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Effect of dietary protein and lipid sources on technical quality of pellets for Atlantic salmon

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I would want to dedicate my thesis to my Uncle Ram Krishna Karki, who died from delta variant of Covid-19.

Abstract

The purpose of the research was to examine technical quality of pellets added protein (P) and oil (O) sources of vegetable (V) or marine (M) origin. Four different diets for Atlantic salmon smolts were produced with 1) marine protein and marine oil (MP/MO), 2) marine protein and vegetable oil (MP/VO), 3) vegetable protein and marine oil (VP/MO/ and 4) vegetable protein and vegetable oil (VP/VO). Additionally, a commercial diet was used as control. The marine protein ingredient was fish meal (61%) while the vegetable protein ingredients were soy protein concentrate (35%), wheat gluten 22%), corn gluten (10%). Fish oil was used in the MO feed and rapeseed oil was used in the VO feed. Wheat was used in all feeds (MP feed 21% and VP feed 8%). The protein and fat content of all feeds were 47% and 22%, respectively.

Measurement of physical quality parameters of pellets included bulk density, durability, fat leakage, hardness, water stability, sinking velocity thickness, colorimetric analysis. Fish feed the feeds were inspected for skeleton development by X-ray as a marker for fish health, in addition to mortality rate.

Plant-based protein and oil sources showed no effect on the velocity of sinking pellets in fresh water as compared to marine-based sources. The average velocity of sinking pellets in saline water was not significantly different between pellets derived from plant and marine sources. Compared to marine-based and commercial meals, plant-based diets (VP/VO) showed lower water stability, stronger durability, and reduced fat leakage in our current study.

In addition, the a-value and b-value of plant-based and marine-based diets differ significantly. The utilization of a plant-based oil source in conjunction with a marine-based protein source has no significant effect on a-value (redness). A similar result can be obtained in the b -value (yellowness) Very low prevalence of spinal deformities were determined and dietary composition did not significantly affect the prevalence. Overall, the plant based diet showed a great potential to substitute the marine based diet in terms of physical pellet quality, further, experiments should examine effects on growth rate, fish weight and bone health condition or deformity concerns later in the sea water.

Keywords : pellet, technical quality, plant diet, marine diet, fat leakage , color, deformity

1. Introduction

The demand for food security is becoming a serious problem, with the global population estimated to exceed $9 billion by 2050. Aquaculture is the fastest-growing food producing sector in the world, with a worldwide production anticipated to expand by over 110 million tons by 2030 (FAO, 2018). It is also anticipated that aquaculture will become the most effective protein source in the world by 2030. When compared to standard terrestrial livestock production, aquaculture can produce large volumes of high-grade protein sources at a lower feed conversion ratio than any other production system, and with less carbon footprint (Hasan, 2019).

Sustainable aquaculture can provide a feasible way to feed the growing population of the globe. Many unsustainable techniques are, nonetheless, employed in fish farming. The high demand in sea food has been linked to harvest of wild creatures from the sea and estimated to harvest 170 billion pounds of creatures every year ("Sustainable Fishing", n.d.). Global consumption of fish meal and fish oil more than doubled between 1995 and 2004 (Hasan and Halwart, 2009). In the past three decades, fishmeal and fish oil consumption has remained constant. Fish meal is a precious source of protein which also contains important vitamins and minerals, whereas fish oil provides polyunsaturated fats as the major source of lipid (PUFA).

The aquaculture sector has been concerned to find good alternative protein and fat sources to minimize dependency on marine protein and lipid sources. The impact of feed resources on physical pellet quality has, however received less attention.

The cost of raw materials and feed components accounts for 80% of the total cost in salmon production. Hence, the economic perspective in the feed formulation process needs to be systematically planned (Wickins, 1988). Rapeseed oil is currently the dominant oil source in salmon diets due to its low cost, availability, and high digestibility, and it has been identified as the most promising replacement to fish oil among the studies being conducted to develop sustainable alternatives (Sargent and Tacon,1999; Turchini, 2011). The production of rapeseed oil with low levels of glucosinolates has been possible by plant breeding and genetic engineering. In addition, a high percentage of PUFA in fish oil makes pellets more sensitive to rancidity which can be overcome by using the rapeseed that is high in monounsaturated fatty acids (MUFA) or other plant based oils instead. Study also shows that some customers enjoy a reduced flavor of fishy smell of fish ; fed diets with lower levels of fish oil (Stead & Laird, 2002). Therefore, the use of use of plant-based oil or in combination can satisfy the consumer's high sensory requirements.

With the great expansion in the salmon and other aquaculture industries, issues have arisen in understanding salmon nutrition and feeding behavior in light of its long-term sustainability as well as environmental and fish health concerns, which could play an essential part in the sector's development. Among the most important health parameters is the normal development of the fish, including the development of the skeleton, as one of the greatest challenges in farmed salmon is the occurrence of bone deformities, which can reduce growth, lower harvest quality, and raise concerns about fish welfare as well as economic and environmental losses (Gjerde et al. 2005; Hansen et al. 2010).

Nutritional and mineral factors could influence the deformities in Atlantic salmon, despite the fact that these factors are variable and irregular within the production environment and can also be changed by production methods (Agyeman, 2019). Deformities can devalue meat at slaughter and, in the worst-case scenario, even cause issues with machines during the filleting process (Sullivan, Guy, Roberts and Manchester, 2007).

According to a study Baeverfjord et al. (2019) the early stages of the salmon's life in fresh water, prior to their transition to sea water, are the most important for the occurrence of deformities in salmonids. The same study also shows that different bone deformities are linked to different life stages, such as the incidence of fusions, hyperdense vertebrae in fresh water, and compressed vertebrae in the seawater phase. Deformities that arise in infancy have been found to have long-term repercussions. Meanwhile, dietary behaviors could be regulated to remedy the deformity problem. Lower phosphorus supplementation in the early feeding diet of salmon (of about 20g) has been reported to have severe impacts on vertebrae and jaw development in the slaughter stage (Baeverfjord et al. 2006). Short tails and compressed caudal vertebrae have been reported to emerge more frequently during the growth period, posing a major welfare issue as well as abnormalities that reduce production value. The frequency of compressed vertebrae increased with fish size in seawater. The data gathered as part of this monitoring study revealed that bone malformations in some early life stages can have long-term consequences. Thus, the dietary factors can play a decisive role in influencing the manifestation of deformities in the later stages of Atlantic salmon such as parr, pre-smolt, smolt, and to a lesser extent in the post-smolt phase. However, Berge et al. (2009) and Gil-Martens et al. (2010) reported that the substitution of fish oil with vegetable oil in the diets of Atlantic salmon in freshwater had no effect on the incidence of vertebral abnormalities at harvest size.

