

The Faculty of Bioscience, Fisheries and Economics

Effect of omega 3 fatty acid on growth in Atlantic salmon (*Salmo salar*) aquaculture

A systematic literature review

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Abstract

Background: The effect of diet on fatty acids in Atlantic salmon, particularly omega-3 longchain polyunsaturated fatty acids (n-3 LC PUFA), has been understudied due to lack of marine raw materials (fish meal and fish oil). A variety of ingredients are used as replacements for fish meal for Atlantic salmon. The aim of this systematic literature review (SLR) is examining the effect of omega 3 fatty acid on growth in Atlantic salmon.

Methods: The present study conducted a systematic literature review to provide a summary of currently available information and to identify the most significant effect of omega-3 levels on growth in Atlantic salmon.

Main finding: There were not so many studies which met the inclusive criteria, therefore only 21 of 844 papers were selected from which to extract the data. Indicators for the growth such as weight gain, feed conversion ratio (FCR), and specific growth rate (SGR) have been collected from those papers. This study combined multiple studies and analyzed the output data, but it was not exactly a meta-analysis as it did not measure outcome variables in response to a general control and lack of data. 14 of the 21 experiments demonstrated that the reduction of omega 3 fatty acid had no effect on the growth of the fish. Contradictorily, there were two papers that demonstrated that the omega 3 fatty acid directly influences growth in salmon. 5 of the 21 papers found that the growth performance in salmon has been slightly reduced when the fish were fed with less or no omega 3 fatty acid levels. Almost all of the papers concluded that there was no significant difference in the final weight between experiment groups.

Conclusion: A potential trend in aquaculture is to include more plant-based ingredients in fish meal to replace the traditional fish meal- and fish oil-based diet. However, digesting plant material may pose a challenge for the fish, influencing the growth performance. Therefore, the minimum requirement for omega 3 fatty acid levels should be upheld during the process of incorporating ingredients into the feed. Further research is required on the ratio of DHA versus EPA to evaluate how each fatty acid affects the growth of Atlantic salmon.

Keywords: Omega 3, fatty acid, growth, health, Atlantic salmon, aquaculture, systematic literature review

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1 Introduction

1.1 Overview

Aquaculture holds a critical role in meeting the world's demand for sea food. The Atlantic salmon (*Salmo salar*) is one of the largest farmed cultured species in marine aquaculture during the last decade. The total supply of all farmed salmonids exceeded 2.65 million tonnes in 2020 Salmon, H., & Handbook, I. (2021). Most farmed salmon come from Norway, Chile, Scotland and Canada. Farmed salmon accounts for 95% of all farmed fish in Norway. The total weight of Norwegian Salmon exported in 2019 was 1.12 million tonnes and the total value in NOK of Norwegian global salmon exports in 2019 was 72.5 billion (Directorate of Fisheries, 2021). Nowadays, salmon consumption is considered to be healthy due to its high content of protein and omega-3 fatty acids and the fact that it is also a good source of minerals and vitamins.

The production of high-quality fishmeal has not kept up with the growth of the industry and the increasing stock densities. The feed demand for aquaculture is huge. In 2018, for example, feed for salmon farmers was 1.65 million tonnes (Fiskeridirektoratet, 2021). As a result, the aquaculture industry might face a shortage of fish feed in the future. Lack of marine raw materials (fish meal and fish oil) has led feed producers to replace these raw materials with other alternative oil sources that have other fatty acids. A variety of ingredients are currently used as replacements for fish meal for Atlantic salmon. When marine oils are reduced in fish feed, the lipid content of the feed also changes through the lifecycle of the fish. Since the 1990s, there have been many studies on the change of fatty acid composition in fish diets, in order to ensure a good performance in terms of growth and health. Since 2000, concerns about fish oil supply have prompted an increasing number of substitutions of fish oil with vegetable (mainly rapeseed oil). Although this can be a cost-effective solution for saving protein to meet the energy requirements of farmed fish and crustaceans, the lack of EPA and DHA in vegetable oils can have serious consequences for the growth and welfare of fish and, furthermore, for the human health benefits of salmon consumption (Shepherd & Bachis, 2014). Therefore, understanding the impact of fatty acids on the healthy growth of salmon has become extremely important in this industry.

1.2 Fatty acids and its importance to fish health

Fatty acids are a part of the lipids class, being important constituents of the membrane cell. They have a methyl group on one end and have long hydrocarbon chain carrying a carboxyl group on the other end. Fatty acids molecules are classified based on the presence and number of double bonds: saturated acids have no double bond, monounsaturated fatty acids have a single double bond, and polyunsaturated fatty acids (PUFA) have two or more double bonds. The number and position of double bonds determine the physical properties of fatty acids (Jane B. Reece et al., n.d.). The fatty acids are named based on how many carbon atoms there are in the carbon chain, how many double bonds there are in the carbon chain, and what position the first double bond is in the 3th position from the methylene end: the naming becomes 22: 6n-3. DHA is an omega-3 fatty acid also called an n-3 fatty acid. There are two kinds of omega-3 fatty acids in fish: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Figure 1 describes the way to name EPA and DHA according to their chemical structure.

n-3 PUFA



Docosahexaenoic acid (22:6n-3)

Figure 1: Name of EPA (20:5n-3) and DHA (22:6n-3)

Fatty acids are indispensable for several biological functions. They have biological activities that act to influence cell and tissue metabolism, function, and responsiveness to hormonal and other signals. An example of the way in which cell membrane are influenced by fatty acids can be seen in phospholipids. A phospholipid has two fatty acids attached to glycerol which

determines the physical properties of the membrane. When the two fatty acid composition have interactions with cholesterol and proteins, this may be enzymes or part of the cytoskeletal material. The capacity of unsaturation of the fatty acids is important in determining the fluidity of the membrane and in providing the correct environment for membrane functions (Calder, 2015). In fish, the degree of unsaturation of membrane fatty acids is also important in the process of adaptation to different environmental temperatures. Furthermore, certain membrane phospholipids and their constituent fatty acids are very active in the metabolism thanks to these to these structural functions. This keeps important roles in the supplying of precursors for prostaglandin¹(Benhamed et al., 2014).

Fats and fatty acids are considered the best source of energy, particularly for carnivorous animals such as salmon and trout, as they burn fat efficiently to produce energy. Recent research on salmon demonstrated that the fatty acids that salmon have in excess in the feed are burned more efficiently (Stubhaug et al., 2007).Furthermore, certain fatty acids, such as saturated fats, are vital for the commencement of bioenergetic reactions. These are known as energy substrates. EPA and DHA have been shown to be substrates for increasing fat burning in mammals (Madsen et al., 1999).

Omega 3 fatty acids are unsaturated fatty acids which have been credited with several health benefits including decreasing inflammation, lowering blood pressure, reducing the risk of cancer, and improving the function of the cells that line the arteries (Calder, 2015; Fabian et al., 2015). EPA and DHA give rise to anti-inflammatory and inflammation-resolving mediators called resolvins, protectins, and maresin (Dyall, 2015; Serhan & Savill, 2005). Animal experiments have shown the benefits of EPA and DHA in a range of models of inflammatory conditions. Human trials demonstrate the benefits of omega 3 fatty acids in rheumatoid arthritis and in stabilizing advanced atherosclerotic plaques. Intravenous n-3 fatty acids may have benefits in critically ill patients through reduced inflammation (Levy et al., 2001; Serhan et al., 2000). However, it is not yet known what role the bioactive components resolvins, protectins, and maresin play in inflammatory diseases in salmon.

Carotenoids (fat-soluble compounds) are produced in plants, fungi and some bacteria and have been shown to have two major functions in photosynthesis. Their main functions in photosynthesizing organisms are to absorb light and to protect against photooxidation.

¹ Prostaglandins (PG): any of a class of unsaturated fatty acids that are involved in the contraction of smooth muscle, the control of inflammation and body temperature, and many other physiological functions.

Astaxanthin and other carotenoids have antioxidant properties that have a number of positive effects on the health and immune system of humans and mammals. Recent research on human found that carotenoids protect against the development of cancer, reduce blood pressure and expression of inflammatory markers, have positive effects on the immune system, increase insulin sensitivity in liver, muscle and adipose tissue, reduce accumulation of fat in the liver and thus counteract metabolic syndrome (Elliott, 2005; Gammone et al., 2015; Sila et al., 2015).

In Atlantic salmon, there is a high level of retention of the n-3 fatty acid (FA) docosahexaenoic acid (DHA, 22:6n-3) relative to the dietary content, whereas saturated FAs never seem to increase above a specified level, which is probably an adaptation to low and fluctuating body temperature. Furthermore, fat-soluble components such as carotenoids, fats, various fatty acids, sterols and bioactive products formed from fatty acids are central to many biological functions, and therefore it is inevitable that changes in the fatty acid composition of salmon and trout feed will have consequences for fish growth, development and health.

1.3 Minimum requirement for EPA and DHA in salmon

The amount of EPA and DHA which is incorporated into the cell membranes is also affected by the amount of omega-6 fatty acids in the feeds, and thus the requirement of EPA and DHA to maintain a robust fish probably increases as the amount of omega-6 fatty acids increases in the feed. According to the previous studies, perhaps an increasing content of omega-6 fatty acids or the balance between omega-6 and omega-3 fatty acids in the feeds, is equally important as the level of EPA and DHA. The current knowledge regarding the requirement for EPA and DHA in salmon has been drawn in the figure 2 based on the results of previous experiments. The effect of omega 3 fatty acid on the fish in long term trials in vessels on land and long term trials in cages at the sea doesn't looks similar. Different environments such as pollution source, wind and wave actions, water quality and exchange etc., between land and sea might be the cause of the different effects of omega 3 fatty acid on the fish. In addition, the seawater phase is known as the period when the fish get enough maturity to observe how the time omega 3 fatty acid effect on the growth of the fish. Thus, omega 3 fatty acid for salmon under the conditions of sea cages becomes more important when compared to those under land conditions (Bou et al., 2017). That is why the thesis focuses on salmon in the seawater phase because of above reasons.

