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DIETARY EFFECT ON SKELETAL DEVELOPMENT OF ATLANTIC SALMON (*Salmo salar*)

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ABSTRACT

The main aim of this thesis was to study the effect of dietary treatments on the skeleton of Atlantic salmon. This experiment was conducted prior to moving the fish to the seawater phase. The starting weight of the salmon in this experiment was 20g.

Four diets were produced and used in this study. The diets were formulated with varying levels of marine protein and oils, vegetable protein and oils and minerals.

The diets were designed Marine diet produced from a 1995 recipe with alterations with the mineral phosphorus (M), Marine diet with low mineral concentration (M-LM), Standard diet with vegetable protein and oils referred to as the Control diet (C) and lastly the control diet with Vitamin K (CK+).

This study was conducted at a NOFIMA research station, Sundalsøra, Norway. These four diets (C, CK+, M, M-LM) were triplicate, were 12 tanks in total. After 16 weeks of experimentation, ten fish per tank were randomly selected. During the sampling, the fish had an average weight of 73g. Calorimetric analysis of the skin were taken and biometric traits and score data were also measured and recorded.

Furthermore, morphological traits /skeletal deformities were also diagnosed or studied using the semi-digital X-ray mammography. X -ray pictures were examined for vertebra fusions, (fusions), compressions, hypo mineralized, and loss of intervertebral spaces.

The mechanical properties of the vertebral column in the (anterior /ANT) and the portion between the posterior end of the dorsal fin and the gut (NQC/Norwegian quality cut). The instrument used was the TA-XT2 analyser. Parameters collected used from the mechanical analyses were compression force (N), Area (total work N*s) and thickness (mm).

Also, the concentration of minerals in the skeleton was determined. Minerals accounted for were calcium (Ca), phosphorus (P), magnesium (Mg), manganese (Mn).

Based on the results of this experiment, the total work (N*sec) required to compress the vertebrae to 50% of total thickness was the most representative parameter. The NQC region/section had the highest thickness, Area and compression force in all the treatments. The NQC had the largest stiffness and strength. In terms of individual treatments, CK+ had the highest Area, Compression force and thickness amongst all the dietary treatments in the anterior section of the skeleton.

The control diet (C) had the highest Area, Compression force in the NQC region but not thickness.

In terms of Mineral Analysis, Marine diet had the highest concentration of Ca, Mg, P, Zn.

Visual score of the skin showed that there was significant difference between treatments. This significant difference was seen in scale loss, red-green, a*, yellow- blue, b* Biometric traits and score data showed that heart weight, cardio somatic index (CSI), visceral fat was significantly different between dietary treatments.

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1. INTRODUCTION

1.1 Background

Atlantic salmon, also well known as the "King of Fish," are anadromous, which means they can live in both fresh and saltwater. (Mills, Read and Hadoke 2003)
Their early years are being spent in freshwater and when their body is big enough, they undergo physical and morphological changes then later migrate to seawater as smolt. Atlantic salmon have a relatively complex life history that begins with spawning and juvenile rearing in rivers. (Mills et al., 2003). The Atlantic salmon do not die after initial spawning and are able to spawn again the coming years, so they are described as iteroparous. (Klemetsen et al., 2003)
The Atlantic salmon prefers temperatures from 4 to 12 degrees Celsius but can withstand short periods of time at lower or upper lethal temperatures from -0.7 to 27.8°C respectively (Bigelow, 1963). Due to the efficiencies of farm husbandry practices, the farming process accelerates the life cycle to 1 year or less in fresh water and from 10 to 15 months at sea (FAO 2019).
The salmon is easy to recognise with the streamlined body shape, the dark blue upper side, the shiny skin with black dots and so-called fat (adipose) fin, located in front of the tail fin. The facilities are carefully planned and located in fjords and other areas with good water replacement that ensures optimal living conditions for the fish. (More et al., 2019)
Atlantic salmon is the world`s dominating fish species for aquaculture for diadromous fishes with a total of 1.5 million tons in 2008. (FAO 2019). In Norway, aquaculture is the second largest industry, and Atlantic salmon cultivation the dominating species that in all contributes to the economic development of the country (FAO 2005).
Skeletons are group of connective tissues, which are embedded in the muscle of vertebrates and are not affected by environmental factors consistently. Skeletons play vital role in supporting the fish, gives shape to the fish and is crucial to the growth and development of Atlantic salmon. (Håstein, 2004). In view of this proper studies should be done to ascertain the dietary effect of skeleton on fish.
A detailed research documented that various extrinsic can affect the quality of fish factors such as pre- and post- slaughter handling procedures (Johnston, 1999). The paramount intrinsic quality features are the colour, texture, processing behaviour, fat content, and chemical content of the fillet. (Periago et al., 2005)

Although there are numerous of nutritional factors that may have a high impact on the skeletal development of salmon, the major and strong factor points towards inadequate dietary supply of mineral as the predominant and vital risk factor in nutrition. This is mostly seen in the impact of sub-optical phosphorus (P) and zinc (Zn) supply. (Grete et al., 2009).
Vitamins most especially vitamin A, C, and D play a crucial role in the proper formation of skeleton in the developmental stages of Atlantic salmon. (Jose' et al., 2008). Even though deformities are variable and irregular within the production environment and hence can be influenced by production methods, deformities in Atlantic salmon are greatly influenced by dietary and mineral factors. (Thomas 2003). The colour of salmon fillets is also a good indicator or hint of the freshness and quality of the fish. Processors and distributers will rate it low or even discard products with too little colour. (Nickell and Springate, 2001)

Skeletal deformities and malformations in worse cases can lead to devalue at slaughter and the degeneration of the musculature due to prolong deformities may impede machine processing of the fillet and raises a lot of concerns. (Sullivan et al., 2007). Major research has shown that various nutrients or minerals in the salmon have been proved to have much effect on chronic diseases such as heart diseases etc. (Børresen, 2008)

1.2 Objectives

The objective of this study was to determine the effect of dietary treatments on:
i) Production parameters a) growth parameters and biometric parameters b) calorimetric traits of the skin
ii) Skeletal mechanical properties
iii) Mineral composition of the skeletons
iv) Morphological traits (skeletal deformities (deviations)

2. LITERATURE REVIEW

2.1 Aquaculture performance

Aquaculture is one of the rapid growing sectors of farmed fish and animal production, which contributes to about 50% of the total fish production (FAO 2012). The present world consumption of fish is predicted to be 20kg of fish yearly per capita, and this is partly supplied by aquaculture. (FAO, 2016).

Commercial Aquaculture development in Norway commenced around 1970, since that era aquaculture has developed tremendously into a major industry majorly around the coastal areas. Atlantic salmon intensification is by a great margin, the paramount activity, accounting for more than eighty percent (80%) of the total Norwegian aquaculture production. In 2003 the volume of production of aquaculture was over 600 000 tonnes, having a value of US 1350 million (Directorate of Fisheries, Statistics 2003). In 2010, the Norwegian aquaculture recorded a sum of four billion Euros in value of Atlantic salmon, which is the largest component of Aquaculture production in Norway. (Torgersen et al., 2014)

The EU is the main market of exports of Norwegian production and approximately about 95% of the production is exported. Salmon products are exported globally, and farmed salmon is now one of the major export commodities from Norway. This contributes immensely to the country’s economy and it is still considered when forecasting about future growth of the economy. The main challenge to the sector is to come up with a profitable aquaculture-based species other than salmon and to develop a sustainable quantity of raw materials to feed the industry. (FAO 2005).

2.2 Life cycle and growth of Atlantic salmon

The life cycle of Atlantic salmon mostly starts in the autumn as a buried egg in the ground mostly in the bottom of rivers and streams like the Tweed. It hatches into tiny alevins in the spring. (Jensen et al., 2014)

At this stage they also have their egg linked to the yolk sac, this provides food and nourishment to them. At this stage they cannot swim very well so they hide underneath the gravel for some weeks approximately 4 to 6 weeks. This hiding phenomenon is also very vital because it also protects them against predators. In the later part of spring and early periods of summer, the alevins will have developed into fry, come out from the stones and start feeding on organisms in and on water. (Thorstad et al., 2012)

After about a year the old fry become parr and begin to eat on remains in fresh water and insects between 1 and 4 years, after this time they become smolts. When they become smolts during their second, third or maybe forth year, they migrate to the sea (Jensen et al., 2014). The mature salmon returns to the river to breed, and this phenomenon is termed as spawning. At this stage the females dig a redd, small nest in the gravel with the aid of its tail. The female then releases the eggs which is then fertilized by the milt been produced by the male salmon. (Dfo-mpo.gc.ca, 2019). This process is repeated for the complete procreation and existence of Atlantic salmon.