According to a study (Gonzalez, 2018), having more phosphorus in the early diet of triploid salmon compared to diploids from their initial feeding to parr stage can result in optimal growth and a reduction in skeletal deformities in Atlantic salmon. This current study also aims to determine whether bone abnormalities are associated with the feed ingredients utilized during the early life stages in freshwater.

2. Aim of the study

The main objective of this thesis is to study the effects of different dietary feed materials on technical quality of pellets for Atlantic salmon. The current study also seeks to determine whether bone deformities are linked to the feed ingredients used during their early life stage in fresh water. In the study, it is hypothesized that different dietary protein and lipid sources from plant- and marine-based diets could have an impact on the pellet quality and health of juvenile salmon during their early life stages.

3. Theoretical background

Population expansion, along with increasing urbanization and higher per capita income in many regions of the world, alters consumption habits and places pressures on available food resources. In 1982, when the world population was 4.5 billion per capita, meat consumption was 15 kg, and it is predicted to rise to 37 kg by 2030 (FAO, 2011a). This will have a significant influence on the environment as well as available land, fresh water, and phosphorus supplies, necessitating immediate action to build food systems that use less energy and produce fewer greenhouse gases. The global food sector consumes around 30% of the world's energy and emits more than 20% of greenhouse gas emissions (FAO, 2011b). Furthermore, the same study reveals changes in land use, primarily deforestation, account for 15% of greenhouse gas emissions.

Meat consumption worldwide is rising at a rate of about 3.6 percent per year, having nearly doubled between 1980 and 2004 and is anticipated to have doubled once again by 2030 (FAO, 2011b). There is also a gradual shift from extensive grazing systems and toward more intensive production methods that rely on more concentrated diets and feed components, that are marketed globally. Moreover, a third of the world's grain crop is presently utilized as animal feed. The usage of phosphorus (P) fertilizer is also critical to global food production. A meat-rich diet requires three times as much P as a vegetarian diet, and a 20% increase in P fertilizers would be required for a world population of 7.7 billion people without any changes to the global diet (Smit et al, 2009).

With limited land area and water, producing food in the sea is an enticing option. Aquaculture is currently over half the entire supply of edible fish and the figure rises annually (FAO, 2012).

This fast development has generated worries among customers, merchants and NGOs regarding the environmental effect and sustainability of fish farming.

The aquaculture feed industries are often invoked as argument against the sustainable use of salmon production and its repercussions for wild fish populations (Deutsch et al., 2007; Tacon and Metian, 2008). Due to the usage of tiny pelagic fish in the feed, salmon farming has been viewed negatively, and it has been argued that salmon farming lowers the quantity of marine protein available for human consumption (Naylor et al., 2009). Aquaculture, like all food production, has environmental impacts, and feed production is an important component contributing to salmon production too (Ellingsen, Olaussen and Utne, 2009). Therefore, it is vital for the strategic decision-making on food production regimes to have a knowledge of the environmental consequences of different feed formulations and how this affects resource use (Åsgård & Austreng 1995; Åsgård et al. 1999).

Norway is now the world's top producer of Atlantic salmon. The national aim, which is 4-5 times the current production, is to raise salmon production up to 5 million tons by 2050. There are several environmental obstacles, including parasites (sea lice), escapees and pollution in the surrounding ecosystem in the water, that need to be managed and regulated to implement this development potential. In the long run, however, sufficient sustainable feed, with a minimal carbon impact, will be the major problem. These issues are strongly concerned by Norwegian fish producers, regulators, researchers, and scientists.

Several projects are now underway to improve feed supply. The harvesting of mesopelagic species, krill, and zooplankton from the seas receives special attention. Alternatives include converting numerous plant materials and Norwegian spruce trees to growing yeast or converting hydrocarbons to bioproteins has also been in trial to find as best alternative for fish feed.

Eventually, with an economic feed conversion rate of 1.2 (kg of edible food / kg of dry feed), up to 5 million tons of salmon will need up to six million tons of dry feed (Almås, 2021). While salmon production in the ocean is one of the most sustainable sources of animal protein due to its abundant water supply and low-temperature use compared to warm-blooded animals such as beef, the feed component is the primary contributor to the carbon footprint of the entire salmon production chain.. The pursuit of sustainable feed materials for salmon farming in Norway is therefore on the strategic agenda.

Fish feed is an important component in the optimal health and performance of fish. Feeds also accompany the biggest contribution to their environmental impact in any life cycle assessment (LCA) of salmon aquaculture. Fish farming is one of the most eco-friendly livestock farming practices. In comparison, chicken has a carbon footprint of 6.2 kg per edible kg, pig has a carbon footprint of 12.2 kg per edible kg, and beef has a carbon footprint of 39.0 kg per edible kg (MOWI, 2021).

Significant CO_2 emissions are minimized by substituting land-based animal protein with farmed salmon. In 2020, 14.0 million tons of CO_2 emissions could have been saved if worldwide salmon production replaced a combination of chicken, pig, and beef production being projected by the same study.

3.1. Changing Feed Composition in Salmon

The wild fishing development and the rapid expansion of aquaculture have led us to rely on over a limited supply of feed, i.e., including fish meal and fish oil, as well as also understanding of the difficulties of replacing other protein and oil (Sargent and Tacon, 1999; Naylor et al., 2000). Thus, as a result, less expensive and cost effective alternatives (such as soybean meal and rapeseed oil) have been explored and progressively substituted in commercial formulae for feed, where they are technically and economically viable and, as appropriate, following additional processing (NRC, 2011).

90% of Norwegian salmon feed was made from fish meal and fish oil in the 1990's (Ytrestøyl, Aas and Åsgård, 2015). The two products now make up around 30 percent of the diet and are still sufficient to ensure adequate concentration of omega-3 fatty acids EPA and DHA in salmon. The balance of the diet comprises of plant components like soya, grown in compliance with principles of sustainability (Ytrestøyl, Aas and Åsgård, 2015) (Figure 1 and 2).