EPA and DHA requirement for salmon through seawater phase, % of the feed



Figure 2: In the seawater period, the EPA and DHA level requirement for salmon divided into too low level (red area), uncertain level (orange area) and safe level (green area). Results from long-term experiments performed in vessels on land are shown above the arrow, and from longterm experiments in cages in the sea below the arrow. There are indications that the need for EPA and DHA can to a certain extent be affected by other components in the feed such as total fat level and level of omega-6 fatty acids. Source: Nofima-2016 (Nofima & Nifes, 2016)

By conducting short-term and controlled trials (terrestrial tanks), Ruyter recently demonstrated that low EPA + DHA levels in salmon feeds generally do not negatively affect health and performance, as salmon has high ability to conserve EPA and DHA in important organs and tissues (Ruyter et al., 2016). However, fish oil cannot be fully replaced with plant oils due to the low level of $\omega 3$ fatty acids and high levels of $\omega 6$ and $\omega 9$ fatty acids. The experiments of Torstensen have demonstrated that saturated fatty acids, monounsaturated fatty acids (MUFA) and PUFA as found in fish oil can be produced from plant oils, but not the high level of unsaturated fatty acids in fish oil (Torstensen et al., 2005). That is why in two long-term trials conducted in terrestrial tanks during the seawater phase, Rosenlund et al. (2016) showed that salmon require approximately 1% EPA and DHA in feed for optimal growth and maintenance of DHA levels in important tissues such as red blood cells, retina and brain (Rosenlund et al., 2016; Sissener, Torstensen, et al., 2016). Salmon fed 1% or less EPA and DHA had significantly higher mortality than salmon fed 1.7% of these fatty acids in the feed at high water temperatures. Across the observational studies on salmon fed a diet with low levels of EPA and DHA, a decreased level of astaxanthin in muscle was shown. This also causes the increase of

lipid accumulation in liver and viscera, the compression in vertebrae and histological change in the mid intestine (Ruyter et al., 2016; Sissener, Waagbø, et al., 2016). Although some previous studies showed higher levels than this can be positive in some disease situations, perhaps EPA and DHA levels of 1.6% of the feed and higher could be free from harm in salmon. Sissener et al., demonstrated that a reduction of dietary EPA and DHA from 2.6 to 1.6% through the seawater phase in a commercial scale production of salmon gave no reduction in growth or survival, despite repeated delousings, as well as an outbreak of pancreas disease and gill infections (Sissener, Waagbø, et al., 2016). Therefore, to ensure that salmon grow well at the sea, it is necessary to meet the minimum requirement of EPA and DHA of >1% of the feed. If salmon receive below this level in the feed, the growth and health will be reduced in performance, and the mortality rates will also increase when exposed to demanding environmental conditions.

1.4 Scope of the research

The aim of this thesis is to investigate the effect of omega 3 fatty acid on the growth of salmon in Atlantic salmon aquaculture using these measurements: weight gain (%), feed conversion ratio (FCR), specific growth rate, protein efficiency ratio (PER), daily growth index (DGI), thermal growth coefficient (TGC) and relative feed intake (RFI). These were determined following the standard methods as mentioned below. A systematic review has been conducted in this thesis to gather and analyze data related to the effect of omega 3 fatty acid on growth in Atlantic salmon.

The research question for this thesis is:

Does the omega 3 fatty acid affect the growth performance of Atlantic salmon?

The purpose is to describe the overall inclination of the omega fatty acid effectiveness – whether weight gain is reduced, whether growth rate is statistically significant, and if there are any negative effects when the dosage of fatty acid is reduced. The question applies to growth performance and health issues of Atlantic salmon in the seawater phase. Growth is a characteristic feature of living beings. This is the process of addition of flesh as a result of protein synthesis. Knowledge of fish growth is of vital importance for obtaining a high yield of fish. The rate of growth varies from species to species, and sometimes varies even among species as well. Rate of fish growth is influenced by many factors such as localities, seasonal effects, availability of food and oxygen, population density and age (Hutchings, 2008). The

thesis is focused particularly on the omega 3 fatty acid content of feed, the growth performance of post-smolt salmon in saltwater (that is, not juvenile salmon).

1.5 Aims of the study

In order to further develop nutritional solutions to enhance the viability of Atlantic salmon aquaculture, both the industry and academia frequently enlist in experimental dietary growth trials. Within the industry, these trials are essential to the uptake of more cost-effective and sustainable aquafeed formulations, yet much of the quantitative data remains in internal organizations and rarely makes its way into peer-reviewed academic journals (Henriksson et al., 2012).

In response, this MSc thesis aims to (i) summarize the extent of published information regarding long-term nutritional growth trials conducted on Atlantic salmon in seawater, and where possible, (ii) describe the most significant effect of omega 3 fatty acid on the growth of Atlantic salmon. The thesis is structured according to the IMRaD format. It consists of the following sections: Introduction, materials and methods, results and discussion/ conclusions.

2 Materials and Methods

2.1 Systematic review

A systematic literature review of peer-reviewed documents has been performed in this thesis. This method is used to gather data about research related to the effectiveness of fatty acid on growth performance in Atlantic salmon.

"A Systematic Literature Review (often referred to as a systematic review) is a means of identifying, evaluating and interpreting all available research relevant to a particular research question, or a topic area, or phenomenon of interest" (Brereton et al., 2007). Therefore, scientific papers relevant to salmon feeding in electronic scientific databases have been mainly searched through Web of science.

In addition, the methods that have been used in this thesis is also applied in other evidencebased studies, including biology and biochemical analyses to understand how the effects of fatty acid content in feed on Atlantic salmon, which have been demonstrated in previous research experiments.

This thesis has been designed for quantitative system review. The first characteristic of quantitative research is the collection of quantitative data. In the quantitative research, the primary importance to start is to focus on hypothesis and then test those hypotheses with empirical data to see whether they are supported (Johnson & Larry Christensen, 2008). The purpose in a quantitative study is frequently declarative statement that identifies type of relationship being investigated and the exact variables to be examined. In this thesis, the data to be collected was the parameters concerning the grow performance of salmon fed with various diets. The dataset should be measurable characteristics of the population, for example we can compare the mean of omega 3 level between groups, the average of weight gain of the fish during the experiment. Furthermore, all occurrences are completely determined by one or more causes in quantitative research (Johnson & Larry Christensen, 2008). This study will try to identify cause-and-effect relationships between omega 3 fatty acids and the growth performance in Atlantic salmon. That may be enable us to make probabilistic predictions and generalizations.

A systematic document review will be used for its advantages: A literature review may be the best methodological tools to provide an overview of a certain issue or research problem. However, depending on the goal of the literature review, the method that should be used has to be flexible (Snyder, 2019). Searching for scientific articles related to salmon feed in electronic

science databases (such as "Web of Science") often provides some hundreds of hits. Therefore, it is expected to find evidence of the effect of fatty acid on the health of salmon in aquaculture and this scientific information can be utilized and integrated in a report. In addition, through the collected results, it is possible to map areas where research is lacking. By using systematic methods when reviewing papers, personal selection bias could be decreased, thus providing reliable results from which conclusion can be draw and decision made (Moher et al., 2009). The process of conducting a literature review typically include four phases; (1) designing the review, (2) conducting the review, (3) analysis and (4) writing up the review. A systematic literature review actually consists of several steps, the number of steps should be influenced by the requirements for a specific research (Snyder, 2019).

2.2 Materials

In order to identify relevant scholarly articles, Web of Science (WoS) and Oria.no were used as databases. These websites contain all the necessary tools for advanced search during the data gathering process. Web of Science is the largest database of peer-reviewed literature and quality controlled web resources. The search was duplicated in the Google Scholar® (GS) database. This was done to find out if there are any search results not covered by the search performed in WoS and Oria.no. Using GS, the retrieved papers were often duplicated since they were from different websites and often composed of newspaper/magazine articles or master theses, while WoS allowed for more precise search (e.g., no newspaper/magazine articles or master theses).

The papers provided by Web of Science included articles, books chapter, reviews, short surveys and conference papers. Documents were then selected by initial criteria and either included or excluded from this systematic literature review. Same papers that were found several times were listed as one source. In order to identify those papers, the authors and year of publication have been considered before those papers are listed in the further step.

2.3 Methods

The methodological approach used for the systematic literature search is based on relevant items from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009). During the first phase (designing the review), a review protocol was developed using the Population, Interventions, Comparisons, Outcomes and Setting framework to define the research question and inclusion criteria. Once the research question has been formulated, a search strategy for selection of relevant literature was identified. Criteria that are considered and are used are year of publication, language of the paper, type of paper

(such as conceptual, randomized controlled trial, etc.). In the phase of conducting the review, a corpus of data in the form of scientific research documents were found using an electronic database. After that, these documents were checked against predetermined criteria related to their content and methodology. The review has been performed through all found published articles and no geographical limitation has been applied. The quality of the documents was then assessed and finally data were extracted for the analysis. A quantitative research focuses on only one or a few causal factors at the same time. That is why a "narrow-angle lens" has been used to describe the process of data searching (Johnson & Larry Christensen, 2008). The overall process is described in the figure below:



Figure 3: Main steps in the systematic literature review. The figure was taken from Guidance on conducing a systematic literature review (Xiao & Watson, 2019).

2.4 Finding sources

The search was performed to identify published studies that reported viable data for inclusion in the data synthesis. The majority of the literature was identified by entering two search terms 1) "Atlantic salmon fatty acid growth" and 2) "Atlantic salmon fatty acid health" into the database 'Web of Science', Google Scholar and Oria.no. Language chosen for the review was English, therefore, documents in other languages have been excluded. Time range for this review is from year 1st of January 2012 until 28th of February 2022, thereby covering

documents of a period of 10 years. Manual searching techniques such as scanning of reference lists and broad searches using Google Scholar® supplemented database searching to retrieve more studies. All found documents through "Web of Science", "Oria.no" and "Google Scholar" were checked by titles, abstracts and full-text. Following the title and the research question, the science journal/article should include: methodologies used in the experiment, characteristics of the sample, outcome – effect on salmon growth: the dosage of omega-3 in the feed, and the rate of weight gain.

2.4.1 Evaluating information resources

The evaluation of source plays an important role and becomes a real problem for conducting of SLR (Rowley & Slack, 2004). Documents were firstly checked by title and abstract. Documents that did not contain data relevant to the research topic were excluded. To be included in further review, the abstract of each document was subjected to analysis according to the following criteria (Table 1):

Inclusion criteriaan	
Language	English
Time period	From 01.01.2012 to 28.02.2022
Population examined	Post-smolt Atlantic salmon in seawater phase
Feed ingredient	Lipid content of feed must be reported (% or mg/g diet)
Presented outcomes on fish	Weight gain rate must be reported (% or g)

Table 1: Criteria used to include scientific articles in the final analysis list.

For some cases, reviewing the abstract alone could not confirm a match with the criteria in the table 1. In those cases, the methodology section was examined in each paper, to look for information about, for example, feed ingredient or the weight gained by the fish.