In early juvenile stages, Atlantic salmon grows very rapidly. Under commercial conditions their growth rates (SGR) is commonly 5-7% per day, combined with a low feed conversion FCR, kg feed fed/per kg gain in body weight). Mineral content of salmon is commonly expressed as whole-body content on wet weight basis. The whole-body analyses are the simplest and the easiest to standardize, and for juvenile salmon there are reliable reference values to compare with (Shearer et al., 1994). Further research has shown that a juvenile with normal mineralization of the skeletal structures should contain 4000mg kg-1 or more of Phosphorus (p) and Calcium (Ca). Mostly the amount of Ca should be balanced to or higher than the level of P. (Aquafeed.com, 2019). "A meaningful knowledge on Atlantic salmon reproduction and physiology does not only allow the life cycle of salmon to be complete, but also helps to even produce in of-season through manipulation of the environment, temperature, enhancing growth rates and ensuring all year-round supply of fresh fillets". (T. Frazer, 2013).

2.3 Feed formulation for Atlantic salmon

Feed formulation is an indispensable and fundamental process in the feed sector, and so plays a pivotal role in the feed industry. It is a very important step needed to meet the standards and requirements of energy and dietary elements. i.e. digestible energy requirement and dietary fat etc. The cost of raw materials and feed ingredients comprises of the major portion in feed formulation process. In view of this we can conclude that the economic aspect or viewpoint cannot be undermined in the feed formulation process. Basically, in the feed sector, the buying of feed ingredients is cost intensive. Meaning it takes up to about 80% of the overall cost and must therefore be really planned and systematic. (Wickins, 1988).

Modern feed formulation is enhanced by commercial linear programming tools and equipment, which is low in cost and maximizes the production of feed efficiently and effectively (Turchini, 2011). Refined fats and oil from both animal and botanic sources i.e. mostly from vegetable sources are used in the modern systems. (Turchini, 2011). Aquafeeds used in feeding salmon basically have two major components, bean oil and protein. Generally, the above listed components emanate from fish and fish oil from farming of fish from the fishing industry and is comparatively low in price and bounty. Due to the tremendous increase in the need of fishmeal and fish oil, whiles there is a rampant increase in human population and the supply of it remains adamant and does not change. As a result of this prices of fish meal and fish oil is always escalating. (Sargent and Tacon,1999). For this main reason, many aquaculture producers have substituted this with plant-based ingredients such as rapeseed oil and soybean oil that is much cheaper and can be sustained. (Sargent and Tacon,1999)

It is a fact that plant-based ingredients have a different constituent than fish-based constituents. An empirical example is plant oils lacking EPA and DHA. Plant based ingredients are mostly composed of higher levels of short chain ALA and linoleic acid (LA) which is basically omega 3 fatty acids. Again, animals synthesize cholesterol and diets composed of plants have low levels of cholesterol implying that the fish must make its inadequacy internally. (Tocher, 2015).

The rise in the application of plant-based ingredients in aquatic feeding for salmon has led to a great upsurge in phytate. Phytate is a binding micro mineral,an antinutrient and a reducing agent which goes along way to reduce the biological availability of nutrients. Research has shown that minerals in a form of chemicals (inorganic or organic) can change the biological availability of these minerals into other forms. This is mostly found in aquafeeds, which are high in the concentration of phytate. (Morales et al., 2016, Gatlin et.,2007).

Hence, it must be well noted that we face challenges when we replace plant oil for marine ingredients.

2.4 Bone structure

Bone is a form of connective tissues in vertebrates which comprises of fibre, matrix and bone cells (Hall, 2015). The cell matrix is the most distinct property of bone, which is made up of high amount of calcium salt deposition that results in a resolute texture. The bone structure is composed of bone matrix, bone marrow and periosteum. The periosteum is tightly attached to the surface of bones and a layer full of dense collagen fibres (Markings, 2004). There are enough nerves and blood vessels, which are distributed in the periosteum, these supply enough nutrients to bones and sensors to nerves (Steele and Bramblett, 1988). One predominant feature vital to the periosteum is that it is made up of osteoblasts and osteoclast that aids in the development, healing and growth and restoration (Mackie, 2003). The Atlantic salmon has an axial skeleton, which fundamentally designed for lateral flexion and undulation and again aid in resisting compression (Stiassny, 2000). A fully developed vertebral column consists of about 57-60 individual vertebrates, which arise by segmental vertebrae of the notochord sheath at post fertilization around 700 day- degrees (Grotmol et al., 2005). The intervertebral space is formed in between the vertebrae which acts as shock absorbers since it is filled with notochord tissue which are formed adjacent at the center (Staissny, 2000). For anatomical purposes the bone is divided into various trunks and caudal regions with trunkcal vertebrae being rib bearing and caudal vertebrae possessing haemal aches. Vertebral body conformation and morphology is mainly reliant on the position of the vertebral body within the vertebral column. (Kacem et al., 1998).

Mechanical properties of the vertebral column are dependent on the region with the mechanically strongest vertebrae located in the anterior caudal region (Fjellanger et al., 2005).

The research into bone is simply branched into two main categories. These are named as “quantity” and “quality” of the bones been examined. The “quantity" of the bone is explained as the volume and number of bones while as the “quality" is the fine structure of bone collagen, mineralized bone matrix and the ability to regenerate, etc.) of the bone which can be seen with a microscope (Rubin *et al.*, 2002).

The sum of the minerals in the bone and the organic matter present is termed as Bone quantity. As at now there is no simple and clear method been used to measure the organic matter content of live bone, in view of this bone mineral content is therefore also regarded as the bone mineral density (BMD). This method has only been used to investigate only in scientific researches and is therefore not regarded as a precise or a definite measurement of bone quantity. With regards to bone strength and bone rigidity, BMD is vital reference point lately. Researchers of our time have realized that BMD can only somewhat show bone strength (Gluer *et al.*, 1994). Bone strength is also dependent on the metabolism and the quality of the bone. Research has shown that BMD can only elucidate fairly 60% - 70% of bone strength. The bone of fish often rated the least among fish by-products is mostly discarded during fish transformation process..

Fish bone is often rich in phosphorus, Calcium and other many essential trace elements vital for the body. Dieticians and specialist in food have referenced or correlated that well ordered or much increase in the intake of fish bone could help prevent many diseases such as osteoporosis etc. (Chiling *et al.*, 2007)

2.5 Bone histomorphometry

This is a continuous thriving field, which deals with the quantitative bone analysis, its changes and architecture. This also aims at investigating the shape or form of bone tissues. It provides valuable information on the amount of bone and its cellular activity thus evaluating the growth and development of cancellous bone and osteoid. This approach has been extensively used in treating diseases of bones. (Mulluche et al., 1982).

2.6 Vertebral body malformations in Atlantic salmon

Malformations of the vertebral column occur in many diverse groups of ray-finned fishes. Malformations are undesirable in the farming of fish. In view of this, there are major on going researches specifically aimed at discovering vertebral malformations recently. (Fisher et al., 2003; Van Eeden et al., 1996).

Vertebral abnormalises in Atlantic salmon has demerits which are detrimental on the fish`s performance, growth and output, this raises eyebrows and interest about the health of animals as far as Atlantic salmon is concerned (Aunsmo et al., 2008). The studies into malformations on farmed Atlantic species have brought a lot of associated risk factors that leads to the emergence of spinal abnormalises. Sum of these basic factors include; parasitic infections, Vitamin c deficiency, Phosphorus (P), egg incubation temperatures, vaccinations, bacteria and lastly environmental pollution. For the sick of this paper the major concern would be on the dietary effect, minerals and vitamins (Berg et al., 2006; Gorman and Breden, 2007; Roy et al., 2002). It is imperative to know that the immense of differences of things in the world must be ordered and put together in a common pool of knowledge before it can be studied and understood. (Mayr and Bock, 2002). In view of this major suggestions and proposals has been made to categorize skeletal abnormalities. There are quite a number of skeletal abnormalities been discovered up to date. In this paper, major concerns are based on the basic malformations in Atlantic salmon. These major deformities are namely; compression of vertebral bodies, fused vertebral bodies and loss of intervertebral space due to shortening of the vertebral column which are mostly compressed. The interest and the requirement of consumers are always paramount and are of vital interest to the aquaculturist. The willingness and the enthusiasm of the buyer to be interested and ready to pay for a product greatly depends on the quality of the product. (Alfnes, et al. 2006). In view of this, skeletal abnormalities are of great concern to the aquaculturist.

