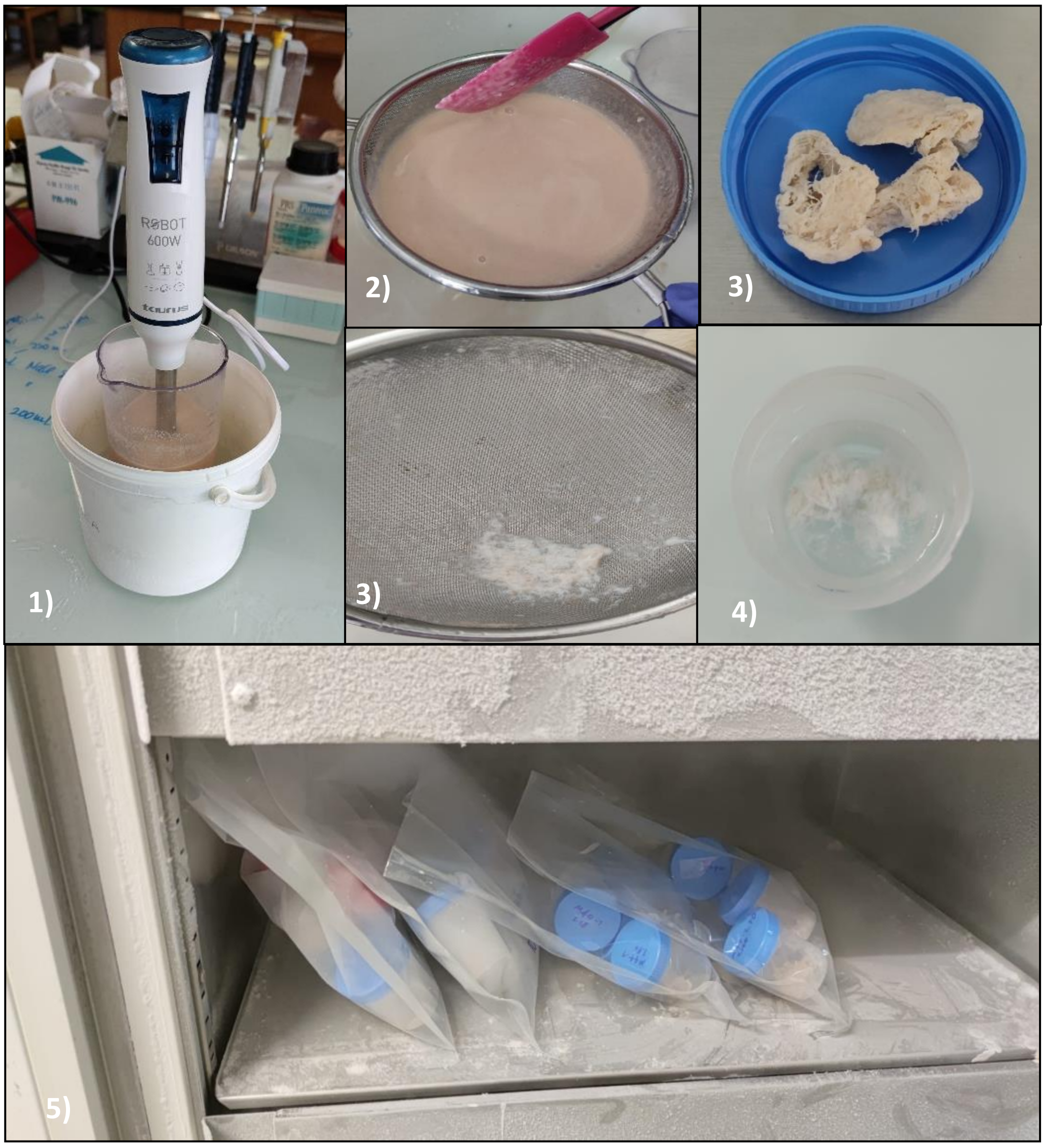
2- Resulting solution is filtrated with a strainer under tap water letting the mixture being washed out