Assessing quality of primary studies is an essential component of systematic reviews. To assess document quality, a specific checklist was created for this review (The Downs and Black Scale-Annex 1). The checklist was completed following the study of systematic review of (O'Connor et al. (2015). The Downs and Black Scale initially consist of 27 questions to evaluate human health care interventions. Many studies in the past have modified the original version by editing the checklist. In order to examine the quality of the data, the checklist in this research has been simplified into 23 questions relating to the quality of reporting (ten questions), external validity

(three questions), internal validity (bias and confounding) (10 questions). Each paper was assigned a grade of "excellent" (24–27 points), "good" (19–23 points), "fair" (14–18 points) or "poor" (<14 points). Documents scaled as "poor" were excluded from the review. During this step, documents that contain the relevant topic but not using any experiment in the method section were also excluded from further review.

2.4.2 Data extraction

The data from the documents that passed the quality control were then extracted independently using a standardized form (Annex 2). The following data were extracted: methodologies used in the experiment, characteristics of the sample, and primary outcome. Data were extracted from studies meeting the pre-determined selection criteria and included: (i) fatty acid composition in the feed and (ii) growth, feed efficiency and biometric data, (iii) physiochemical parameters. Where the same growth trial data was presented in multiple studies, the study best meeting the selection criteria or with the most complete dataset was included. Within studies, individual treatment groups that did not meet all requirements of the selection criteria were omitted. To accommodate for changes to dietary proximate composition was reported as the average fed to experimental fish based on the percentage of total fish growth at each dietary proximate composition level. If no information was given on fields that do not cover primary outcome, the item was filled out with "Not-Application" (N/A).

2.4.3 Data analysis

The PRISMA statement was used to present the number of documents found. The chart shows an overall process of data searching in which relevant papers were searched, irrelevant ones were removed, and papers analyzed according to some pre-defined categories.

Due to the present study's focus on how the effect of omega 3 fatty acid vary with different populations with different intervention characteristics such as dose and duration, subgroup analyses and meta-regression is a reasonable method (Higgins et al., 2019). Grouping data was performed by tabulating and describing data. Tables include descriptions of: dosage of DHA and EPA (%), fish oil (%), initial weight, final weight, and weight gained during study... (see Annex 3). Data in each table were organized in chronological order. Data were also displayed graphically. The papers were separated into three groups according to the main question asked: does the omega 3 affect the growth in salmon? The results are grouped into: growth shown in all salmon, in a few salmon, and in no salmon. By comparing the papers in each group, the

effect of omega 3 on the health of the fish can be evaluated and the direction for further studies could be identified.

3 Results

3.1 Data collection and extraction results

The first search of data took place on 13.01.22 in "Scopus" and retrieved 706 results. Besides, the searches were performed with different search terms, thus 74 results provided by PubMed; 256 results found in Web of Science. However, after a first review by title and abstract, the found documents were excluded since they were irrelevant to the goal of the thesis.

The last search was performed on 28.02.22 on Web of Science with 585 publications found relevant to the key words. The search on Google Scholar and other sources such as Oria.no, Pubmed and Frontier was also done on the same day in order to find papers that are not covered on Web of Science. There were 259 publications from other sources. The "Scopus" search was dropped as the UiT has cancelled the subscription to Scopus one year ago (from January 1st, 2021).

After remove 55 duplicated papers, the review by title and abstract excluded 639 documents were excluded as irrelevant to the inclusion criteria.

In term of quality control, most of the documents were classified as "good" or "excellent" based on the checklist for measuring study quality. There were five documents (3,3%) that were ranked as "fair". Those papers mostly lacked actual probability values for statistical analysis or did not include the confidence level within the methods section (normally that should be concluded 95%). The "good" papers were determined to be 25,3% (38 papers) and the "excellent" paper were 71% (107 papers). Some papers did not have a full description in the methodology on such as sample, location of study. However, none of the documents were excluded because of poor quality. After the full-text review, 44 documents have been removed due to lack of primary data and after the next steps, another 85 documents were excluded because the studies did not focus only on growth and health of salmon. Those papers concentrate on topics such as transgenesis, breeding, triploid.

A PRISMA flow diagram covering information about the data gathering process from sources is shown in figure 4. The results of both first and last search was finalized in the chart that included the documents from Web of Science, Google Scholar and Oria.no. Among twenty one papers were selected, there were nine papers that have been published before 2012 and have taken from Oria.no.



Figure 4 The PRISMA flow diagram, depicting the flow of information through the different phases of the review.

3.2 Data analysis results

3.2.1 Summary of studies

This section will briefly describe the general information about included documents, from 844 documents at the first search until the 21 documents remaining in the last review.

According the first search, the number of publications per year increased between 2012 to 2021 (Figure 5). The highest number of reports were published in 2021. However, in 2022, the data was collected on 28th Feb 2022, so the number of relevant publications will probably be even higher this year.



Figure 5. Number of publications per year from 2012 to 2022 (Data source from Web of Science)

As the graph shown in the Figure 6, the type of document is mostly article (94.87%), the rest is review articles, early access, proceeding papers, reprints and editorial materials. The results had a match with the debut expectation of this thesis because in order to access the quality of the document, it's better if literatures review are same type (Snyder, 2019). That is why all 21 papers that have been selected for analysis are research articles, the authors of which are working in reputable establishments.



Figure 6. Document types found in Web of Science (Data source from Web of Science)

After comparing the lists with reports based on empirical work, there were 21 papers passed the selection criteria. The studies included consisted of a variety of cage and laboratory-based studies. A brief summary of the relevant data extracted from the selected studies is given in table 2.

Table 2: Summary of information from 21 studies on Atlantic salmon

Paper	Concern	Samples	Experiment	Year	Country	Duration	Reference
A1	Growth	Smolt: 139–232 g	EDA+DHA: 0,09; 1;1,4%	2018	Canada	14 weeks	Minimizing marine ingredients in diets of farmed Atlantic salmon (Salmo solar): Effects on growth performance and muscle lipid and fatty acid composition
A2	Growth	Adult	70-80% of the FO replaced by three Rapeseed oil, Olive oil, Soybean oil, Palm oil, Linseed oil	2010	Norway	28 weeks	Net production of Atlantic salmon (FIFO, Fish in Fish out < 1) with dietary plant proteins and vegetable oils
A3	Growth/ intestinal health	Smolt	17,5% CO vs 12,25% fish oil	2020	Norway	60 days	Growth, Chemical Composition, Histology and Antioxidant Genes of Atlantic Salmon (Salmo salar) Fed Whole or Pre-Processed Nannochloropsis oceanica and Tetraselmis sp
A4	Growth/digestibility	1.7kg: random 10 fish/tank	40% tallow inclusion	2013	Australia	195 days	<u>Viability of tallow inclusion in Atlantic salmon diet, as assessed by</u> an on-farm grow out trial
A5	Growth/Flesh adiposity/ FA composition	Post-smolt, 3kg: random 25fish/pen	Reduce 100% FO (FAT) or 100% VO (Lean) and CAL (Mix diet)	2004- 2005	Scotland	24 weeks	Growth, flesh adiposity and fatty acid composition of Atlantic salmon (Salmo salar) families with contrasting flesh adiposity: Effects of replacement of dietary fish oil with vegetable oils,
A6	Growth	Fish of 2053 g: random 1/93 fish in cage	RO comprised 60% of the total added oil	2010	Norway	10 weeks	Interactive effects of dietary protein/lipid level and oil source on growth, feed utilisation and nutrient and fatty acid digestibility of Atlantic salmon,
A7	Growth/ FA synthesis	From salmon parr (25g) to smolt	Investigate Atlantic salmon, a high intake of stearidonic acid (SDA) from Echium oil (EO)	2011	Australia	208 days	Effect of feeding Atlantic salmon (Salmo salar L.) a diet enriched with stearidonic acid from parr to smolt on growth and n-3 long- chain PUFA biosynthesis
A8	Growth/ innate immune response	Post-smolt: 940g	Six different feeds with different levels of FM and FO substitution	2010- 2011	Norway	195 days	Effects of marine protein, marine oil and marine free diets on the growth performance and innate immune responses of Atlantic salmon (Salmo salar, L.) post smolts
А9	Growth, Mucosal Barrier Status, and Activity of Leucocytes from Head Kidney	Initial weight 72.7 ± 1.2 g	Fishmeal and fish oil (BG1), soybean meal (BG2), fishmeal and rapeseed oil (BG3), iv) a mix of plant protein and fish oil (BG4), plant and marine ingredients in the ratio 70:30 (BG5)	2019	Norway	65 days	Nutrient Digestibility, Growth, Mucosal Barrier Status, and Activity of Leucocytes From Head Kidney of Atlantic Salmon Fed Marine- or Plant-Derived Protein and Lipid Sources
A10	Growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality	Fish of 0,16 kg	Replacing of 20% pea protein to fish meal	2008	Norway	12 weeks	Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (Salmo salar)—Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality