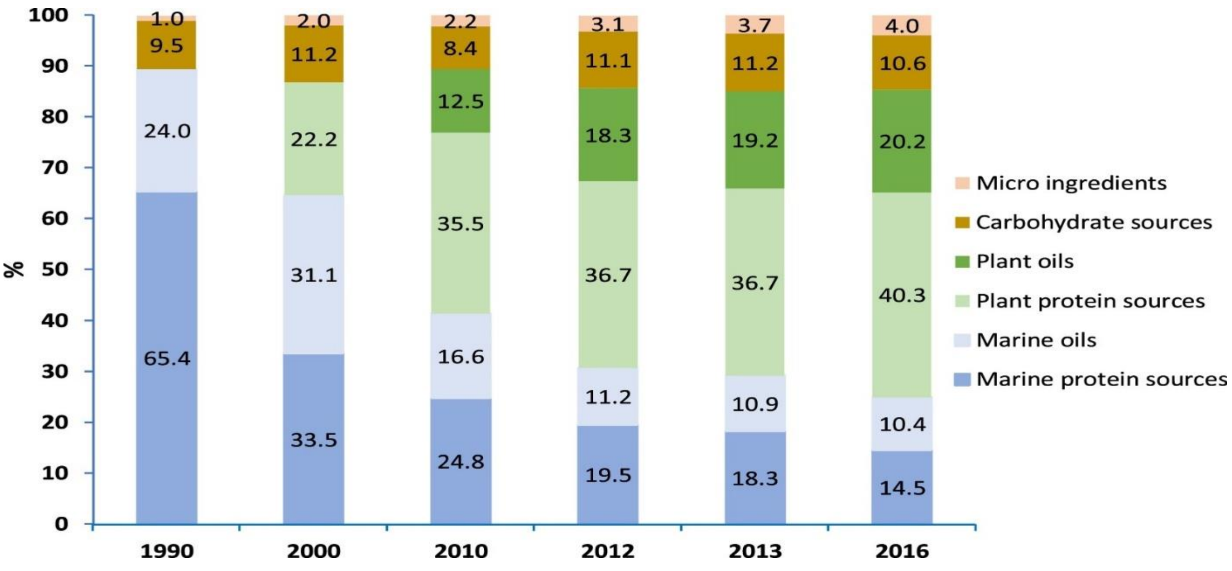


Figure 1. Changes in the ingredient sources in Norwegian salmon feed (percentage of feed) in 2016 compared to prior years (Adapted from Aas et al., 2019)

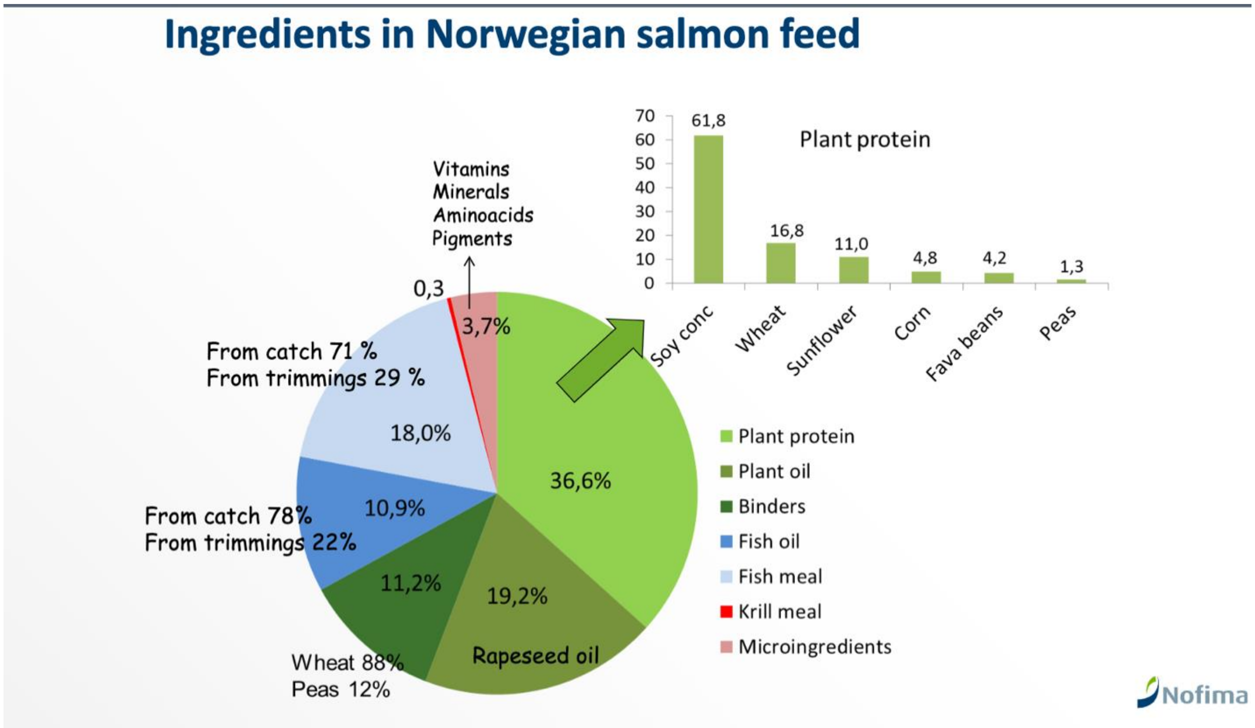


Figure 2. Feed Ingredients in the Norwegian Salmon *Source Nofima.no*

However, plant based ingredients lacking EPA and DHA and in the diet with plants accompanying the lower fat or cholesterol level, fish should take it externally (Tocher, 2015). Increase in the content of phytate in the plant-based diet has affected the availability of nutrients acting as anti-nutritional properties affecting the fish performance.

Furthermore, according to research published in 2015 by Ytrestøyl, Aas & Åsgård, the comparable marine oil reliance ratio was 2.8 in the 1990s, but in 2013, just 0.5 kg of marine oil was required to produce 1 kilogram of fat in salmon with the reduction of 82.15 %. The fatty acid composition of the salmon, on the other hand, mirrors that of the dietary oil. Thus, reducing the amount of marine fat in salmon feed diet reduces the levels of the long-chained unsaturated marine fatty acids EPA and DHA while raising the levels of n-6 fatty acids. Thus, nutritional dependence rations for EPA and DHA may be a more accurate indicator of marine fat dependence, so long as these fatty acids are obtained from marine sources rather than other alternative sources.