2.7 Radiography/x-rays

Radiography, or the use of X-rays for analysis is the preferred method for fish skeletal deformity diagnoses. This is because X-rays have enough energy to penetrate soft tissues. It allows the evaluation of the development and identification of pathology in bones. (Aquafeed.com, 2019). Usually the quality of X-rays is treated to be fluctuating due to the size of the specimens been used, preparation of the specimen, setting of X-ray equipment and the images been processed. The insensitivity for mostly calcified bodies tends to make digital images more variable than analogue images.

The main limiting factors in fish radiography are the object size and low density of the fish skeleton and these limitations are challenged constantly by the need for early diagnostics, therefore leading to the sampling of very small fish. (Kirsti and Grete (as cited in Control of malformations in fish aquaculture. (Aquafeed.com, 2019)

In early juvenile stages, Atlantic salmon grows very rapidly. Under commercial conditions their growth rates (SGR) is commonly 5-7% per day, combined with a low feed conversion FCR, kg feed fed/per kg gain in body weight). Mineral content of salmon is commonly expressed as whole-body content on wet weight basis. The whole-body analyses are the simplest and the easiest to standardize, and for juvenile salmon there are reliable reference values to compare with (Shearer et al., 1994). Further research has shown that a juvenile with normal mineralization of the skeletal structures should contain 4000mg kg-1 or more of Phosphorus (p) and Calcium (Ca). Mostly the amount of Ca should be balanced to or higher than the level of P. (Aquafeed.com, 2019)

2.8 Mechanical analysis of bone

Mechanical properties of bones can be explained by their reaction under pressure or the application of force (Fa-Hwa Cheng, 1997). Basically, materials are measured based on certain factors or criterion. These factors are fracture resistance, impact, hardness, ductility, and strength. There is a major challenge with regards to measuring bone properties; this is as a result of the skeletal structure forming part of an integrated system. The central factors affecting the mechanical properties of bones are namely: 1) Bone status to a certain degree a result of the organism`s interaction with its immediate surrounding. 2) Differences in bone shape and size. 3) Differences in age, sex, lifestyle, developmental stages and growth.

The early investigation of the mechanical properties of bone originates from Galileo. A renowned scientist who did further studies in the seventeenth century (Ascenzi, 1993). Galileo did so well, investigated and proposed that larger vertebrates have disproportionately more strong bones that adapt to mechanical loading, and this is not solely as a result of the size of the vertebrates (Martin, 1999). He also proposed that with relatively the same amount of stress, smaller bones are harder and stronger than large bones. This is factual because the increased weight caused the larger bones to be hollow. These assertions or views many people were ignorant about it. In the 1830s, Wyman described the architecture of trabecula bones. Wyman also propounded the architecture of trabecula bones. Bourgery and Ward also tried to explore the hypothesis, but their explanations later turn out to be deficient and inadequate (Von Meyer, 2011, Wolff, 2012).

3. MATERIALS AND METHODS

3.1 Experimental diet

Four diets were produced and used in this study. The diets were formulated with varying levels of marine protein and oils, vegetable protein and oils, and minerals. The diets were designated i) Marine (**M**) diet produced from a 1995 recipe with alterations with mineral phosphorus, ii) Marine diet with low mineral content (marine with ½ mineral content, **M-LM**), iii) vegetable protein and oils diet (referred to as a control diet, **C**) and iv) The control with additional Vitamin K+ (CK+).

3.2 Experimental design

To study the effect of diets on growth and welfare indicators (deformities in skeletal formations, etc), an experiment was setup at a NOFIMA Research station, Sundalsøra, Norway. Atlantic salmon smolts obtained from Norway were used in this study; the average starting weight was 20g. There were four diets and each diet was replicated three times. Therefore, there were twelve tanks used in this study. Fish and were fed with the corresponding diet as represented in figure 1 below.

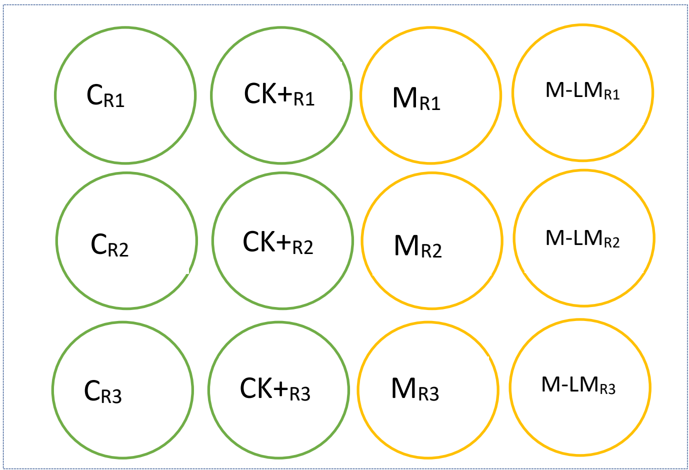

Figure 1: Experimental setup for the study. The underscore R1, R2 and R3 are the replicate for each treatment.

3.3 Sampling protocol

After 16 weeks of experimentation, five fish per tank were randomly selected from 12 tanks (a total of 60 fish) were used for further analysis. The fish had an average weight of approximately 73g. The fish was gutted on the same day, and various calorimetric analysis of the skin were determined and recorded using the instrument Minolta chroma meter (CR-400, KONICA MINOLTA SENSING, INC. JAPAN). Biometric parameters of the fish were also measured. Growth parameters and maturation of the Atlantic salmon were measured accordingly. Visual score of the skin were also measured using the colour hue. The Atlantic salmon were filleted by hand. This is done by a transversal cut at the dorsal border of the opercula to the top of the spine. The knife is curved along the spine and the knife pulled through to the tail end. Cut through the spine and curve the knife, following the line to the tail end to get the second fillet

3.4 Growth related measurement

We measured, round weight with an electronic scale, fork-length (from the tip of the snout to the indentation of the caudal fin), condition factor (CF), carcass yield, organ index, fillet yield and Calorimetric analysis of the skin (skin colour) using the salmofan. The following were how some off the above derived phenotypes were calculated:

- Condition factor is calculated as, CF = live weight (g) / length (cm) ^{3} * 100.
- Organ index: organ weight (g) / body weight (g) * 100.
- Fillet Yield: fillet weight * 2 (g) / body weight (g) * 100.
- Slaughter Yield: gutted weight (g) / body weight (g) * 100.

3.5 Health related traits

Health indicators were measured on all the 60 fish that were slaughtered. The weights of both the heart and liver were taken using the electric balance. We measured the weight of the heart removing bulbous and atrium, and visually scored the heart from 0 to 3 (representing normal to deformities), the weight of the liver, colour of the liver (ranging from 1to 5) and also scored for soft liver (0 to 1). We also computed the heart index (CSI) and liver index (HIS) as follows:

- HIS (liver %): (liver mass / body mass) * 100
- CSI (heart %): (heart mass / body mass) * 100

3.6 Detection of skeletal abnormalities through X-RAY INSPECTION

X-rays were taken of the bone formation of Atlantic salmon (Salmo salar L.). The major aim of the x-rays was to detect skeletal abnormalises of the fish. The X-rays were done using the semi-digital system, using a mammography X-ray source machine. We counted the number of fused (fusion), compressed, hypo-mineralized and vertebral spaces and losses of each fish.

3.7 Mechanical analysis of the vertebrae

In order to present a more understandable, clear and comprehensible support for the process of the optimization of bone, the investigation of mechanical properties of bone has a more practical significance. The mechanical properties or strength of the bones were measured at different points. The main aim of this was to determine how strong the bones are at different points. These points were identified or chosen based on the visual assessment of bones by the X-ray. The two main points chosen were Norwegian quality cut (NQC) which is the area from directly behind the dorsal fin to the anus) and the anterior (ANT).