Paper	Concern	Samples	Experiment	Year	Country	Duration	Reference
A11	Growth	Post-smolt: 215g	Replacing of 20% alga	2016	Norway	84 days	Nannochloropsis oceania-derived defatted meal as an alternative to fishmeal in Atlantic salmon feeds
A12	Growth/fillet quality	Fish of 800g	10% (FM) and 1–1.25% total n-3 LC-PUFA levels: (1) fish oil (FO), (2) Schizochytrium limacinum biomass (ScB), or (3) a mix of the two (FO/ScB).	2015	Norway	11 months	Microalgal Schizochytrium limacinum Biomass Improves Growth and Filet Quality When Used Long-Term as a Replacement for Fish Oil, in Modern Salmon Diets
A13	Growth	Post-smolt: 150g- >5kg	The control diet contained 8% EPA + DHA of total FAs (FAs, 26 g kg-1 feed), the low n-3 diet contained 6% EPA + DHA of FAs until 1200 g body weight and 4.5% in following feeds, averaging out to 5% of FAs (16 g kg-1 feed)	2012	Norway	6 weeks	Reduced n-3 long chain fatty acid levels in feed for Atlantic salmon (Salmo salar L.) do not reduce growth, robustness or product quality through an entire full scale commercial production cycle in seawater
A14	Growth / FA retention	Post-smolt (111 \pm 2.6 g; mean \pm S.)	Dietary inclusion levels (1, 5, 10, 15 and 20 g kg-1) of DHA (22:6n-3)	2014	Scotland	9 weeks	Interactions between dietary docosahexaenoic acid and other long- chain polyunsaturated fatty acids on performance and fatty acid retention in post-smolt Atlantic salmon (Salmo salar).
A15	Growth / FA retention	Initial weight 1.30± 0.1 kg	35% protein and 28% lipid were formulated with a low level of FM that was replaced: 25/45 (% FM/% PP, F25), 18/50 (F18), 11/55 (F11) and 5/60 (F5).	2007- 2008	Scotland	19 weeks	Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (Salmo salar L.), Aquaculture,
A16	Growth/ FA composition	Initial weight: 1168 g	Crude rapeseed oil (RO) comprised 0, 30 or 60% (R0, R30, R60, respectively) of the added oil	2004	Norway	12 weeks	Effects of dietary protein, and fat level and rapeseed oil on growth and tissue fatty acid composition and metabolism in Atlantic salmon (Salmo salar L.) reared at low water temperatures
A17	Growth/ liver	Fish of 179 ± 29 g	Vegetable oil (ABP); a fish meal/fish oil (MAR) and a plant protein/vegetable oil(VEG)-table	2018	Canada	14 weeks	Changes in the liver transcriptome of farmed Atlantic salmon (Salmo salar) fed experimental diets based on terrestrial alternatives to fish meal and fish oil
A18	Growth	initial average weight of 229 g	Replacing algae to FM at 0/10, 10/5, 20/2.5% (CT, SCE 10 and SCE 20)	2018	Norway	65 days	Microalgae Scenedesmus sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (Salmo salar L.)
A19	Digestibility, growth and utilization of feed	Initial weight of 452 g	P. tricornutum replacing fishmeal in the ratios 3%, 6% and 12%	2016	Norway	82 days	Microalga Phaeodactylum tricornutum in feed for Atlantic salmon (Salmo salar) —Effect on nutrient digestibility, growth and utilization of feed
A20	Growth/ FA composition	25 fish of average weight 167.6 g.	Control without algae, 10% and 20% algae in feed	2016	Norway	70 days	Defatted biomass of the microalga, Desmodesmus sp., can replace fishmeal in the feeds for Atlantic salmon
A21	Growth/digestibility, digestive enzymes, gut morphology	Initial weight: 0.421 kg	Groups: fish meal (FM), soybean meal (SBM), raffinose (RA), stachyose (ST), affinose and stachyose (RA–ST), soya-saponins (RA–ST–SA)	2010	Norway	68 days	Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (Salmo salar, L)

3.2.2 Diet fish oil percentage and omega 3 fatty acid composition

The table 3 displays the data of omega 3 fatty acid and fish oil percentage in twenty one studies. In total, there were 98 diets in the review. It was sometimes necessary to calculate the sum of DHA and EPA levels from the diet formula. Overall, it is readily apparent that only a few diets contained over 30% of DHA and EPA. Levels of both 20:5n-3 (EPA, eicosapentaenoic acid) and 22:6n-3 (docosahexaenoic acid DHA) (express as percentage added fatty acid) varied from 0% to 52.7% and averaged 7.95%. However, this parameter was not always reported in some papers (Gong et al., 2019; Metochis et al., 2017; Pratoomyot et al., 2010; M. Sørensen et al., 2016; S. L. Sørensen et al., 2021). Fish oil (expressed as percentage of added oil) varied from 0 to 100% and averaged 40%.

With respect to the original goal of this study, an extensive search of peer reviewed literature, including the full-text assessment of 150 published articles, uncovered only 21 studies with recorded the duration of experiments using post-smolt Atlantic salmon at the seawater phase. The duration of experiments was expressed in day and week, the unit was then converting in days to compare easily between the studies. The duration varied from 42 days to 330 days and averaged 70 days.

Paper	Study/Year	EPA and DHA (%)	Fish oil (% of total lipid)	Duration (day)	Name samples
A1	Foroutani et al., 2020	0,09	0,00	98	ω3LC0
A1	Foroutani et al., 2020	1,00	5,00	98	ω3LC1
A1	Foroutani et al., 2020	1,41	7,00	98	ω3LC1.41
A2	Liland et al., 2013	18,1	100,00	196	FO
A2	Liland et al., 2013	4,2	20,00	196	OO-80% olive oil
A2	Liland et al., 2013	5,5	20,00	196	RO-80% rapeseed oil
A2	Liland et al., 2013	4,4	20,00	196	SO-80% soy oil
A3	Sørensen et al., 2021	18,60	17,50	60	СО
A3	Sørensen et al., 2021	20,80	12,25	60	NU-Nannochloropsis diet
A3	Sørensen et al., 2021	17,60	12,25	60	NE-pre-extruded Nannochloropsis diet
A3	Sørensen et al., 2021	21,30	12,25	60	TU-Tetraselmis diet,
A3	Sørensen et al., 2021	16,00	12,25	60	TE-pre-extruded Tetraselmis diet

Table 3: Fish oil (%), EPA and DHA (%) of 98 diets from 21 studies fed to Atlantic salmon during experimental trials

Paper	Study/Year	EPA and DHA (%)	Fish oil (% of total lipid)	Duration (day)	Name samples
A4	<u>Emery et al., 2016</u>	31,50	20,00	195	CD
A4	Emery et al., 2016	32,95	20,00	195	TD
A5	<u>Bell et al., 2010</u>	18,60	91,70	168	Lean FO
A5	<u>Bell et al., 2010</u>	2,70	0,00	168	Lean VO
A5	<u>Bell et al., 2010</u>	13,60	100,00	168	Fat FO
A5	<u>Bell et al., 2010</u>	18,60	0,00	168	Fat VO
A5	<u>Bell et al., 2010</u>	2,70	100,00	168	CAL FO
A5	<u>Bell et al., 2010</u>	13,60	0,00	70	CAL VO
A6	Karalazos et al., 2011b	9,90	100,00	70	HP-FO
A6	Karalazos et al., 2011b	10,00	100,00	70	MP-FO
A6	Karalazos et al., 2011b	9,90	100,00	70	LP-FO
A6	Karalazos et al., 2011b	3,90	40,00	70	HP-RO
A6	Karalazos et al., 2011b	3,90	40,00	70	MP-RO
A6	Karalazos et al., 2011b	3,60	40,00	70	LP-RO
A7	Codabaccus et al., 2011	0,70	0,00	208	EO
A7	Codabaccus et al., 2011	38,10	100,00	208	FO
A7	Codabaccus et al., 2011	1,50	0,00	208	RO
A8	Metochis et al., 2017	7,45	47,50	195	MB
A8	Metochis et al., 2017	7,50	51,50	195	MBABP
A8	Metochis et al., 2017	9,60	0,00	195	MFABP
A8	Metochis et al., 2017	3,90	56,11	195	VP
A8	Metochis et al., 2017	9,80	0,00	195	VO
A8	Metochis et al., 2017	10,15	0,00	195	VP/VO
A9	Solveig L. Sørensen et al., 2021	N/A	100,00	65	BG1-fishmeal and fish oil
A9	Solveig L. Sørensen et al., 2021	N/A	100,00	65	BG2-soybean meal
A9	Solveig L. Sørensen et al., 2021	N/A	15,20	65	BG3-fishmeal and rapeseed oil
A9	Solveig L. Sørensen et al., 2021	N/A	100,00	65	BG4-mix of plant protein and fish oil
A9	Solveig L. Sørensen et al., 2021	N/A	28,00	65	BG5-plant and marine(70:30)
A10	Øverland et al., 2009	N/A	100,00	84	FM
A10	Øverland et al., 2009	N/A	100,00	84	SBM-soybean meal

Paper	Study/Year	EPA and DHA (%)	Fish oil (% of total lipid)	Duration (day)	Name samples
A10	Øverland et al., 2009	N/A	100,00	84	PPC, 35% CP-Pea protein
A10	Øverland et al., 2009	N/A	100,00	84	PPC, 50% CP-Pea protein
A11	M. Sørensen et al., 2017	N/A	100,00	84	1C- control
A11	M. Sørensen et al., 2017	N/A	100,00	84	1L-10% algal meal
A11	M. Sørensen et al., 2017	N/A	100,00	84	1H-20% algal meal
A12	Katerina et al., 2020	4,5	17,69	330	FO3
A12	Katerina et al., 2020	4,3	9,48	330	FO/ScB3
A12	Katerina et al., 2020	4,5	0,00	330	ScB3
A12	Katerina et al., 2020	3,63	13,25	330	FO4
A12	Katerina et al., 2020	3,63	0,00	330	ScB4
A13	Sissener et al., 2016	8,10	34,5	42	Cage 2
A13	Sissener et al., 2016	8,10	34,3	42	Cage 11
A13	Sissener et al., 2016	8,30	35,2	42	Cage 12
A13	Sissener et al., 2016	5,10	33,7	42	Cage 3
A13	Sissener et al., 2016	5,10	33,6	42	Cage 4
A13	Sissener et al., 2016	5,40	34	42	Cage 10
A14	Glencross et al., 2014	0,00	0,00	63	D1
A14	<u>Glencross et al., 2014</u>	2,60	0,00	63	D5
A14	Glencross et al., 2014	0,80	0,00	63	D10
A14	<u>Glencross et al., 2014</u>	34,40	0,00	63	D15
A14	Glencross et al., 2014	31,40	0,00	63	D20
A14	Glencross et al., 2014	52,70	0,00	63	D10A
A14	Glencross et al., 2014	0,20	57,69	63	D10E
A14	Glencross et al., 2014	0,00	30,00	63	D5E
A15	Pratoomyot et al., 2010	7,10	40,00	56	F25
A15	Pratoomyot et al., 2010	7,20	40,00	56	F18
A15	Pratoomyot et al., 2010	6,40	40,00	56	F11
A15	Pratoomyot et al., 2010	6,20	40,00	56	F5
A15	Pratoomyot et al., 2010	7,10	40,00	56	F25
A15	Pratoomyot et al., 2010	7,20	40,00	56	F18