Because Atlantic salmon have a limited capacity to synthesize the essential fatty acids (EFA) 18:2n-6 (linoleic acid) and 18:3n-3 (α-linolenic acid); EPA (20:5n-3) and DHA (22:6n-3) must be added to their diet (Waagbø et al., 2001). These long-chain fatty acids are critical for maintaining the fluidity of cell membranes, which is crucial for cold-water marine organisms like salmon.

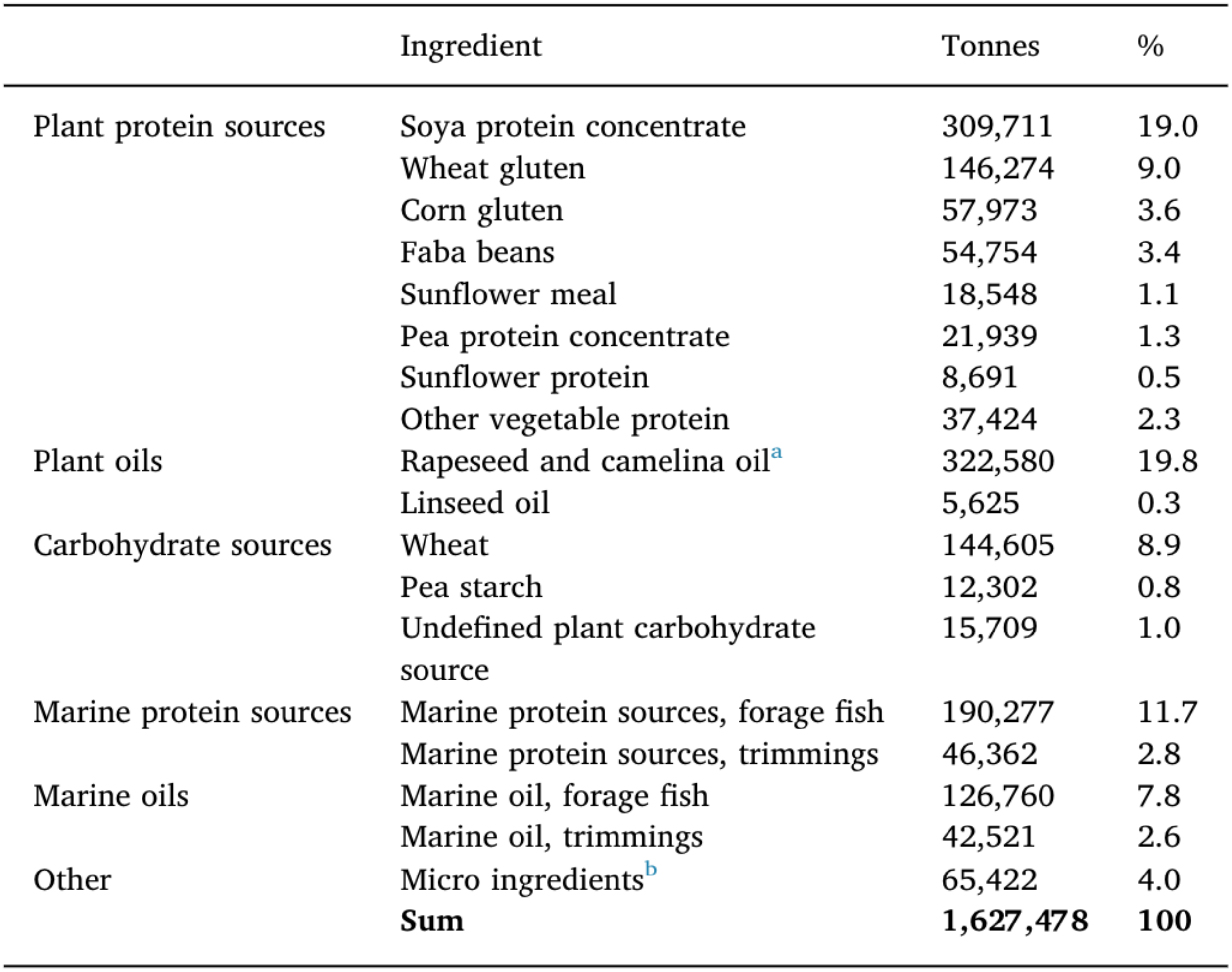
The marine harvest of wild caught fish for fish meal and oil production, on the other hand, is heavily reliant on anchovy fisheries, and their quantity varies with structural changes and temperature change (Moron et al., 2019). Using higher tropic marine resources even raises problems about sustainability and the need for finding alternative sustainable materials.

Some mesopelagic fish, krill, and copepods from lower tropic level species may be able to replenish some quantities, but this is a finite and unsustainable resource too. There has been a lot of continuing study into the use of bacteria, fungus, yeast, algae, and other insects as sustainable marine sources (Bellona, 2021).

Soy protein concentrate (SPC) is the most important plant ingredient in Norwegian Atlantic salmon aquaculture production. Almost 20% of all substances used in 2016 were made of it.

Wheat gluten (9%) and maize gluten (3.6%) were the second and third most important sources of plant protein after soy protein concentrate. Rapeseed and camelina oil make up 19.8% of the total amount of components used. In comparison to other plant-based oils, linseed oil accounts for barely 0.3 %. Wheat is also a key source of carbohydrates (Aas et al., 2019). Micronutrient supplementation accounts for 4% of total feed components (Table 1), which is critical when plant-based ingredients are also utilized in greater proportions than marine-based diets in salmon feed (Vera et al., 2020)

Table 1. Ingredients used in Norwegian Atlantic salmon aquaculture production, 2016