Basically, to be able to measure the strength of the bone, which is embedded in the muscle, these stages were followed;

- Randomly select a fish
- Cut the tail
- Removed the skin

    In order to remove the skin, the fish was heated in a microwave. One predominant factor in the use of microwave is to get the right temperature /heat treatment and so a heat treatment scale was formulated for the heating. A scale was developed based on the weight of the fish, in other words, the weight of the fish in grams were used to determine the amount of time to heat the fish and this is actually crucial in the sense that we want to denature proteins, not to cook the fish, this also helps to keep the parts of the fish i.e. skeleton intact and to be able to easily remove the skin. We heated the fish at 300W for a) 50 seconds for fish that were < 50g, b) 65 seconds for fish that were between 50 – 65 grams and finally, c) 80 seconds for fish that were between 65 – 80 g.

- Locate the NQC and cut that part of the skeleton
- Locate the anterior part
- Clean the skeleton- separate other parts from the skeleton
- The skeleton is been placed on the texture board, and then a command is been made for the texture analyser to automatically measure the texture at the ANT region and the NQC region.

In this experiment the fish sizes were small, so the texture analyser calibrations were adjusted especially with the speed and force of the knife for efficient measurement of the texture. This texture analyser applies this principle by performing the procedure automatically and indicating the results visually on a digital numerical display.

The texture /strength of both the ANT and NQC were measured using (TA-XT2 texture analyser stable micro systems, Surrey, UK) by compressing a guillotine knife with 70mm width, of 3mm thickness) into the middle position of the vertebra with trigger force of 1N at a speed of 0.5mm/sec until it broke the rib. The force required to puncture the rib surface can also be described as stiffness. This was recorded. The system was programmed to record forces at;

i. 15% and Forces at 50% respectively.
ii. Area at 15% and Area at 50% respectively.
iii. Thickness at both ANT and NQC were measured respectively.

The breaking force is most usually the indicator of texture quality of fillet. The effect of texture on quality, measurement procedures is readily analysed into details. The required force N to puncture the surface of the bone is considered to measure the breaking force. The samples were kept in the freezing room for some time after measuring the textures at various portions.

3.8 Mineral analysis of skeleton

The samples of the vertebral column were collected by cutting two representative portions ANT. This stands for the anterior portion and the NQC stands for the Norwegian quality cut column. The samples were labelled accordingly. The flesh was removed from the vertebrae manually, and the bone tissue was thoroughly cleaned with a soft, smooth flexible cleaning brush, with NaCl solution of 0.9%. The samples were later dried with paper towels. The samples for each portions ANT and NQC were mashed and homogenized with the help of a blender (Waring Commercial blender, Waring Commercial, CT, USA) by adding solid carbon dioxide. Samples were instantly frozen and stored at kept at -70 degrees Celsius (-20°C) before it was analysed in dry ice.

For Laboratory analyses of minerals, the homogenized samples were digested in 5mL of ultrapure concentrated nitric acid at 260 degrees Celsius in an ultraclave from Milestone and diluted to 50.0 mL with DI water after digestion. Blank samples and certified reference material of bone meal (NIST 1486 Bone meal) were digested at the same time. Quantified samples will be on an ICP-OES OR AN ICP-MS depending on the amount of concentration. Minerals analyzed for in this experiment were Calcium (Ca), Magnesium (Mg), Zinc (Zn), Phosphorus (P) and Manganese (Mn).

3.9 Statistical analysis

The phenotypes recorded were summarised into means, minimum, maximums and standard deviations. The results were presented in tables.

To analyse the growth-related measurements, i.e. (traits presented in section 3.4 and 3.5), the following statistical analysis was undertaken.

$$y = Diet + sex + e$$

y were the growth-related measurements, $Diet$ was the fixed effect of the four (4) different diet used to feed the fish, sex was the sex of the fish and e was a residual term of the model. Significant differences between diets were determined at P<0.05. The analysis was undertaken with the help of GLM procedure in SAS (version 9.4 TS level 1M2, SAS Windows version; SAS institute, Cary, NC, USA).

The model used to analyse the mechanical properties and mineral composition of the bones was as follows

$$y = Diet + Zr + e$$

y was mineral content in bone (Area 50% in mm, Force at 50% and thickness), $Diet$ was the fixed effect of the four (4) different diet used to feed the fish, Zr was the incidence matrix (Z) and a random effect (r) of the twelve (12) replicated tanks used in the study, 3 replicate for each diet ($= 4 \times 3$) and e was a residual term of the model. Significant differences between diets were determined at P<0.05 or P<0.10. The analysis was undertaken with R statistical analysis software using the *lmer* and *glht* function.

For ribs /skeletal information using the X-ray. The data for individual vertebrae were statistically analysed using GLM procedure in SAS.

4. RESULTS

4.1 Visual score of the skin and scale damage of whole fish

Table 1 shows the visual scoring of the whole fish on skin colour and scale loss. The average skin colour score ranged from 0.50 to 0.30. The standard control diet (C) had the highest score while the standard marine diet had the lowest, however, these differences were not significant. For lightness (L*) of the skin, although none of the differences was significant, the largest mean value was recorded for the control diet with vitamin K (65.8) and the smallest values was recorded for the standard marine diet (55).

For red-green colour, a* the average value ranged from -0.20 to 0.70. A negative red-green value depicts more of greenness and a positive value depicts redness. Therefore, the results show that adding Vitamin K to the standard control diet increased the colour of the fish from green to red (i.e. from -1.7 to 0.70). This increase of 1 unit from the standard control diet was significant. Using a marine diet with low mineral content also increased the redness of the fish. The average red-green value for the standard marine diet was -2.7 while that of the marine diet with low mineral content was -0.2 and this difference was statistically significant.

There was no significant difference between all the diet for the yellow-blue, b* colour except for the standard control diet with additional vitamin K. The addition of vitamin K to the standard control diet caused the fish to be less yellow.

There was significantly less scale loss on the fish with the marine diets than those feed the two standard control diets (C and CK+ diets).

Table 1: Visual score of skin colour (quality trait), colorimetric parameters (L*, a*, b*) and scale loss of Atlantic salmon fed four different diets: 1a control diet (C), 2a the same control diet plus vitamin k (CK+), 3a diet based only on marine ingredients (M) or 4the same marine diet with low mineral level (1/2 concentration; M-LM)

Trait	Control diet		Marine diet	
	Standard (C)	+Vitamin K (CK+)	Standard (M)	Low mineral (M-LM)
Skin colour, score	0.46 ± 0.12	0.40 ± 0.11	0.32 ± 0.13	0.42 ± 0.12
Scale loss score, c	1.23 ± 0.15[a]	0.46 ± 0.14[a]	0.43 ± 0.11[b]	0.96 ± 0.17[b]
Lightness, L*	57.37 ± 3.96	65.97 ± 4.13	55.77 ± 2.57	56.42 ± 4.28
Red-green, a*	-1.70 ± 0.93[b]	0.73 ± 0.46[a]	-2.72 ± 0.81[b]	-0.17 ± 0.59[a]
Yellow-blue, b*	3.81 ± 0.79[a]	1.56 ± 0.80[b]	3.80 ± 0.63[a]	3.64 ± 0.70[a]

Values in the same line with different superscripts are significantly different ($p < 0.05$).

4.2 Growth and slaughter traits

The average body weight ranged from 71.6g to 74.6g with the highest weight recorded for the standard control diet plus additional vitamin K (Table 2). The differences in average weight, length of the fish, condition factor, gutted weight and slaughter yield were not significantly between all treatments.

The standard marine diet had the lowest fat level of all the diets (2.3) and this was different from all other diet except the standard control diet plus additional vitamin K. The increase in fat levels ranged from +0.50 to +0.30 with the standard marine diet with low mineral content having the largest value (2.8).