Paper	Study/Year	EPA and DHA (%)	Fish oil (% of total lipid)	Duration (day)	Name samples
A15	Pratoomyot et al., 2010	6,40	40,00	56	F11
A15	Pratoomyot et al., 2010	6,20	40,00	56	F5
A16	Karalazos et al., 2007	22,30	100,00	84	HP-R0
A16	Karalazos et al., 2007	14,70	70,00	84	HP-R30
A16	Karalazos et al., 2007	8,20	40,00	84	HP-R60
A16	Karalazos et al., 2007	23,80	100,00	84	LP-R0
A16	Karalazos et al., 2007	16,10	69,80	84	LP-R30
A16	Karalazos et al., 2007	7,80	39,60	84	LP-R60
A17	Caballero-Solares et al., 2018	N/A	66,70	98	MAR
A17	Caballero-Solares et al., 2018	N/A	26,00	98	ABP
A17	Caballero-Solares et al., 2018	N/A	22,76	98	VER
A18	<u>Gong et al., 2019</u>	12,47	50,00	65	СТ
A18	<u>Gong et al., 2019</u>	12,14	50,00	65	SCE 10
A18	<u>Gong et al., 2019</u>	11,42	50,00	65	SCE 20
A19	M. Sørensen et al., 2016	19,2	100,00	82	FA0 0% algae
A19	M. Sørensen et al., 2016	20,2	100,00	82	FA3 3% algae
A19	M. Sørensen et al., 2016	20,4	100,00	82	FA6 6% algae
A20	<u>Kiron et al., 2016</u>	N/A	100,00	70	4C
A20	<u>Kiron et al., 2016</u>	N/A	100,00	70	4L
A20	<u>Kiron et al., 2016</u>	N/A	100,00	70	4H
A21	M. Sørensen et al., 2011	N/A	68,20	68	FM
A21	M. Sørensen et al., 2011	N/A	89,25	68	SMB
A21	M. Sørensen et al., 2011	N/A	62,00	68	RA
A21	M. Sørensen et al., 2011	N/A	62,70	68	ST
A21	M. Sørensen et al., 2011	N/A	64,46	68	RA-ST
A21	M. Sørensen et al., 2011	N/A	63,80	68	RA-ST-SA

3.2.3 Growth and performance of Atlantic salmon fed the experimental diets

The growth performance that has been reported was taken from the outcome of the studies. A summary of experimental feed intake and biometry measures are presented in Table 4.

The fish were weighed individually at the start and at the end of the experiment. All the data are presented as the average of each experiment group. Final weights of fish ranged from 82.6g to 5155g and the average across all of the included studies was 750.5g. The average food conversion ratio across the studies was 0.97. The specific gain rate expressed as percentage per day varied from 0.43 to 2.06% and averaged 0.92. Similarly, to other parameters, this parameter was not always reported in all the papers used.

Table 4: Feed intake and biometry of Atlantic salmon fed 98 different diets from 21 studies.

Paper	Study/Year	Initial weight (g)	Final Weight (g)	Weight gain (g)	Specific Gain rate % per day	RGI %	Feed intake (g)	FCR	Final length (cm)	Duration (day)	Name samples
. 1	F	154 40	200.20	122 50	0.50	27/4	145.40	1.10	26.60	00	21.00
Al	Foroutani et al., 2020	176,60	309,30	132,70	0,56	N/A	145,40	1,10	26,60	98	@3LC0
Al	Foroutani et al., 2020	179,30	339,70	160,40	0,64	N/A	159,00	0,99	30,60	98	ω3LC1
Al	Foroutani et al., 2020	178,10	341,90	163,80	0,66	N/A	161,50	0,99	30,60	98	ω3LC1.41
A2	Liland et al., 2013	815,00	3398,00	2558,00	0,75	N/A	2982,00	1,17	60,20	196	FO
A2	Liland et al., 2013	815,00	3459,00	2655,00	0,78	N/A	2748,00	1,04	59,30	196	OO-80% olive oil
											RO-80% rapeseed
A2	Liland et al., 2013	815,00	3475,00	2683,00	0,79	N/A	2759,00	1,03	59,50	196	oil
A2	Liland et al., 2013	815,00	3267,00	2442,00	0,74	N/A	2573,00	1,05	58,30	196	SO-80% soya oil
A3	<u>Sørensen et al., 2021</u>	154,40	307,80	99,40	1,15	N/A	N/A	N/A	N/A	60	CO NU-
A3	Sørensen et al., 2021	154,00	288,90	87,60	1,05	N/A	N/A	N/A	N/A	60	Nannochloropsis diet
											NE-pre-extruded Nannochloropsis
A3	Sørensen et al., 2021	153,90	292,90	90,30	1,07	N/A	N/A	N/A	N/A	60	diet
۸3	Sørensen et al. 2021	154.40	282.90	83 30	1.01	N/A	N/A	N/A	N/A	60	TU-Tetraselmis
AJ	<u>Sorensen et al., 2021</u>	1,77,70	202,70	05,50	1,01	11/74	11/71	11/71	11/71	00	
A3	Sørensen et al., 2021	154,30	285,10	84,80	1,02	N/A	N/A	N/A	N/A	60	TE-pre-extruded Tetraselmis diet

Paper	Study/Year	Initial weight (g)	Final Weight (g)	Weight gain (g)	Specific Gain rate % per day	RGI %	Feed intake (g)	FCR	Final length (cm)	Duration (day)	Name samples
A4	Emery et al., 2016	1714,00	3892,00	132,10	0,43	N/A	N/A	1,22	N/A	195	CD
A4	Emery et al., 2016	1725,00	4016,00	137,90	0,44	N/A	N/A	1,30	N/A	195	TD
A5	Bell et al., 2010	80,60	3120,00	3039,40	0,92	N/A	N/A	1,23	65,30	168	Lean FO
A5	Bell et al., 2010	89,50	3030,00	2940,50	0,91	N/A	N/A	1,39	63,70	168	Lean VO
A5	<u>Bell et al., 2010</u>	92,10	3180,00	3087,90	0,93	N/A	N/A	1,08	63,90	168	Fat FO
A5	<u>Bell et al., 2010</u>	84,30	2840,00	2755,70	0,89	N/A	N/A	1,19	62,50	168	Fat VO
A5	Bell et al., 2010	52,80	2750,00	2697,20	1,00	N/A	N/A	1,08	63,10	168	CAL FO
A5	Bell et al., 2010	51,60	2890,00	2838,40	1,01	N/A	N/A	1,01	62,80	70	CAL VO
A6	Karalazos et al., 2011b	2031,7	3340,2	1308,50	0,86	N/A	N/A	1,07	N/A	70	HP-FO
A6	Karalazos et al., 2011b	2097	3491,1	1394,10	0,88	N/A	N/A	1,1	N/A	70	MP-FO
A6	Karalazos et al., 2011b	2031,7	3352,9	1321,20	0,86	N/A	N/A	1,06	N/A	70	LP-FO
A6	Karalazos et al., 2011b	2065,3	3591,8	1526,50	0,95	N/A	N/A	0,99	N/A	70	HP-RO
A6	<u>Karalazos et al., 2011b</u>	2055,7	3664,2	1608,50	0,99	N/A	N/A	1,02	N/A	70	MP-RO
A6	Karalazos et al., 2011b	2038,3	3405,7	1367,40	0,88	N/A	N/A	1,09	N/A	70	LP-RO
A7	Codabaccus et al., 2011	96,30	204,40	108,10	0,90	N/A	N/A	N/A	N/A	208	EO
A7	Codabaccus et al., 2011	103,50	223,00	119,50	0,90	N/A	N/A	N/A	N/A	208	FO
A7	Codabaccus et al., 2011	109,40	252,80	143,40	1,00	N/A	N/A	N/A	N/A	208	RO
A8	Metochis et al., 2017	992,30	2608,70	1616,40	N/A	N/A	8,38	0,91	N/A	195	MB
A8	Metochis et al., 2017	901,80	2417,50	1515,70	N/A	N/A	6,93	0,85	N/A	195	MBABP
A8	Metochis et al., 2017	924,80	2415,60	1490,80	N/A	N/A	6,70	0,84	N/A	195	MFABP

Paper	Study/Year	Initial weight (g)	Final Weight (g)	Weight gain (g)	Specific Gain rate % per day	RGI %	Feed intake (g)	FCR	Final length (cm)	Duration (day)	Name samples
		8/	8/	8***** (8/		, .	(8/		()	(20)	
A8	Metochis et al., 2017	940,70	2528,90	1588,20	N/A	N/A	7,90	0,90	N/A	195	VP
A8	Metochis et al., 2017	983,90	2626,90	1643,00	N/A	N/A	7,60	0,85	N/A	195	VO
A8	Metochis et al., 2017	892,90	2381,70	1488,80	N/A	N/A	6,95	0,85	N/A	195	VP/VO
A9	(Solveig L. Sørensen et al., 2021)	72,40	152,30	110,20	1,10	N/A	N/A	N/A	N/A	65	BG1-fishmeal and fish oil
A9	(Solveig L. Sørensen et al., 2021)	71,30	138,30	93,80	1,00	N/A	N/A	N/A	N/A	65	BG2-soybean meal
A9	(Solveig L. Sørensen et al., 2021)	72,90	158,40	117,20	1,20	N/A	N/A	N/A	N/A	65	BG3-fishmeal and rapeseed oil
A9	<u>(Solveig L. Sørensen et</u> <u>al., 2021)</u>	73,50	150,70	105,10	1,10	N/A	N/A	N/A	N/A	65	BG4-mix of plant protein and fish oil
A9	Solveig L. Sørensen et al., 2021	73,50	150,30	104,70	1,00	N/A	N/A	N/A	N/A	65	BG5-plant and marine (70:30)
A10	Øverland et al., 2009	159,50	421,30	262,00	1,18	N/A	208,50	0,80	N/A	84	FM
A10	Øverland et al., 2009	157,50	385,40	228,00	1,09	N/A	203,50	1,00	N/A	84	SBM-soybean meal
A10	Øverland et al., 2009	158,10	427,70	270,00	1,21	N/A	228,70	0,86	N/A	84	PPC, 35% CP-Pea protein
A10	Øverland et al., 2009	157,60	432,00	274,00	1,23	N/A	210,40	0,77	N/A	84	PPC, 50% CP-Pea protein
A11	M. Sørensen et al., 2017	214,50	429,00	100,20	0,82	N/A	0,68	0,81	N/A	84	1C- control
A11	M. Sørensen et al., 2017	213,80	420,20	96,30	0,80	N/A	0,70	0,86	N/A	84	1L-10% algal meal
A11	M. Sørensen et al., 2017	218,00	407,80	86,90	0,74	N/A	0,75	1,00	N/A	84	1H-20% algal meal
A12	Katerina et al., 2020	270,00	870,00	600,00	N/A	N/A	1,15	0,82	N/A	330	FO3
A12	Katerina et al., 2020	277,00	845,00	568,00	N/A	N/A	1,04	0,79	N/A	330	FO/ScB3
A12	Katerina et al., 2020	273,00	889,00	616,00	N/A	N/A	1,0	0,77	N/A	330	ScB3
A12	Katerina et al., 2020	865,62	2818,00	1952,38	N/A	N/A	N/A	1,51	N/A	330	FO4
A12	Katerina et al., 2020	867,75	3299,00	2431,25	N/A	N/A	N/A	1,42	N/A	330	ScB4