3- Repeat step 1 and 2 as it is necessary to obtain a whitish CT possible

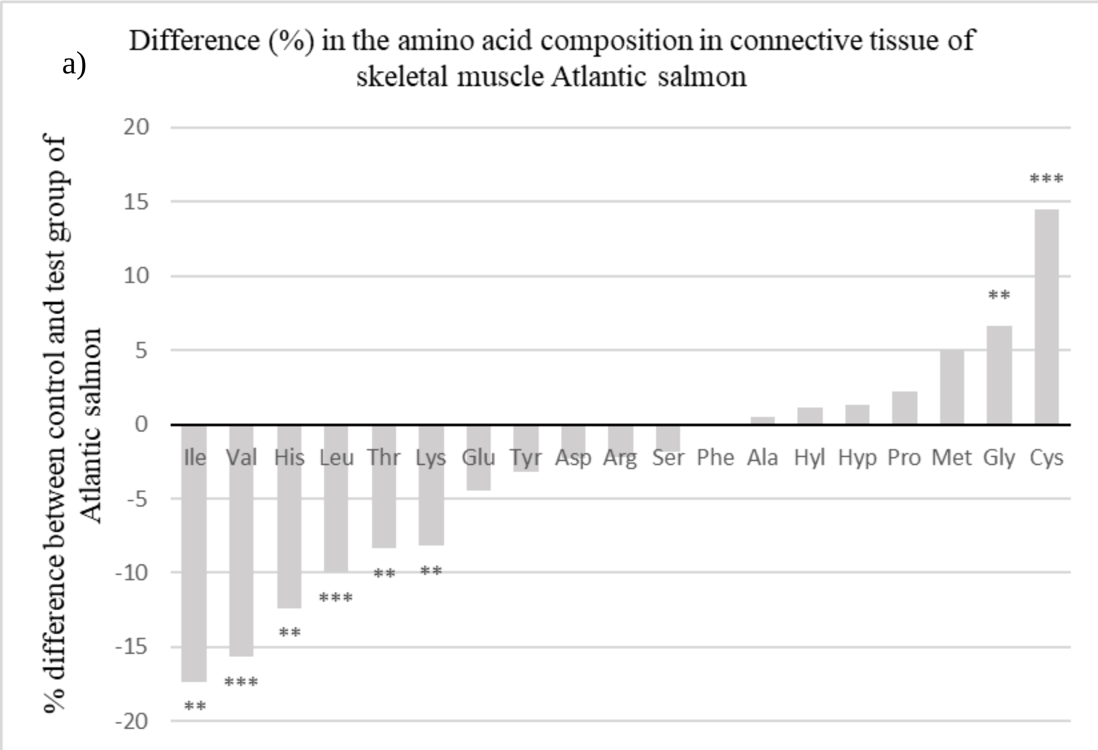
4- Add Butanol 10: 1 CT (v:p ) to remove fat at 5 °C/1h and then wash properly with distilled water

5-Frozen at-80°C

*All the steps done at 0-5 °C (using Ice to control the temperature)



## 8.2 Difference (%) in the amino acid composition



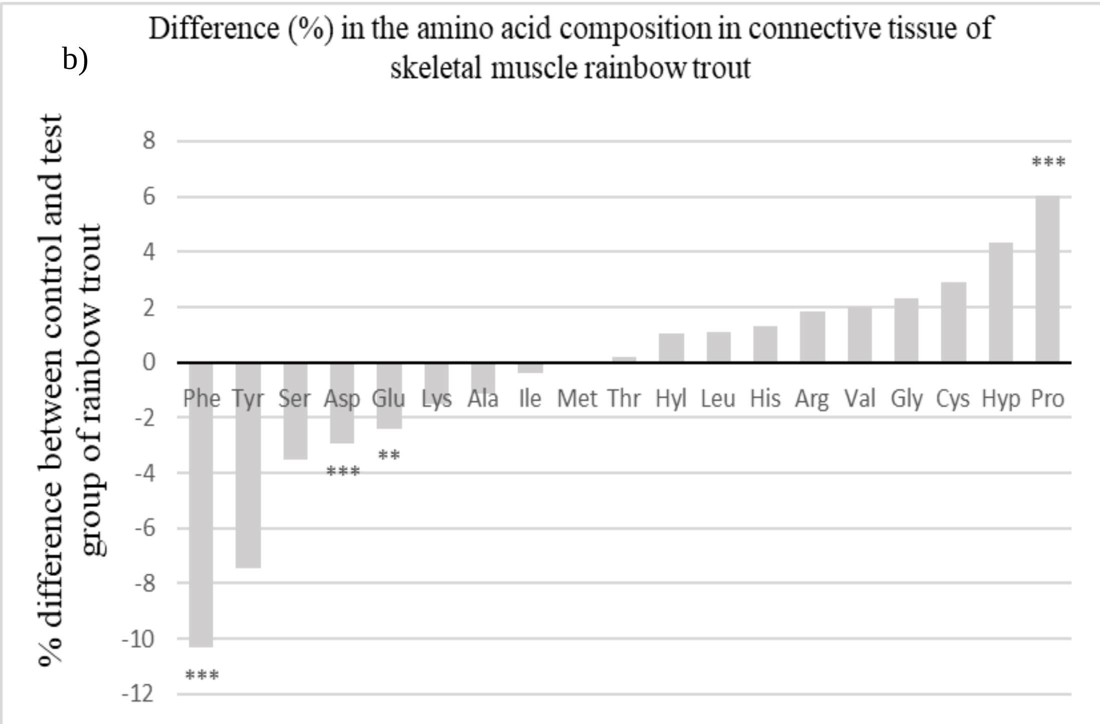




*Figure 15. The percentage difference in amino acid composition (residues/1000 amino acids) of the fillet connective tissue of Atlantic salmon(a) & rainbow trout (b) fed a commercial diet compared to the diet with 4% inclusion of Black Soldier Fly (Hermetia illucens) is shown, with statistical significance denoted by *p<0.05, **p<0.01, and ***p<0.001.*