Table 2: Biometric traits and score data for all four diets

Trait	Control diet		Marine diet	
	Standard C	+Vitamin (CK+)	Standard (M)	Low mineral (M-LM)
Body Weight(g)	74.27 ± 1.68	74.62 ± 2.85	72.12 ± 2.20	71.62 ± 2.25
Length(cm)	18.15 ± 0.13	18.09 ± 0.22	18.04 ± 0.15	17.94 ± 0.20
Gutted Weight(g)	64.69 ± 1.51	65.51 ± 2.51	62.99 ± 1.92	62.71 ± 1.97
Heart Weight(g)	0.12 ± 0.00^{a}	0.11 ± 0.01^{ab}	0.12 ± 0.00^{a}	0.10 ± 0.01^{b}
Liver Weight(g)	0.67 ± 0.02	0.68 ± 0.02	0.67 ± 0.03	0.64 ± 0.04
Calculated traits:				
Condition Factor (CF)	1.24 ± 0.01	1.24 ± 0.01	1.22 ± 0.01	1.23 ± 0.01
Slaughter yield (%)	87.15 ± 0.67	87.81 ± 0.30	87.37 ± 0.40	87.57 ± 0.31
Heart /CSI (%)	0.16 ± 0.00^{ab}	0.14 ± 0.00^{bc}	0.16 ± 0.00^{a}	0.14 ± 0.01^{c}
HSI (%)	0.90 ± 0.02	0.93 ± 0.02	0.92 ± 0.03	0.88 ± 0.04
Score data:				
Visceral fat (score 0-5)	2.70 ± 0.10^{a}	2.60 ± 0.09^{ab}	2.30 ± 0.12^{b}	2.80 ± 0.13^{a}
Heart Score (0-2)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Soft Liver (score 0-1)	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.53 ± 0.09^{a}
liver Colour (score1-5)	3.87 ± 0.06	3.91 ± 0.06	3.98 ± 0.09	3.81 ± 0.07

Values on the same line with different superscripts are significantly different (P < 0.05).
In all the parameters measured, sex was not statistically significant, meaning sex had no effect on all the traits.
Control diet (C), the same control diet plus vitamin K (CK+), marine ingredients (M) and marine diet with low mineral level (1/2 concentration; M-LM).

4.3 Health indicators

4.3.1 Weight of the liver and heart

The average liver and heart weight ranged from 0.64g to 0.68g and from 0.09g to 0.12g, respectively (Table 2). The highest liver weight was recorded in the control plus vitamin K diet (0.7) and the lowest was recorded in the marine with low mineral diet (M-LM). However, this difference was not significant (Table 2). The lowest heart weight was obtained for fish on the standard marine diet with low mineral content (M-LM) and the highest average value recorded for the standard control diet (C). The reduction of mineral in the standard marine diet (M-LM) resulted in a significantly (P<0.05, Table 2) low heart score than the standard marine diet (M). There was no difference in heart value between all the other 3 diets (C, CK+ and M).

4.3.2 Heart score, colour of liver and soft liver

There was no significance difference between diets for heart score and colour of liver. However, for soft liver, reduction in the mineral content of the marine diet (M-LM) resulted in significantly higher softer liver than all other diets (Table 2).

4.3.3 Heart and liver index

The average CSI value ranged from 0.14 to 0.16. Among the marine diet, the lowest CSI was recorded for fish on the marine diet with low mineral content. The difference between the two marine diet was statistically significant. Among the control diet, the control diet with additionally vitamin K had the lowest CSI, however this difference was not statistically significant. Furthermore, there was no difference between the standard marine and control diet, and between the control diet plus vitamin K and the marine diet with low mineral content.
For liver index, there were no significance differences between the diets.

4.4 Mechanical properties of the skeleton

Table 3 shows the summary information about the mechanical properties of the skeleton of the anterior and the Norwegian quality cut sections of the fish. Figure 2a-c also shows bar graph of the least square means and the significant differences between the dietary treatments.

4.4.1 Area at 50% of the skeleton

For the anterior section, the lowest mean area at 50% value was recorded for the marine diet with low mineral content (8.76mm) and the highest value was recorded for the control diet with additional vitamin K (9.8mm) (Table 3). The difference in area at 50% between diets was not significant (Figure 2a).

For the Norwegian quality cut section, the average values ranged from 9.9mm to 14.6mm (Table 3). The marine diet had the lowest area at 50% value and was statistically significant from all treatments except the marine diet with low mineral content (Figure 2a).

Table 3: Summary statistics of the anterior quality cut (ANT) and the Norwegian quality cut (NQC) for the mechanical properties of salmon skeleton.

Parameters	Diet	ANT					NQC				
		N	mean	SD	min	max	N	mean	SD	min	max
Area 50% (mm)	C	12	9.484	2.53	4.246	13.773	12	14.624	2.948	10.547	19.002
	CK+	15	9.864	2.89	3.077	15.329	15	13.175	3.595	7.948	20.20
	M	14	9.009	1.443	6.188	11.077	16	9.913	3.555	4.852	17.71
	M-LM	15	8.764	2.18	5.549	12.099	15	12.937	3.397	4.383	16.982
Force 50%	C	12	13.331	4.586	4.546	20.185	12	19.830	2.997	14.766	24.064
	CK+	15	14.586	4.515	3.078	22.972	15	18.692	5.032	7.131	26.08
	M	14	11.754	3.707	3.382	16.633	16	13.024	7.01	3.841	24.59
	M-LM	15	11.849	4.663	3.496	17.994	15	18.246	5.942	2.419	26.451
Thickness	C	12	2.108	0.185	1.807	2.477	12	2.197	0.155	1.929	2.379
	CK+	15	2.143	0.225	1.682	2.484	15	2.262	0.248	1.839	2.754
	M	14	2.075	0.199	1.802	2.462	16	2.162	0.182	1.877	2.499
	M-LM	15	2.092	0.192	1.699	2.559	15	2.260	0.219	1.962	2.714

Control diet (C), the same control diet plus vitamin K (CK+), marine ingredients (M) and marine diet with low mineral level (1/2 concentration; M-LM).

4.4.2 Compression force of the skeleton at 50%

The two marine diet had the lowest force at 50% value (13.0 and 18.2; Table 3) compared to the standard diet (18.7 and 19.8; Table 3). The compression force required for the fish feed with the marine diet was 34% ((13.0-19.8)/19.8=0.34) lower than the standard control diet. The difference in force between the standard marine diet and all the other diet was statistically significant (Figure 2b).

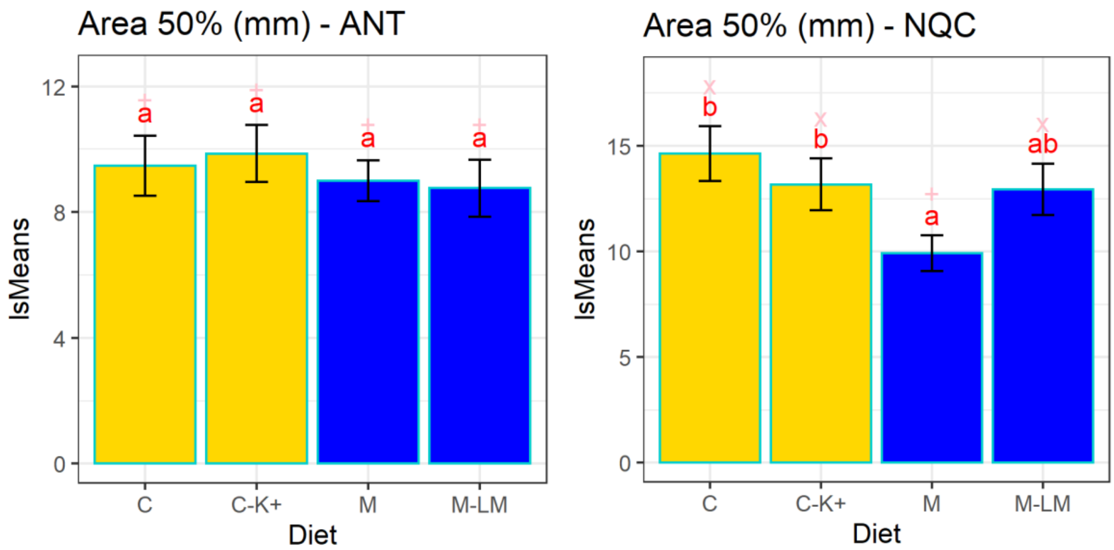

*Figure 2 a**:** Least square means of Area 50% (mm) of the mechanical properties of salmon vertebrates. for four (4) diets (C – standard control diet, C-K+ - Control diet + Vit K, M – standard marine diet, M-LM – marine diet with low mineral diet) feed to the fish. Different alphabet at the top of the bars means that the diets were significantly different with P-value < 0.05. Different symbols at the top of the bars means that the diets were significantly different with P-value < 0.10.*