Paper	Study/Year	Initial weight (g)	Final Weight (g)	Weight gain (g)	Specific Gain rate % per day	RGI %	Feed intake (g)	FCR	Final length (cm)	Duration (day)	Name samples
		(8/	(8/	8. (8/	IJ		18/	-		(***))	
A13	Sissener et al., 2016	150,00	5305	5155,00	N/A	98,20	N/A	1,17	N/A	42	Cage 2
A13	Sissener et al., 2016	150,00	5305	5155,00	N/A	100,7 0	N/A	1,18	N/A	42	Cage 11
A13	Sissener et al., 2016	150,00	5305	5155,00	N/A	97,60	N/A	1,18	N/A	42	Cage 12
A13	Sissener et al., 2016	150,00	4753	4603,00	N/A	97,50	N/A	1,17	N/A	42	Cage 3
A13	Sissener et al., 2016	150,00	4753	4603,00	N/A	97,30	N/A	1,17	N/A	42	Cage 4
A13	Sissener et al., 2016	150,00	4753	4603,00	N/A	98,70	N/A	1,14	N/A	42	Cage 10
A14	Glencross et al., 2014	110,80	226,80	116,00	1,87	N/A	106,30	0,95	N/A	63	D1
A14	Glencross et al., 2014	112,50	226,70	114,20	1,84	N/A	105,90	0,96	N/A	63	D5
A14	Glencross et al., 2014	110,70	233,10	122,50	1,98	N/A	108,50	0,90	N/A	63	D10
A14	Glencross et al., 2014	113,70	232,10	118,50	1,91	N/A	105,30	0,90	N/A	63	D15
A14	Glencross et al., 2014	109,20	231,40	122,10	1,97	N/A	107,30	0,90	N/A	63	D20
A14	Glencross et al., 2014	111,80	231,90	120,10	1,94	N/A	105,00	0,91	N/A	63	D10A
A14	Glencross et al., 2014	111,00	238,90	127,90	2,06	N/A	107,40	0,86	N/A	63	D10E
A14	Glencross et al., 2014	108,00	229,60	121,60	1,96	N/A	106,10	0,87	N/A	63	D5E
A15	Pratoomyot et al., 2010	1310,00	2470,00	1160,00	1,27	N/A	N/A	1,04	N/A	56	F25
A15	Pratoomyot et al., 2010	1300,00	2370,00	1050,00	1,19	N/A	N/A	1,06	N/A	56	F18
A15	Pratoomyot et al., 2010	1300,00	2160,00	850,00	1	N/A	N/A	1,17	N/A	56	F11
A15	Pratoomyot et al., 2010	1320,00	1980,00	660,00	0,81	N/A	N/A	1,29	N/A	56	F5
A15	Pratoomyot et al., 2010	2470,00	3840,00	1370,00	0,57	N/A	N/A	1,03	N/A	56	F25
A15	Pratoomyot et al., 2010	2370,00	3640,00	1260,00	0,55	N/A	N/A	1,02	N/A	56	F18

Paper	Study/Year	Initial weight (g)	Final Weight (g)	Weight gain (g)	Specific Gain rate % per day	RGI %	Feed intake (g)	FCR	Final length (cm)	Duration (day)	Name samples
A15	Pratoomyot et al., 2010	2160,00	3350,00	1230,00	0,56	N/A	N/A	1,00	N/A	56	F11
A15	Pratoomyot et al., 2010	1980,00	3000,00	1010,00	0,53	N/A	N/A	1,03	N/A	56	F5
A16	Karalazos et al., 2007	1168,40	1711,30	542,90	0,49	N/A	N/A	0,86	50,50	84	HP-R0
A16	<u>Karalazos et al., 2007</u>	1184,60	1772,00	587,40	0,52	N/A	N/A	0,84	50,90	84	HP-R30
A16	Karalazos et al., 2007	1152,40	1784,30	631,90	0,56	N/A	N/A	0,81	50,70	84	HP-R60
A16	Karalazos et al., 2007	1162,80	1721,70	558,90	0,50	N/A	N/A	0,86	50,70	84	LP-R0
A16	Karalazos et al., 2007	1171,90	1760,30	588,40	0,52	N/A	N/A	0,87	49,90	84	LP-R30
A16	Karalazos et al., 2007	1168,40	1767,70	599,30	0,53	N/A	N/A	0,85	51,80	84	LP-R60
A17	<u>Caballero-Solares et al.,</u> 2018	176,80	342,50	165,60	N/A	N/A	178,70	N/A	N/A	98	MAR
A17	<u>Caballero-Solares et al.,</u> 2018	179,20	316,30	137,10	N/A	N/A	146,60	N/A	N/A	98	ABP
A17	Caballero-Solares et al., 2018	177,20	332,90	155,70	N/A	N/A	167,40	N/A	N/A	98	VER
A18	<u>Gong et al., 2019</u>	228,40	473,60	107,10	1,12	N/A	0,86	0,76	N/A	65	СТ
A18	Gong et al., 2019	230,80	451,00	95,40	1,03	N/A	0,90	0,88	N/A	65	SCE 10
A18	Gong et al., 2019	228,10	416,70	82,60	0,93	N/A	0,89	0,97	N/A	65	SCE 20
A19	M. Sørensen et al., 2016	325,20	539,40	214,20	0,62	N/A	N/A	0,83	N/A	82	FA0 0% algae
A19	M. Sørensen et al., 2016	323,20	562,10	238,90	0,68	N/A	N/A	0,81	N/A	82	FA3 3% algae
A19	M. Sørensen et al., 2016	325,20	550,70	225,50	0,64	N/A	N/A	0,82	N/A	82	FA6 6% algae
A20	Kiron et al., 2016	166,61	358,60	191,99	N/A	N/A	N/A	0,81	N/A	70	4C
A20	Kiron et al., 2016	165,90	343,90	178,00	N/A	N/A	N/A	0,86	N/A	70	4L
A20	Kiron et al., 2016	170,30	359,80	189,50	N/A	N/A	N/A	0,90	N/A	70	4H

Paper	Study/Year	Initial weight (g)	Final Weight (g)	Weight gain (g)	Specific Gain rate % per day	RGI %	Feed intake (g)	FCR	Final length (cm)	Duration (day)	Name samples
A21	M. Sørensen et al., 2011	421,00	656,00	237,00	N/A	N/A	N/A	0,84	N/A	68	FM
A21	M. Sørensen et al., 2011	421,00	564,00	142,00	N/A	N/A	N/A	1,05	N/A	68	SMB
A21	M. Sørensen et al., 2011	421,00	598,00	180,00	N/A	N/A	N/A	0,88	N/A	68	RA
		101 00	(21.00)	100.00				0.04		<i>c</i> 0	
A21	<u>M. Sørensen et al., 2011</u>	421,00	621,00	198,00	N/A	N/A	N/A	0,86	N/A	68	ST
A 21	M. Samana et al. 2011	421.00	(28.00	214.00		NT/A		0.05	N T/ A	(9	DACT
A21	M. Sørensen et al., 2011	421,00	038,00	214,00	N/A	IN/A	IN/A	0,95	IN/A	08	KA-51
A 21	M. Saransan at al. 2011	421.00	605.00	183.00	NI/A	NI/A	NI/A	0.90	NI/A	68	ΡΑ ST SA
A21	wi. Sørensen et al., 2011	421,00	005,00	165,00	1N/A	1N/A	1N/A	0,90	1N/A	00	NA-01-0A

3.2.4 The three group studies categorized base on the results of experiments

The three groups were categorized by reviewing the conclusion regarding the effect of each diet on the growth performance of salmon. There are only two papers that found that there was significantly lower growth performance in Atlantic salmon when they are fed feed with reduced EPA and DHA levels (Table 5). Of the 21 papers, 5 have been categorized as the showing the effect of omega 3 "in few" fish; see table 6. 14 of 21 studies in the review showed that the change of omega 3 fatty acid levels in the feed had no effect on growth and health in Atlantic salmon; see table 7.

Table 5- List of studies found that the change of omega fatty acid has significant effect to the growth performance in Atlantic salmon

Paper	Study/Year	Effect of omega 3 fatty acid on Atlantic salmon
A1	Foroutani et al., 2020	Final weights were lowest when using the diet with the lowest fish meal and fish oil content (ω 3LC0). The reduction of 10% marine resource affected on growth, lipid class, and fatty acid composition of muscle tissue
A15	Pratoomyot et al., 2010	Atlantic salmon showed lower growth performance when dietary FM inclusion was reduced from 25% to 5% by increased substitution with PPs

Table 6- List of studies found that the change of omega 3 fatty acid gave slightly effect to the growth performance in Atlantic salmon

Paper	Study/Year	Effect of omega 3 fatty acid on Atlantic salmon		
A11	M. Sørensen et al., 2017	The results indicate that the defatted microalgae N. Oceania can be used at modest inclusion levels–a level close to 10%–without negative effects on weight gain and specific growth rate and health parameters. But algae group got the lower weight compared to control fish ($p=0.09$)		
A13	Sissener et al., 2016	Reducing dietary EPA + DHA from 8 to 5% of FAs (26 to 16 g kg-1 feed) during the entire production cycle in seawater of commercially farmed Atlantic salmon does not affect fish performance, health or robustness. However, reduced EPA + DHA or increased n-6/ n-3 ratio seems to increase the prevalence of melanin spots in the fish fillet.		
A14	Glencross et al., 2014	Atlantic salmon were not highly sensitive to dietary LC-PUFA manipulation and could perform relatively well with only low dietary levels of these fatty acids. However, the finding showed that EPA created a futher improvements to growth in the fish.		
A17	<u>Caballero-Solares et al.,</u> 2018	Fish growth is only slightly affected by omega 3. There was different between groups: the best growing in fishmeal diet following is VEG and ABP (p = 0.069)		
A21	M. Sørensen et al., 2011	A slight reduction on growth in the fish, fish fed the FM performed best $(p=0.07)$		

Table 7 - List of studies found that the change of omega 3 fatty gave no effect on growth performance in Atlantic salmon