	Ingredient	Tonnes	%
Plant protein sources	Soya protein concentrate	309,711	19.0
	Wheat gluten	146,274	9.0
	Corn gluten	57,973	3.6
	Faba beans	54,754	3.4
	Sunflower meal	18,548	1.1
	Pea protein concentrate	21,939	1.3
	Sunflower protein	8,691	0.5
	Other vegetable protein	37,424	2.3
Plant oils	Rapeseed and camelina oil[a]	322,580	19.8
	Linseed oil	5,625	0.3
Carbohydrate sources	Wheat	144,605	8.9
	Pea starch	12,302	0.8
	Undefined plant carbohydrate source	15,709	1.0
Marine protein sources	Marine protein sources, forage fish	190,277	11.7
	Marine protein sources, trimmings	46,362	2.8
Marine oils	Marine oil, forage fish	126,760	7.8
	Marine oil, trimmings	42,521	2.6
Other	Micro ingredients[b]	65,422	4.0
	Sum	**1,627,478**	**100**

a : rapeseed oil and camelina oil

b : vitamin, mineral complexes, amino acids, phosphorus and astaxanthin

(Aas et al., 2019)

Since 1990, the salmon feed has transformed considerably, with a growing number of marine ingredients substituted by plant ingredients (Ytrestøyl et al., 2015). Same study reveals that, in 2016, marine protein sources made for 14.5% of feed, a drop over 2013. The plant protein sources have increased accordingly. Marine oil accounted for 10.4% of feed, which has reduced slightly since 2013, and a slight increase in plant oils has occurred (Ytrestøyl et al., 2015). The same study depicts that, in terms of marine sources, forage fish account for 11.7 percent of marine protein and 7.8 percent of marine oil, respectively, whereas trimmings account for 2.8 percent and 2.6 percent of marine protein and marine oil sources, respectively. The primary sources of carbohydrate such as wheat are included as binders, and also, use of micronutrient elements has progressively increased in the diet (Table 1).

The increase in the use of the plant-based ingredients and its replacement in the diet of salmon could lead to a inflammatory effect on the intestine (Waagbø et al., 2013). Moreover, the antinutrient factors present in plant-based ingredients can be very harmful. In addition, plant based oil sources lack the omega 3 long chain fatty acids, which the salmon requires and is fully available in marine based sources. Moreover, there are promising results documenting production of omega 3 from genetically modified plants (Napier et al., 2019).

As a more affordable, infinite, and sustainable alternative to marine-based sources, plant-based ingredients for aquafeed raise questions about their long-term viability in the face of rising population and competition with human food and land resources. A life cycle assessment of these ingredients is therefore necessary which likely can change the feed ingredients for salmon in future (Taelman et al 2013).

In general, fish oil prices are more variable than vegetable oil prices, owing to unpredictable supply as a result of fisheries quota regimes. The average price of fish oil in 2020 was about USD 2,275 per tone, while the price of rapeseed oil has also been declining in recent years (MOWI 2021).

The rise in supply, notably in expanding production in Brazil, has put soya and maize prices reducing the price per ton to USD 455 (MOWI, 2021). Although prices of fish meal have remained steady on an annual basis, there have been significant fluctuations across years (Figure 3).

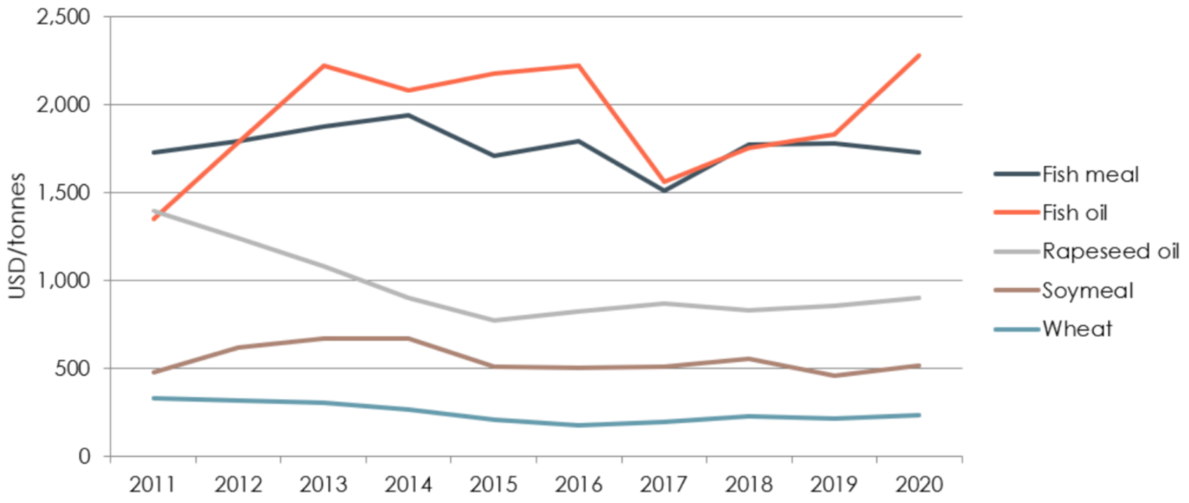


Figure 3.Comparison of feed Ingredient Prices in respective years

(MOWI, 2021).

Raw material sources for aquaculture from the wild fishing industry have limited development potential due to sustainability problems (Michael & Wijkström, 2002). Because of the shortage of such raw ingredients and the reduced availability, price rises might lead to strong pressures on the aquafeed sector to find an alternative and sustainable sources of fish meal and fish oil.

The major focus regarding alternative feed sources is to identify easy-to-process ingredients, make minimal losses before and after processing, supply important nutrients needed for fish, improve fish's health, and ingredients should be cost effective and easily accessible throughout the year.

3.2. Fish Quality and Other Aspects

Quality of fish is very important in the aquaculture production industry as it has a direct influence on the consumer level in regard to their health and nutrition concerns. To maintain proper quality, fish flesh should meet the certain quality parameters in terms of appearance, freshness, firmness, texture, juiciness, smell, taste and other hygienic properties which must be guaranteed for improved consumer satisfaction (Nguyen, 2011; Skrede & Wold, 2008). Moreover, fillet should meet hygienic, technical and ethical quality parameters in the production line before reaching the customers, who are an ultimate judge for the quality. However, quality assessment differs in different countries and also varies with different marketing chains (Adhikari, 2016).

Overall, various factors from breeding of species (Gjedrem, 1997), to its feed composition (Bell et al. 2002), metabolism, stress (Mørkøre et al., 2008), temperature fluctuation (Nguyen, 2011), period of starvation prior to harvesting (Mørkøre et al., 2008), handling, transportation , chilling and also the storage conditions (Sigholt et al. ,1997) can have significant impact on flesh quality of fish.