### 8.3 Sensory evaluation questionnaire

**Please write the plate number here:....**

**Gender:**

**Age:**

**Nationality:**

**Study Program:**

**How Often do you eat fish:**

-Once a month or less ☐

-Two times a month ☐

-Once per week ☐

-More than once a week ☐

**Dear Participant,**

You will receive three fish pieces; each piece is marked with a code (A, B, C). Please try each piece of the fish and rate the relevant parameters based on the table below. It is recommended to have some sips of water and a small bite of cracker between samples to cleanse your palate. This will ensure the smell and taste of one sample don't affect the next one.

| **Parameters** | **Scores** | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| **Colour** | Dislike | Acceptable | Likes slightly | Good | Excellent |
| **Odor** | Dislike | Acceptable | Likes slightly | Good | Excellent |
| **Tastiness** | Dislike | Acceptable | Likes slightly | Good | Excellent |
| **Firmness** | Soft | Acceptable | Medium | Good | Excellent |
| **Juiciness** | Dry | Acceptable | Medium | Good | Excellent |
| **General Acceptability** | Dislike | Acceptable | Likes slightly | Good | Excellent |

Please write the score (1-5) below:

1) Sample A:

Colour: ................. Odor: ............... Tastiness: .................

Firmness: ................ Juiciness: ............... General Acceptability: ...............

2) Sample B:

Colour: ................. Odor: ............... Tastiness: .................

Firmness: ................ Juiciness: ............... General Acceptability: ...............

3) Sample C:

Colour: ................. Odor: ............... Tastiness: .................

Firmness: ................ Juiciness: ............... General Acceptability: ...............

4) Order the samples according to your preference.

| 1 (The best) | 2 | 3 (the worst) |
|---|---|---|
| | | |

5) Please write any personal comments; If you feel anything significant for example; off flavour or unusual taste. (please remember to mention the sample code) .......................................

***Thanks for your cooperation.***

1

## 8.4 The best choice of fish groups in sensory evaluation

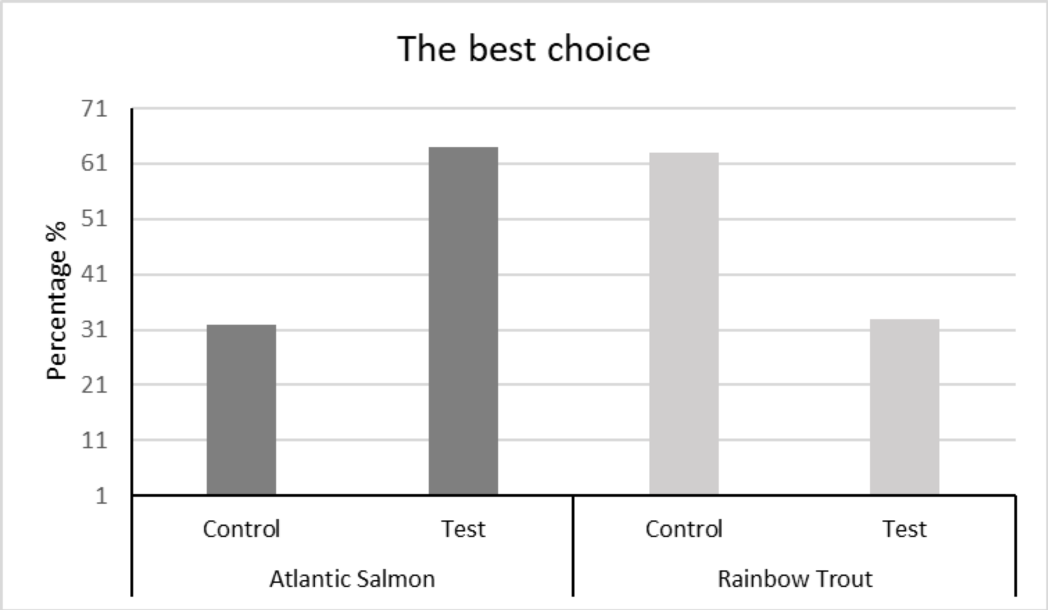



*Figure 16. Sensory assessment of cooked fillets of Atlantic salmon and rainbow trout fed commercial feed and commercial feed with 4% inclusion of Black Soldier Fly (Hermetia illucens).*

## 8.5 Representative image of fish using in the experiment


*Figure 17. Representative image of rainbow trout (Oncorhynchus mykiss) used in the study.*

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