Paper	Study/Year	Effect of omega 3 fatty acid on Atlantic salmon
A2	Liland et al., 2013	No effect but SO affects long-term feed intake ($p=0.02$)
A3	Sørensen et al., 2021	No effect but the algae group get lower weight compared to control group
A4	<u>Emery et al., 2016</u>	No effect at 40% replacement FO with tallow
A5	<u>Bell et al., 2010</u>	No effect when reduce up to 65% n-3 LC-PUFA compared to fish fed FO but there was different growth between groups due to diet and interactions between fish group and diet. Genetic background had an influence on lipid and metabolism.
A6	Karalazos et al., 2011b	Low protein/high lipid diets can be used with no negative effects on the growth, FCR and chemical composition of Atlantic salmon reared at high water temperature

Paper	Paper Study/Year Effect of omega 3 fatty acid on Atlantic salmor	
A7	Codabaccus et al., 2011	No effect but although an EO diet increased the n-3 LC-PUFA biosynthesis, EPA and DHA contents in both fresh and seawater fish were still lower compared with in those fed the FO diet
A8	Metochis et al., 2017	No effect but longer adaptation periods might be required for salmon to fully accept these diets. Moreover, dietary FO substitution seems to be easier than FM replacement. No differences in final body weight, weight gain, specific growth rate
A9	<u>Solveig L. Sørensen et al.,</u> 2021	and thermal growth coefficient were noted for fish belonging to the different dietary treatments. But soybean meal impact on gut health in salmon over the long term.
A10	Øverland et al., 2009	No effect at 20% replacement FM with pea protein
A12	Katerina et al., 2020	No effect if the requirement for EPA is covered (> 1.3%)
A16	Karalazos et al., 2007	No effect when fish were fed with lower protein feeds where RO replaced FO up to 60% of the total oil (p< 0.10)
A18	<u>Gong et al., 2019</u>	No effect with 10% in low fishmeal but at 20% in low fishmeal significantly reduce the digestibility, nutrient retention efficiency and feed conversion.
A19	M. Sørensen et al., 2016	No effect with replacement FO with under 3-6% algae biomass in diets
A20	<u>Kiron et al., 2016</u>	No effect with replacement under 20% algae

4 Discussion

4.1 Geographical origin of papers

Most of the papers mentioned the geographical location of studies in the methodology section. For the ones that did not show the location of study, the institutional connection of the first author has been investigated. Some of the paper have two locations of study because the first period of the study was carried out in one country and in the second period the samples have been moved to another country. Among the 21 papers analyzed,14 articles had their first author affiliated to a Norwegian institution, followed by Scotland (3), Australia (2) and Canada (2). The pie chart in figure 7 details the percentage of studies in this review according to nation. Most of the studies on Atlantic salmon have a first author associated with Norway, except two studies from Australia (A4, A7in table 2) and one study from UK (A14 in Table 2).

Norway, together with Chile, are the leading countries when it comes to Atlantic salmon aquaculture (Guenard, 2021). The two countries account for over 50% of the world's total production of the fish (The Federation of Norwegian Industries, 2017). This proves that Norway as the most producing aquaculture country is also the one doing most research on the effect of omega 3 fatty acid in Atlantic salmon.

Norway is the most dominant country in the findings of this thesis. Norwegian researchers focus on including more plant-based ingredients to replace the traditional fish meal and fish oil based diet. This is also the trend of present circumstance in the industry.



Figure 7. Geographical origin of 21 analyzed papers (Data source from table 2)

4.2 Dosage of omega 3 in the feed and weight gain rate

According to the findings of this thesis, the average of omega 3 levels in the combined experiments was 7.95%. The result is in line with the State of World Fisheries and Aquaculture 2020 (a part of The state of the world series of the FAO) in which the FAO has reported that the composition of omega 3 fatty acid in the diets for farmed Atlantic salmon tends to reduce less than 10 percent (Guenard, 2021). The FAO also confirmed that fish meal and fish oil are still maintaining a critical role in the ingredients for farmed fish, as well as the major source of omega-3 fatty acids (EPA and DHA). However, the high costs and the limited source have shown a variety of disadvantages in salmon feeding. Thus, the reduction of omega 3 levels in fish feed is an inevitable trend today.

There were contradictory results among the 21 studies in the review as to whether the omega 3 fatty acids affect growth in salmon. The pie chart in figure 8 shows the percentage of the three groups of studies base on the results of the experiments.



Figure 8- The percentage of the three groups studies classified based on the results of experiments. (Data source from table 5,6,7)

There are two studies (9% of total the studies) which show that the change of omega 3 fatty acid levels in the feed had considerable influence on growth and health in Atlantic salmon. They show that the fish fed with lower omega 3 diet get slower progress in growth compared to the fish fed with full omega 3. Both two studies have shown that there was significant difference in growth among groups (Foroutani 2020; Pratoomyot 2010). The decreased growth

was associated with decreased feed consumption, as the level of FM inclusion decreased (Foroutani et al., 2020). The finding of those papers had agreed with the study of M. Bou that low levels of very-long-chain n-3 PUFA in Atlantic salmon diet reduce fish robustness under challenging conditions in sea cages (Bou et al., 2017). Moreover, lipid accumulation in liver and viscera can increase with decreased EPA and DHA in the feed (Pratoomyot et al., 2010). Similarly, that was the conclusion of Rosenlund during their research (Rosenlund et al., 2016).

In addition, 5 of 21 studies (24% of total the studies) found that omega 3 fatty acid had a slight effect on the growth of salmon. The addition of EPA further improved growth response while addition of ARA had no effect on growth (Sørensen et al., 2017). The mortality was higher in the group fed the low n-3 diet (16%) compared to the group fed the standard diet (12%), but the difference was not statistically significant (A13 in table 6). Both of five studies showed that there was a significant effect of dietary fatty acid composition on growth (as final weight, weight gain and gain rate) but the differences among group mean values were not statistically significant (p < 0.5) (A11, A13, A14, A17, A21 in table 6).

In contrast, 14 of 21 studies (67% of total the studies) demonstrated that omega 3 had no effect on growth in salmon. Of the 14 studies, six studies found that there was a significant effect of oil source on the growth performance (SGR and TGC) (A3, A6, A9, A18, A19, A20 in table 7). However, they finally concluded that the experimental feeds did not differ significantly in term of statistic. Twelve of the fourteen papers showed that the fish fed with fish oil got the best progress in growth. In spite of that, Karalazos had the same conclusion in the two different studies: salmon fed with rapeseed oil resulted in higher SGR compared to the groups fed with fish oil (A6, A16 in table 7). Almost all studies in this group have demonstrated that fish oil and fish meal could be replaced with other proteins in certain levels. However, it is apparent that we cannot completely remove omega 3 fatty acids from the ingredients in feed.

Despite of limitation of dataset, the present review found that omega 3 had both negative and positive effects on the growth and health of Atlantic salmon. The study of Sissener and colleagues shows that the fish fed food with high lipid content (50-60%) had significantly lower final body weights than fish fed with low lipid content (45%) (Sissener, Waagbø, et al., 2016). As opposed to that, Sørensen et al. (2017) found that fish fed the FM performed best (p= 0.07), regarding the weight gain during experiments. With respect to the studies of Hixson and Rosenlund, the diet of n-3 LC PUFA has been reported to be required for optimal growth in seawater reared Atlantic salmon (Hixson et al., 2017; Rosenlund et al., 2016). According to the review, the average of omega 3 fatty acids in g "in all" group was 5.12% and 11.59%, 12.61% in "in few" and "no in fish" respectively. Obviously, the growth rate of the most affected

experimental group was reared with the lowest omega 3 levels and vice versa. However, the effect might be in all metabolism process or a part of this. The factors such as genetics, temperature and other components in the feed also play a role. The results of this review do not clearly show how the dosage of omega 3 influences on the growth of Atlantic salmon. Consequently, further study of the biological relationship between omega 3 and the growth performance in salmon is needed.

4.3 The ratio of DHA vs EPA

In this review, most of studies reported the level of EPA and DHA in combination while both of the fatty acids are known to have independent biological actions.

In fact, EPA becomes the most important of the omega-3 fatty acids to reduce cellular inflammation for a number of reasons. First, EPA is an inhibitor of the enzyme delta-5-desaturase (D5D) that produces arachidonic acid (AA) that are the primary mediators of cellular inflammation (Calder, 2012)(Connor, 2000). Furthermore, EPA can also be a competitor with AA for the enzyme phospholipase A2. This is necessary to release AA from the membrane phospholipids. That is because if AA cannot be released from the cell membrane, then inflammatory eicosanoids cannot be produced. In term of structure, DHA is not a good competitor for the enzyme phospholipase A2 due to greater spatial size of this fatty acid. Thus, DHA again has little effect on cellular inflammation whereas EPA can have a powerful impact.

Actually, DHA has biological functions that differ to the ones of EPA. With the advantages from the structure, DHA has the extra double bond (six in DHA vs. five in EPA) and increased carbon length (22 carbons in DHA vs. 20 in EPA) means that DHA takes up a lot more space than does EPA in the membrane. This can make membranes (especially those in the brain) a lot more fluid as the DHA sweeps out a much greater volume in the membrane. The expansion of membrane fluidity is critical for synaptic vesicles and the retina of the eye as it allows receptors to rotate more effectively thus increasing the transmission of signals from the surface of the membrane to the interior of the nerve cells. That is why DHA is a vital component of these highly fluid portions of the nerves (Connor, 2000).

From a holistic perspective, it is clear that DHA and EPA do different things. In spite of this, there are no studies that directly compared the effects of physiological doses of EPA vs DHA in this review. Therefore, defining the mechanisms underpinning how EPA and DHA alter growth performance of Atlantic salmon is an interesting topic worthy of future work.

4.4 Duration of experiments

With respect to the present study, an extensive search of peer reviewed literature, including the full-text assessment of 150 published articles, uncovered only 21 studies which recorded the duration of experiments in post-smolt Atlantic salmon at the seawater phase. The duration of experiments was expressed in day and week, the unit was then converted into days for easy comparison between the studies. The duration varied from 42 days to 330 days and averaged 70 days.

Generally, fish should be given time to recover from handling before being used in experiments. The amount of time needed may vary with species and conditions; therefore, preliminary tests should be conducted to establish the appropriate recovery period (Jenkins, 2014). A shorter trial may risk Type II error, due to incomplete absorption of the pre-trial fatty acid. The influence of the diet may be partially hidden during the process of sampling observation. Additionally, the study of Tocher showed that ontogenetic change concurrent with a seawater life stage in Atlantic salmon affects fatty acid uptake and metabolism (Tocher, 2010). In order to ensure the reliability of the study, experiments should be long-term growth trials in salmon populations.