Adult Atlantic salmon with marketing quality are distinguished by their streamlined body form, glossy skin with black spots, dark blue upper part, and fat fin in front of the tail fin, and the fish also should not have deviating appearance due to spinal abnormalities responsible for causing cartilage in fillets and consequently poor quality. The pink-red color, firm texture of the fillet and delight of sea fragrance are other key sensory quality features which need to be maintained for good sensory quality.

3.3. Vertebral Malformations in Salmon

Mismanagement and insufficient attention on technical factors of quality might lead to vertebral column abnormalities, that can decrease productivity. Vertebra defects can prevent the normal development of farmed salmon, which can pose ethical questions regarding the welfare of animals

The typical Atlantic salmon has 56-60 vertebrae. The vertebrae are a lightweight or fragile structures that have evolved to a life in water. It has four layers and are made up of cells, minerals, proteins, and lipids (Lall, 2002).

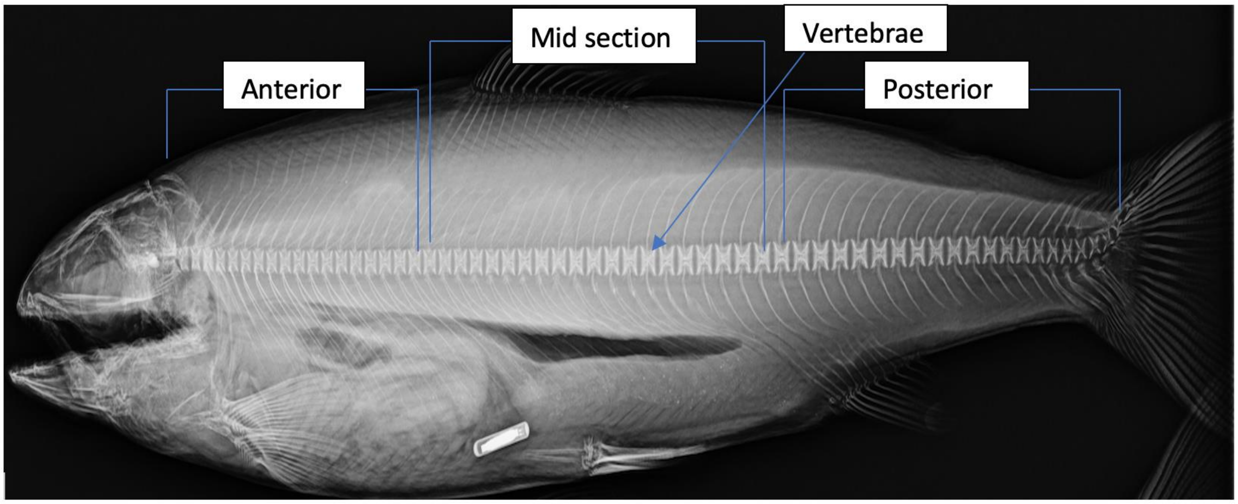


Figure 4. Image showing the major areas (anterior, mid and posterior section) and vertebras (1-60) of Atlantic salmon (Photo: Turid Mørkøre, 2021)

Deformities are anomalies that can impede one's ability to function. This reduces productivity but also raises worries about fish welfare (Huntingford et al., 2006). Researchers, aquaculture professionals, businesses, quality assurance systems and NGOs and consumers are increasingly interested in the subject of fish welfare (Huntingford et al., 2006). Skeletal quality has been found to be major downgrading factor in many of the hatchery production accompanying 7-20 % on the produced juveniles (Georgakopoulou, et al., 2010). This type of abnormalities in fish will have an impact on production costs, the look of fish, their survival and eventual growth (Berillis, 2015). Some studies think that these distortions are caused throughout embryonic and post-embryonic lifetimes, as well as influenced by abiotic and dietary or nutrient factors (Cobcroft & Battaglen, 2009; Georgakopoulou, et al., 2010).

Atlantic salmon pose a high risk of developing deformities in the early seawater phase and also during the period of smoltification, major changes in vertebral column could also be prevalent through out the growing period in varied life stages (Chidakwa, 2019).

Vertebral malformations such as lordosis, scoliosis, kyphosis, platyspondyly, and vertebral fusion; jaw malformations such as bent, reduced lower jaw, harelip, front, and downwards protuberance of jaw; spinal malformations such as compressed snout and neck bend; reduced or uneven fins, short jaw, and branchial arches deformity are all common in Atlantic salmon (Berillis, 2015).

Only a few vertebrae may fuse in the case of vertebral abnormalities, without causing substantial or visible physical alterations in the fish. A short front-back vertebra column can lead to a hunch back or a shorter tail when a large number of vertebrae are fused. Nodal densification may also occur at the fusion location (Witten, et al., 2005). Some smolts may have squeezed a vortex during the fall by decreased dietary mineral intake. Swirl compression is especially apparent between vertebra 31 and 49 which can be addressed by ensuring that in the early water stage these smolts satisfy their mineral requirements (Fjelldal, et al., 2006). Furthermore, other abnormalities of the jaw may develop. The fish might possess opercular shortening, snout shortening and other lower or bending jaw deformities. unequal fins, short jaw, arch deformity, adhesions, curving, lordosis, scoliosis, kyphosis and platyspondyly are some types of deformity which can also be more prevalent (Chidakwa, 2019).

In the early juvenile phases, where P is inadequately supplied, the vertebral and jaw abnormalities during the harvest period are frequently observed. Furthermore, at the later growth stage, the predominance of compressed vertebras might be distinctive.

Rapidly growing Atlantic salmon may develop low-mineralized soft bones in comparison to fish with lower growth rates (Fjelldal et al., 2006). The likelihood of vertebral deformations has risen in fast growing salmon due to increased pressure of new muscle growth mass under calcified bones ((Fjelldal, Nordgarden and Hansen, 2007; Fjelldal, 2005), causing the intermediate tissue and malformation (Witten et al., 2005). For bone mineralization requirements, rapidly developing animals must absorb a greater share of the mineral content (Hernandez et al., 2000). However, further investigations are required about the absorption of mineral products and through its transit or passage of fish intestines. The present shift in the development of fish feed, by changing to the lipid diet based on vegetables, might further affect mineral, vitamins and other amino acid intestine absorption (Gebreselassie, 2017).