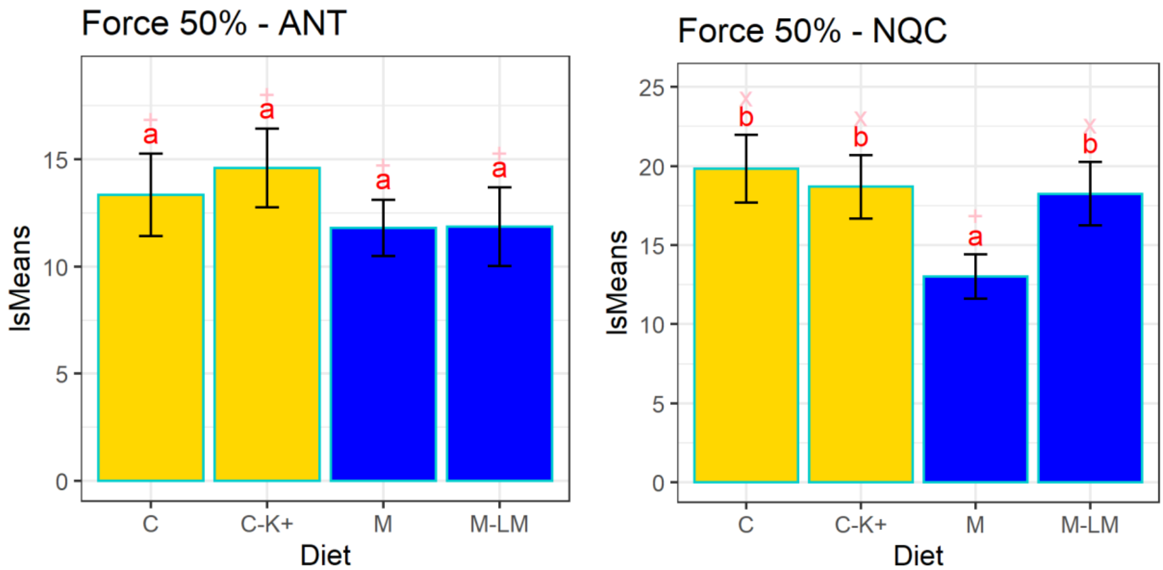

Figure 2 b: Least square means of Force 50% of the mechanical properties of salmon vertebrates or ribs for four (4) diets (C – standard control diet, C-K+ - Control diet +vit K, M – standard marine diet, M-LM – marine diet with low mineral diet) feed to the fish. Different alphabet at the top of the bars means that the diets were significantly different with P-value < 0.05. Different symbols at the top of the bars means that the diets were significantly different with P-value < 0.10.

4.4.3 Thickness of the skeleton

The average thickness of the vertebrate ranged from 2.07 mm to 2.14 mm for the anterior quality cut and from 2.16mm to 2.26mm for the Norwegian quality cut side of the fish (Table 3). For the anterior quality cut side of the fish, the marine diet had the lowest value, while the control diet plus vitamin K had the highest values. However, there was no significant difference between all the diets (Figure 2c). The same trend was observed for NQC and the difference between diet were not also significant.

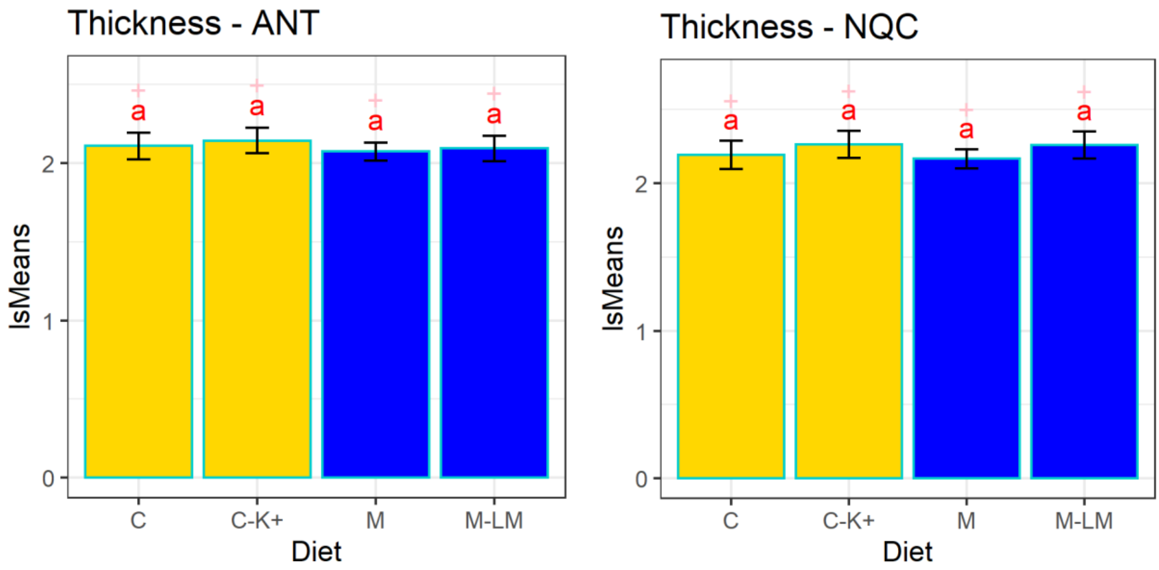

Figure 2 c: Least square means of thickness of the mechanical properties of salmon vertebrates for four (4) diets (C – standard control diet, C-K+ -Control diet + vit K diet, M – standard marine diet, M-LM – marine diet with low mineral diet) feed to the fish. Different alphabet at the top of the bars means that the diets were significantly different with P-value < 0.05. Different symbols at the top of the bars means that the diets were significantly different with P-value < 0.10.

4.5 Mineral contents of the skeleton

Table 4: summary of the mineral composition of the vertebrates and ribs of the salmon for each of the diets

Mineral*	Diet	N	mean	SD	min	Max
Ca	M	10	48.70	8.73	34.00	67.00
Ca	CK+	11	40.45	3.80	34.00	46.00
Ca	C	16	39.06	7.51	26.00	49.00
Ca	M-LM	12	38.83	5.81	32.00	46.00
Mg	M	10	1.12	0.18	0.79	1.50
Mg	CK+	11	0.96	0.09	0.83	1.10
Mg	C	16	0.95	0.15	0.67	1.20
Mg	M-LM	12	0.91	0.12	0.77	1.10
P	M	10	28.50	4.93	20.00	39.00
P	CK+	11	24.09	2.12	21.00	28.00
P	C	16	23.31	4.36	15.00	29.00
P	M-LM	12	23.17	3.10	19.00	27.00
Zn	M	10	127.80	16.59	98.00	150.00
Zn	CK+	11	103.09	10.74	79.00	120.00
Zn	C	16	104.56	21.86	68.00	140.00
Zn	M-LM	12	96.75	11.76	83.00	120.00
Mn	M	10	8.08	1.82	6.00	12.00
Mn	CK+	11	10.82	1.06	9.20	13.00
Mn	C	16	10.14	2.54	6.00	14.00
Mn	M-LM	12	8.10	1.28	6.40	9.60

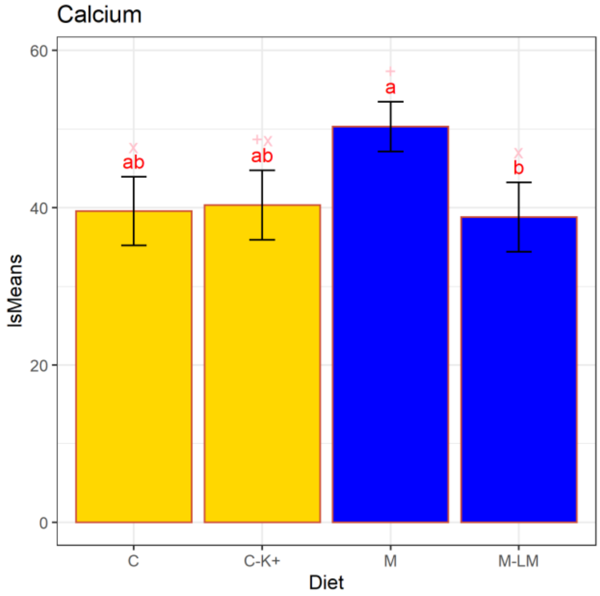

Figure 3 a: Least square means of Calcium in the skeleton for four (4) diets (C – standard control diet, C-K+ - Control diet + vit K, M – standard marine diet, M-LM – marine diet with low mineral diet) feed to the fish. Different alphabet at the top of the bars means that the diets were significantly different with P-value < 0.05. Different symbols at the top of the bars means that the diets were significantly different with P-value < 0.10.