Almost all studies in this review have a shorter duration of experiment, but still warranted the criteria of this review. Based on the results of the review, the average duration of the "no effect" group was longest with 133 days following 77 days in the "in all" group and 71 days in the "in few" group. Recently, Rosenlund et al. (2016) found that if the experiment is short term, the reduction of EPA+DHA levels in salmon feed generally do not affect health and performance. This result seems opposite to the previous study of Rosenlund. However, in my opinion the duration of experiment should be long enough in order to access the accurate data. That should be more than 6 months for all the trials because salmon have a long life cycle (from 2 to 7 years)- Life of a Salmon - Science World. (n.d.).

4.5 Evaluation of research question

The study showed that the present trend in the salmon industry where the source of omega 3 fatty acid is limited and where fishmeal and fish oil are replaced by other alternative oils is a potential solution. Many studies have demonstrated that the reduction of omega-3 fatty acid in the feed had no effect on growth and health in Atlantic salmon. However, twenty one studies found that there was a limitation on the reduction of omega 3. That is why we cannot completely remove omega 3 fatty acid out from the ingredients in the feed. The results of this

study can be combined with the studies of Rosenlund and Sissener as mention in the Introduction section, all showing that the minimum requirement for DHA and EPA levels in salmon should be higher than 1% in the feed. Therefore, the focus must be on reasonable ways to replace omega 3 fatty acid in the diet.

In addition to the effects of omega 3 on growth, it is also important to note that in this review, two studies have shown the effect of soybeans on the intestinal health of Atlantic salmon (S. L. Sørensen et al., 2021)((Liland et al., 2013). The findings match those of a paper that was written by Metter Sørensen in 2011 in which they looked at growth, digestibility and intestinal histology, and found reduced growth, impaired feed utilization, and development of intestinal inflammation in the fish fed SBM – soybean meal (Sørensen et al., 2011)

There are not only contradictory results among the studies in this review, but there are also the contradictory results between one paper in the review and the other studies not included in the review. That is the conclusion in the study of Glencross et al., (2014) in this review and Rosenlund et al., (2016). Glencross has shown that Atlantic salmon were not highly sensitive to dietary LC-PUFA manipulation and could perform relatively well with only low dietary levels of these fatty acids. While it has been demonstrated that long-term growth trials are needed to ensure the effects of dietary lipid manipulation are accurately reflected in the fillet tissue of post-smolt Atlantic salmon (Rosenlund et al., 2016; Sissener et al., 2016). Additionally, the result of study conducted by Øveland did not agree with the study by Penn et al., (2010). Øveland demonstrated that there is no effect at 20% replacement FM with pea protein. While Penn found that intestinal inflammation may be caused by a high amount of pea protein concentrate, which is interesting because our plant protein diets (BG4 and BG5) contain some pea protein concentrate. They also had diets with corn gluten and soy protein concentrate, similar to our plant protein diets.

Lack of data can be considered a limitation in this thesis. The statistical analysis could be performed in more depth to increase the reliability of the study. However, this doesn't work because many parameters are not always reported in the studies and there is not a common control.

According to the above analysis, the demand for omega 3 fatty acid by the aquafeed industry is continuously increasing. The solution is to replace it with other material. The conditions such as dosage of omega 3, ratio between DHA vs EPA, and the duration of experiment have a certain effect on the growth of Atlantic salmon. Therefore, those conditions should be

considered carefully when we evaluate the effect of omega 3 acid on the growth of the fish. In my point of view, the owner of salmon farms should increase the use of omega 3 selectively at specific stages of production, such as for hatchery, broodstock and finishing diets, and decrease the incorporation of fishmeal and fish oil in grower diets. Although the review did not focus on factors such as temperature, genetics, and stress, the review has shown the initial conditions for the growth performance in the fish.

5 Conclusion

A systematic review was conducted and the data from long-term nutritional growth trials focusing on seawater-reared Atlantic salmon was analysed. This has provided an overview of the current body of research and has described the most significant effects of omega 3 fatty acid on the growth of Atlantic salmon. The majority of the studies have shown that the reduction of omega 3 fatty acid has not significantly affected the growth and health of the fish. However, the conclusiveness of these findings is limited by a relatively small dataset and incomplete or inconsistent data reporting in some studies. Possible further research can be conducted to examine the effect of ratio DHA vs EPA on the growth and health of Atlantic salmon, which is an important task for the future development of the industry and especially for aquaculture in Norway.

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Appendix

Annex 1: List of questions for quality accessibility

Measuring the quality of articles

1. Is the hypothesis/aim/objective of the study clearly described?

yes 1 no 0

2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?

If the main outcomes are first mentioned in the Results section, the question should be answered no.

yes 1 no 0

3.Are the characteristics of the population included in the study clearly described?

In cohort studies and trials, inclusion and/or exclusion criteria should be given. In casecontrol studies, a case-definition and the source for controls should be given. yes 1 no 0

4. Are the interventions of interest clearly described? yes 1 no 0

5.Are the distributions of principal confounders in each group of subjects to be compared clearly described? *A list of principal confounders is provided.* yes 2 partially 1 no 0

6. Are the main findings of the study clearly described?

Simple outcome data (including denominators and numerators) should be reported for all major findings so that the reader can check the major analyses and conclusions. This question does not cover statistical tests which are considered below. yes 1 no 0

7. Does the study provide estimates of the random variability in the data for the main outcomes?

In non-normally distributed data, the interquartile range of results should be reported. In normally distributed data the standard error, standard deviation or confidence intervals should be reported. If the distribution of the data is not described, it must be assumed that the estimates used were appropriate and the question should be answered yes. yes 1 no 0

8. Have all important adverse events that may be a consequence of the intervention been reported? *This should be answered yes if the study demonstrates that there was a*

comprehensive attempt to measure adverse events. A list of possible adverse events is provided.

yes 1 no 0

9. Have the characteristics of population lost to follow-up been described? *This should be answered yes where there were no losses to follow-up or where losses to follow- up were so small that findings would be unaffected by their inclusion. This should be answered "no" where a study does not report the number lost to follow-up.* yes 1 no 0

10. Have actual probability values been reported (e.g., 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001? yes 1 no 0

External validity

All the following criteria attempt to address the representativeness of the findings of the study and whether they may be generalized to the population from which the study subjects were derived.

11. Were the subjects in the study representative of the entire population from which they were recruited?

The study must identify the source population and describe how the sample were selected. Sample would be representative if they comprised the entire source population, an unselected sample, or a random sample. Random sampling is only feasible where a list of all members of the relevant population exists.

yes 1 no 0 unable to determine 0

12. Were those subjects' representative of the entire population from which they were recruited? *Validation that the sample was representative would include demonstrating that the distribution of the main confounding factors was the same in the study sample and the source population.*

yes 1 no 0 unable to determine 0

13. Were the staff, places, and facilities where the sample were treated, representative of the treatment the majority of population receive?

For the question to be answered yes, the study should demonstrate that the intervention was representative of that in use in the source population.

yes 1 no 0 unable to determine 0

Internal validity - bias

14. If any of the results of the study were based on "data dredging", was this made clear? *Any analyses that had not been planned at the outset of the study should be clearly indicated. If no retrospective unplanned subgroup analyses were reported, then answer yes.* yes 1 no 0 unable to determine 0

15. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of sample, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls? ves 1 no 0 unable to determine 0

16. Were the statistical tests used to assess the main outcomes appropriate?

The statistical techniques used must be appropriate to the data. For example, nonparametric methods should be used for small sample sizes. Where little statistical analysis has been undertaken but where there is no evidence of bias, the question should be answered yes. If the distribution of the data (normal or not) is not described it must be assumed that the estimates used were appropriate and the question should be answered yes.

yes 1 no 0 unable to determine 0

17. Were the main outcome measures used accurate (valid and reliable)?

For studies where the outcome measures are clearly described, the question should be answered yes. For studies which refer to other work or that demonstrates the outcome measures are accurate, the question should be answered as yes. yes 1 no 0 unable to determine 0

Internal validity / confounding (selection bias)

18. Were the samples in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population? yes 1 no 0 unable to determine 0

19. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time? yes 1 no 0 unable to determine 0

20. Were study subjects randomized to intervention groups? yes 1 no 0 unable to determine 0

21. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn? *In non-randomized studies if the effect of the main confounders was not investigated or confounding was demonstrated but no adjustment was made in the final analyses the question should be answered as no.*

yes 1 no 0 unable to determine 0

22. Were losses to follow-up taken into account? yes 1 no 0 unable to determine 0

23. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%? Insufficient power 0 Medium power 3 Sufficient power 5

Data to be extracted					
Item	Description	Note during the review			
Paper	Coding of paper from A(1) until A(n)				
Study/Year	Publishing year				
Concern	Growth/health/diseases	Topic that the paper focus on			
Samples	Samples size, age of the fish	If not smolt stage, exclude			
Experiment	Describe the diets in brief	If no, exclude			
Country	The place where the trials have been performed				
Duration	How long have the experiments taken place				
Reference	Title of paper with the link to the content				
Source	Full-text has been downloaded from which website				

Annex 2: The form for the first full-text review

Summary of growth performance in the 21 studies						
Item	Description	Note during the				
		review				
Paper	Coding of paper from A(1) until A(n)	Set up again the code after first full-text review				
Study/Year	Publishing year					
EPA and DHA (%)	Find out in the method and materials section	It should be calculated from diets formular if that no show				
Fish oil (% of total lipid)	Find out in the method and materials section	It should be calculated from diets formular if that no show				
Initial weight (g)	Find out in the results section					
Final Weight (g)	Find out in the results section					
Weight gain (g)	Find out in the results section					
Special Gain rate % per day	Find out in the results section					
RGI%	Find out in the results section					
Feed intake (g)	Find out in the results section					
FCR	Find out in the results section					
Final length (cm)	Find out in the results section					
Duration (day)	Find out in the method and materials section	It should be converted in day				
Name samples	Find out in the method and materials section	It should present all sample groups in the study				
Effect of omega 3 on growth in Atlantic salmon	Describe the finding of the study in brief (focus on the effect of omega 3 on the growth)	Combine both results section and conclusion section				
Category	In all, in few and no effect	For the studies get both negative and positive effect on the fish, those can be categorized in "in few" group				

Annex 3: The form for the second full-text review