There is consequently a need to regulate the several elements that influence proper development and growth throughout the early phase of life in the achievement of the predictable production of quality fish later in life stage. In order to be able to balance diet and properly utilize the feed ingredients available, it is essential that we understand the relationships between dietary mineral levels, n−6/ n− 3 fatty acid ratios, bioavailability, growing rate, temperature conditions, and intestine uptake and absorption of nutrients.

Also, the potential of radiography or X-ray to penetrate deep into the tissues has developed the potential to study the pathology of bones allowing for better diagnosis of deformity.
The size of the specimens used, the preparation of the specimen, the configuration of the X-ray equipment, and the pictures processed are all factors that affect the quality of X-rays. The major limits in fish radiography are the size of the fish skeleton and its low density, and this continuously undermines the requirement for early diagnosis leading to the sampling of extremely small fish (Aquafeed.com, 2019). Atlantic salmon develops fast in early juvenile fresh water phases. Salmon mineral content is often measured in the wet weight as whole-body content. Furthermore, studies have indicated that a juvenile salmon with proper skeletal structural mineralization should have phosphorus (p) and calcium 4,000mg kg-1 or more (Aquafeed.com, 2019).

4. Materials and Methods

4.1. Fish Feed Materials

Feeds pellets used for Atlantic salmon (*Salmo salar, L.*) were prepared by extrusion method at Nofimas Feed Technology Center and later used in the fish feeding trail at Sunndalsøra (Nofima Forskningsstasjon for bærekraftig akvakultur).

The marine diet was predominantly composed of fish meal (61%), wheat (20.7%) (pellet binder), and fish oil (15.4 percent). Soy protein concentrate, SPC (34.6%), wheat gluten (22%), and rapeseed oil (20.4%) constituted the bulk of the plant diet, with corn gluten (10%) and wheat gluten (8.4%) serving as supplements in plant based diet.

The composition of feed used in the experiment is shown in Table 2. Both plant based and marine based diets were added with vitamins and mineral premix in the tiny amount as shown in the Table

3. Those feed samples was stored at the cold storage at 4°C and tested for physical and chemical analysis.

4.2. Experimental Framework

As of December 2019, eyed Atlantic salmon eggs were hatched in Sunndalsøra, Norway. They came from the same genetic source (Bolaks) and were all from the same batch. When the fish reached the fry stage after 861 d°, in January 2020, it was time to start feeding them. A flow-through system was then set up for the fish that were used, in this study. The fish were put into six circular tanks and kept there. At 30g weight on average, they were tagged and then moved to 15 500L tanks between July 7th and September 16th 2020, 110 fish per tank (15 tanks in total; 3 tanks per dietary treatment). The fish were exposed to a light regime that mimicked a natural summer-winter-spring daylength signal (12:12) from July 13th, they began to smoltify. The fish were vaccinated from August 26th to August 27th (when average weight was 83g). During the 16-17th of September, all fish in each tank were weighed and length was measured

Table 2. Composition of feed used in the experiment

Treatment	Composition
T1	Marine protein/Marine oil (MP/MO)
T2	Marine protein/Vegetable oil (MP/VO)
T3	Vegetable protein/Marine oil (VP/MO)
T4	Vegetable protein/Vegetable oil (VP/VO)
T5	Commercial feed

Table 3. Formulation of feeds used in the Experiment

Diet Composition	**Marine protein/Marine oil**	**Marine protein/Vegetable oil**	**Vegetable protein/Marine oil**	**Vegetable Protein/Vegetable oil**
Fish meal	61	61	-	-
SPC	-	-	34.6	34.6
Wheat gluten	-	-	22	22
Corn gluten	-	-	10	10
Wheat	20.68	20.68	8.37	8.37
Fish oil	15.4	-	20.4	-

Rapseed oil	-	15.4	-	20.4
MgSO4 (500ppm ekstra)	0.2	0.2	0.2	0.2
K2CO3 (500ppm ekstra)	0.1	0.1	0.1	0.1
Vitamin premix	0.5	0.5	0.5	0.5
Monosodiumphosphate	2.5	2.5	2.5	2.5
Astaxanthin	0.05	0.05	0.05	0.05
Yttrium oxide	0.01	0.01	0.01	0.01
Mineral premix	0.5	0.5	0.5	0.5
Water adjustment	-0.98	-0.98	0.73	0.73
Sum	100	100	100	100

4.3. Chemical composition of the diets

Protein was the primary component of both the marine and plant diets, followed by lipids, carbohydrate, and ash. The marine diet had 47% protein, 22% lipids, 12.4% carbohydrate, and 11.7% ash. On the other hand, the plant-based diet contains 47% protein, 22% lipids, 5% starch, and 5.9 % ash. Concentration of EPA and DHA varies in Marine based diet and plant based diet.

The marine diet had a total of 4% EPA and DHA, whereas the marine protein and vegetable oil mix diet contained 1.1 % EPA and DHA. The entire plant-based diet included no EPA or DHA, but the plant protein and marine oil mixture contained 3.8 % (Table 4).

Table 4. Nutrient profile of the experimental diets

Diet Composition	**Marine protein /Marine oil**	**Marine protein /Vegetable oil**	**Vegetable protein /Marine oil**	**Vegetable Protein /Vegetable oil**
Protein	47.0	47.0	47.0	47.0

Lipid g/kg	22.0	22.0	22.0	22.0
Starch	12.4	12.4	5.0	5.0
Ash g/kg	11.7	11.7	5.9	5.9
Water	6.5	6.5	6.5	6.5
Energy. MJ/kg	22.1	22.1	20.8	20.8
EPA + DHA	4.0	1.1	3.8	0.0

For dry matter calculation, feed samples were dried at 105 °C to constant weight, then analyzed for ash by combustion at 550 °C to constant weight, crude protein by nitrogen x 6.25 (Kjeldahl Auto Analyzer), and crude lipids by SOXTEC hydrolyzing and extraction systems.

Bomb calorimetry was used to determine gross energy (Parr 1271 Bomb calorimeter). Inductively coupled plasma mass spectroscopy (ICPMS, Eurofins, Moss, Norway) was used to examine minerals and a marker (yttrium).