4.5.1. Calcium concentration in vertebrae

The average mean of Calcium in the vertebras ranged from 38.83 – 40.70. The highest concentration of calcium was obtained in the marine diet. (M) Followed by Control + vit. K(CK+) diet as shown in fig. 3a.

However, there were significant differences between treatment means.

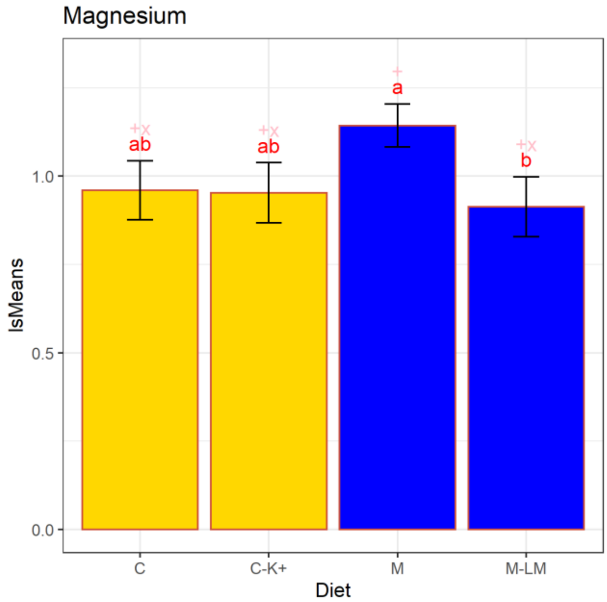

Figure 3 b: Least square means of Magnesium in the skeleton for four (4) diets (C – standard control diet, C-K+ - Control diet + vit K, M – standard marine diet, M-LM – marine diet with low mineral diet) feed to the fish. Different alphabet at the top of the bars means that the diets were significantly different with P-value < 0.05. Different symbols at the top of the bars means that the diets were significantly different with P-value < 0.10.

4.5.2 MAGNESIUM CONCENTRATION IN VERTEBRAE

The standard Marine diet (M) recorded the highest mean in all the treatments with Marine low mineral having the least Mg content. These means were statistically significant from each other as shown in figure 3b.

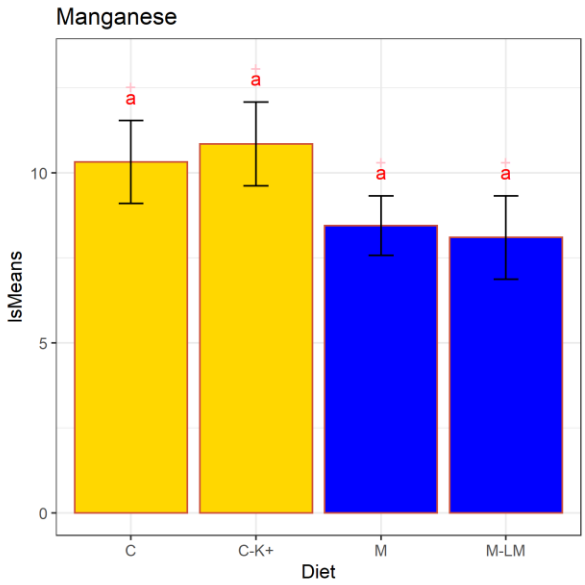

Figure 3 c: Least square means of Manganese in the salmon skeleton for four (4) diets (C – standard control diet, C-K+ - Control +vit K, M – standard marine diet, M-LM – marine diet with low mineral diet) feed to the fish. Different alphabet at the top of the bars means that the diets were significantly different with P-value < 0.05. Different symbols at the top of the bars means that the diets were significantly different with P-value < 0.10.

4.5.3 MANGANESE CONCENTRATION IN VERTEBRAE

Numerically, Control + vit K(CK+) diet recorded the highest mean in terms of Manganese content as 10.82. The range of means for Manganese was recorded as 2.74. It is interesting to note that the control diets (C, CK+) and the control + vit K diet had the highest means. There was no significant difference between dietary treatments statistically.

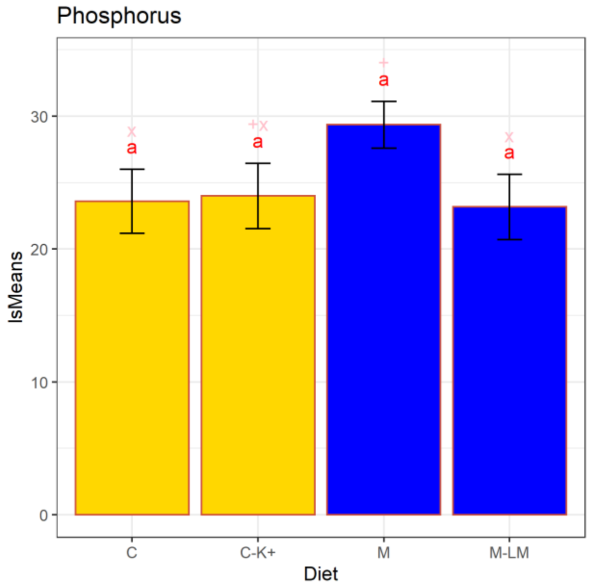

Figure 3 d: Least square means of Phosphorus in the skeleton. for four (4) diets (C – standard control diet, C-K+ - Control diet + vit K, M – standard marine diet, M-LM – marine diet with low mineral diet) feed to the fish. Different alphabet at the top of the bars means that the diets were significantly different with P-value < 0.05. Different symbols at the top of the bars means that the diets were significantly different with P-value < 0.10.

4.5.4 PHOSPHORUS CONCENTRATION IN VERTEBRAE

The average Phosphorus concentration in this analysis ranged from 23.17 to 28.50 with Standard Marine diet having the highest concentration of phosphorus.

However, there were no significant differences between the various treatments means statistically.

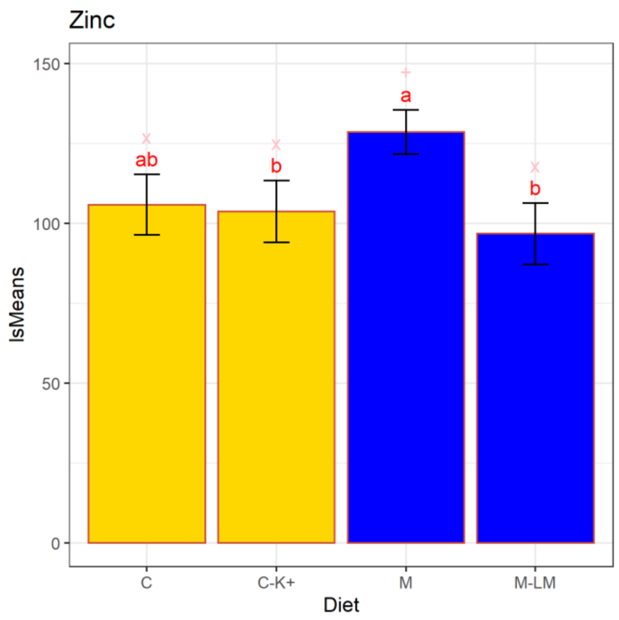

Figure 3 e: Least square means of Zinc in the salmon skeleton for four (4) diets (C – standard control diet, C-K+ - Control diet + vit K, M – standard marine diet, M-LM – marine diet with low mineral diet) feed to the fish. Different alphabet at the top of the bars means that the diets were significantly different with P-value < 0.05. Different symbols at the top of the bars means that the diets were significantly different with P-value < 0.10.

4.5.5 ZINC CONCENTRATION IN VERTEBRA

Among the marine diets, the standard marine diets recorded the highest Zn concentration than the Marine low mineral diet as shown in fig 3e. The range between treatments recorded was from 96.75 -127.80. with an average of 31.05. The differences in averages of Zinc (Zn) concentration treatments were statistically significant.

4.6 Malformation of the vertebrae based on x-ray analysis.

Because only a few fillets that were analysis exhibited any form of malformation, the results are presented by describing the malformations. Four fillets out of the 120 fillets examined had vertebrae that were fused (fusion). Two (i.e. 2 fillet out of 30 fillet) were fed control diet with vitamin K (in both cases, it was one vertebrae that was fused), one each from the fish fed the standard control diet (two vertebrae were fused) and the marine diet with low mineral content (three vertebrae were fused). Two fillets from fish (one fillet each) that were fed the control diet and marine diet with low mineral content had loss of vertebrae (also referred to as space in vertebrae). Four and three vertebrae were lost from the fillet with the control diet and marine diet with low mineral content, respectively. Only one fillet from the control diet with addition of vitamin K exhibited compressed vertebrae. And finally, there were no hypomineralization of the vertebrae for any of the samples, thus no dietary effect was observed.

5. DISCUSSION

In recent times, many other forms of expertise or modern means are been exploited, used to analyse or examine the mechanical properties of bones in general. Such modern methods can be 3D imaging, non-identification etc.

The enormous intricacies in examining mechanical properties has to do with the material composition of bones been examined. Bear in mind that these materials are of different nature, structure, composition and quality. The material feature of bones, its differences and diversities make measurement of bones vary as a result of direction of measurement. (Jameson R. J, 2014).

In this chapter, I will summarize the treatments and its effect on the mechanical properties of the salmon. From the current research, the main aim was based on the effect of various dietary treatments on the vertebras of the salmon.

First, I will summarize the various treatments and its effect on the calorimetric, biometric traits and score data of the salmon.

Over the years, Marine diet has been proven to be the best diet for feeding salmon, but it is faced with major challenges. These challenges are i) very expensive ii) ethical concerns.

The ethical concerns have to do with harvesting marine products to feed marine fish or using fish products to feed fish. Some researchers argue that if we are to feed salmon with marine diet, meaning we have to harvest half of the ocean, which has tremendous effect in the long run.

In view of this, there is the need to replace marine proteins and oils with processing by products and plant substitutes and alternatives which will improve the sustainability farmed fish species such as Atlantic salmon as elucidated in Gatlin et al., 2007;Tacon & Metian, 2008; Naylor et al., 2009; 2011; 2010; Hardy, 2010; Welch et al., 2010; Stone et al., 2011a, b).

BIOMETRIC TRAITS AND SCORE DATA

The present study showed that feeding the salmon with all the dietary treatments did not affect body weight, length, gutted weight, liver weights significantly. However, the numerical values displayed that the average body of the Control diets were higher than that of the marine diets which co-related what Fountoulaki et al., postulated in 2009).

VISCERAL FAT

Visceral fat was high in Marine low mineral (M-LM) diets than the other treatments. Accumulation of fat around the viscera was as a result of the low deposition of mineral. In other words, the availability of minerals around the viscera contributed to low accumulation of fat. Again, Marine diet that is rich in nutrients had the lowest fat score, which is a good recommendation. Increased in fat concentration resulted in reduced nutrients which relates to what Berg et al., (2006), Berg et al., (2007)., Hillestad et al. (1998)., Fjellanger et al., 2000)

CSI

Basically, it shows the ratio of liver to heart weight correlated to full body weight. The control diets presented a significant higher value of CSI during the fresh water sampling stage, than the control + vit K and the marine low mineral diet. There were however much significant difference between treatments. Again, it is interesting to note that the marine diet had a higher CSI value than all the treatments. This agrees to what Larsen et al., reported in 2012

Bigger livers may indicate an increase in fat that goes a long run to reduce metabolic activities. A higher CSI value may be as a result of accumulation of fat.

HEART WEIGHT

The treatments were significantly different from each other. However, there were no significant difference between the Control diet and the Marine diet, rather there were difference between Control + vit k(CK+) marine diet and M-LM diet.

It was clear that both the marine diet and the standard diet had no significant difference between them. And the difference between both diets (Marine and standard control diet) and other treatments was not that huge. This is in contrast to what Rørvik et al., documented in 2008). In their experiment the standard diet had a higher heart weight than the marine diet. Not forgetting the fact that they were in seawater phase.

SCALE LOSS

Scale loss was very high in the control standard diet (C) than all the other dietary treatments followed by marine low mineral diet (M-LM). However, there were significant difference between the control diets and the Marine diets. This may occur in fish as a result of smoltification phenomenon or process. During this stage, the salmon undergo physiological and morphological changes prior to been transported to sea water. This agrees to what Lekang, 2008) documented.

MECHANICAL PROPERTIES

THICKNESS

In terms of thickness, the NQC increased in all the diets than the Ant portion/section. According to the results in section 4.4.3 shown clearly on fig 2c, the thickness of the salmon vertebraes was not significantly different. This applies to both the different portions/ sections of the vertebrae, precisely the NQC, and the anterior.

According to the results shown on fig. 2b. The breaking force of salmon vertebraes was increased from the anterior to the tail section (NQC). In other words, the vertebrae strength of salmon was much stronger at the portion nearest to the tail which was described as the NQC in this paper.

From the graph in Fig 2b.

There were no significant differences in force between diets in the ANT. region.

A comparison of the maximum compression force attained in all the diet, and its corresponding thickness showed that the maximum thickness value among the diets showed the maximum compression force. In other words, the thickness corresponded to its strength.

The maximum compression force can occur at different depth within the same vertebrae as compression force continued until 50 per cent thickness.

Although the method of division of the vertebrae region was not in trunks as outlined in what Kacem and co. postulated in 1998 this was not adopted in this research paper.

The part which had the greatest stiffness and length was the NQC or the part closest to the tail this reaffirms what Fjelldal et al, documented in 2004, 2006) as vertebrae length and thickness increased at the tail region.

Although compression force was able to reflect a clear mechanical property of the salmon vertebrae. The total work (represented by the area under the graph, N*s)

Area at 50% showed a more detailed variation of mechanical properties.

AREA

The marine diets had the lowest area, but the Control diets increased appreciably in terms of area. It was vital to note that there was significant difference between treatment means in the NQC but not significant in the ANT.

FORCE

The marine diets had the lowest compression force. Control diet specifically CK+ had the highest area.

THICKNESS

The control diets increased in thickness than the marine diets but there was no significant difference between treatments.

The implication of the statements above shows clearly that the Marine diets had the softest vertebrae and that the control diet had a stiffer vertebra. In a nutshell, the control diet had the highest vertebrae strength.

MINERAL CONCENTRATION OF SKELETON

The marine diet had the highest Calcium, Magnesium, Phosphorus and Zinc concentration followed by the Control + Vit k diet (CK+). With the exception of Manganese, CK+ recorded the highest concentration of Mn.

Phosphorus(P) is a vital mineral for fish which is a major constituent of bone. The majority of phosphorus in plant sources is bound to a phytic acid making it more or less unavailable to the fish. This may lead to a very odd situation, because it makes dietary content of phosphorus not available leading to higher levels been discarded to the environment. In this study, the Standard marine diet had the highest concentration of Phosphorus(P). There was no significant difference between treatment means statistically.

Marine diet did not have an effect on most of the parameters discussed in this paper, but it was interesting to note that the marine diet was the highest in terms of mineral concentration. This may be due to the architecture of the skeleton. This is in line with what Parnell and Grimal discovered in 2009).

6. CONCLUSION

- Marine diet had the lowest strength
- Control diet had the greatest strength and had high stiffness
- With regards to higher mineral concentration, Marine diet was the better diet comparatively.
- Control diet proved to be useful and economical and is recommendable.
- More feed trials should be done in further studies.
- Since this sampling was taken prior to fish been transferred from fresh water to sea water phase, it will be interesting to see the dietary effect of the fish after harvest.

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