4.4. Physical properties of the feeds

4.4.1. Durability Test

Durability of the experimental feeds was examined by using LignoTester Serial N^0 LT110 Borregaard Lignotech, Norway at Laboratory of IHA building, NMBU. All the samples were passed through sieve shaker machine (Retsch AS200 Control “g”, Germany) for separation of the dust and broken particles present in pellet before using it in lignotester. Particle size of various samples were also examined through this sieve machine. Approximately 30 grams of dust free sample was then added to hopper of tester. The hopper has diameter of 2.5 mm and was covered with filter paper to avoid any of the broken pellet to escape while running lignotester machine. In addition, any oil content present in the feed can also stick to filter paper during the test. For each test, hopper was run for 2 minutes and test was done for 3 times for each sample. During the run, air pressure of 100 mbar was maintained. Sample was sieved automatically in 2 minutes run for each test, meanwhile dust, broken , smashed or sieve particles are also collected in perforated hole present in the bottom of machine. This automatically sieve samples were weighed to know the final weight of sample after going through tester. After each test, machine was cleaned and wiped up to reduce any error for next test. With the known initial weight of pellet before testing and final weight after ligno testing, pellet durability index (PDI) is calculated by the formula

$$\text{PDI (\%)} = \frac{\text{Final Weight of sample after lignotesting}}{\text{Initial weight of sample before lignotesting}} * 100$$

4.4.2. Particle Size of pellet

Table 5. Particle size analysis of pellet

The particle size analysis showed no any significant difference among the treatments.

		Pellet size (100 gm weight)	
Sample	<1mm	1mm	2mm
Marine protein/Marine oil	0.18g	0.12g	99.7g
Marine protein/Vegetable oil	1.01g	0.24g	98.74g
Vegetable protein/Marine oil	0.4g	0.07g	99.53g
Vegetable Protein/Vegetable oil	0.31g	0.05g	99.64g
Commercial feed	0.14g	0.04g	99.82g

In regard to measurement of other physical properties, an electronic caliper was used to measure the pellets diameter and length. Furthermore, the bulk density was determined by pouring the feed through a funnel into a 1000 ml measuring cylinder loosely. Prior to registering the weight, the top was slightly levelled.

4.4.3. Fat leakage using Texture analyzer

Fat leakage analysis was carried instrumentally using a Texture analyzer (TA-XT2, Stable Micro System, Surrey, UK) (See Appendix 10.2). Ten pellets of each of the feed sample were used as replicates.

4.4.3.1. Sample preparation for fat analysis

· All the pellets will be brought to same room temperature & left it for 4 hours

· 589 ^{3} Blue ribbon ashless S & S Filter Paper circles of 125 mm diameter (Ref. no. 300211) to be used

Ten pellets of each sample were placed in a horizontal position at the top of filter paper and test was performed according to below test mode parameter. After that, broken pellets can be removed gently and later filter paper can be used to dry at 105 ^{0}C for 3 hours and weights are measured

4.4.3.2. Test mode of analyzer for analysis

The trigger force of 1N was applied. Test speed 1 mm/s with 80% strain for 90 seconds.

Fat leakage can be calculated as the

(Final weight of paper-initial weight of paper)/Weight of pellet used for analysis *100

The test speed for the load arm was assigned to 1mm/sec. The load arm was also equipped with the flat aluminum probe where the pellets of each replicate of feed samples gets broken in between the probe and bottom plate and the force required to break the pellets (hardness of the pellet) were recorded in Newton along with other parameter to be record will be area under from the force time graph and oil leakage.

Table 6. Assigned Testing Mode of Texture Analyzer

Caption	**Value**	**Units**
Test Mode	Compression	
Pre-Test Speed	5	mm/sec
Test Speed	1	mm/sec
Post-Test Speed	10	mm/sec
Target Mode	Strain	
Strain	80	%

Hold Time	90	sec
Trigger Type	Auto (Force)	
Trigger Force	1	N
Advanced Options	Off	

For, floating and sinking pellet analysis, methodology of Pandey, 2018 is practiced. A cylindrical tube of 1 liter was filled with fresh water and for making salt water, five liters of fresh water were used to make sea water at a concentration of 3.5 percent by volume. 175g salt was added to five liters of water for making salt water. Following that, the salt water and fresh water was left for 24 hours at room temperature. Each test sample consisted of 60 pellets chosen at random. The pellets were then dropped using a tweezer from the tube's top (5cm above the water level), and the time taken to travel from the first to the last mark (33.5 cm) was recorded using a timer.

Pellets that do not sink after 15 seconds were classified as Floating pellet and that sinks were classed as sinking pellet. For salt water, a similar process was followed and additionally, the time required to travel the known distance 33.5 cm was measured in seconds using a stopwatch. For every five pellets dropped into the fresh or salt water, the water was replaced as recommended by (Pandey, 2018)

4.4.4. Water Stability Test

The methods and techniques used in study were according to Baeverfjord et al.(2006) as a basis for measuring the stability of feed pellets in the water. For the test, 20 gm of pellet sample is used for each replicate. Each test sample was then placed in cylindrical wire guaze container (diameter: 8cm, net mesh size :3mm) which was placed in a beaker of 600 ml with 300ml distilled water (fig). Later, the beaker with the cylindrical wire container were placed in a shaker SW22 Shaking water bath (temperature of water bath in shaker maintained at 23^0 C). The shakers were carried out in the speed of 100 shakings per minute and was shaked for 30 and 60 minutes for each replicate test sample. After the respective time of shaking, shaking bath was turned off followed by gently drying of the wize guaze with some papers and weighing was carried out. The wire guaze was then dried in the heating oven chamber at 105^0C for 18 hours and residual drymatter weight (%) was calculated. The test for 30 minutes and 60 minutes water bath shake was carried out three times per feed sample (Appendix 10.4).

Finally, the water stability was calculated through a difference in the dry matter weight of feed before and after the incubation from the oven chamber (Baeverfjord et al., 2006)

4.4.5. Bulk Density

Measurement is carried out by filling up the measuring cylinder with a known specified volume of the pellets. The surplus feed from the surface is gently scraped away and process is repeated three times for each sample treatment. The bulk density of feed pellet is calculated as the mass of feed to the known volume of the space the feed occupied. As the feed pellet bulk density is one of its most important features as it is a crucial factor for determining the floating and sinking characteristics of the feed pellets and can also be regulated during the processing of the pellets as per the behavior of fish.

4.4.6. Dry matter content test

The dry matter content of feed samples was measured in accordance with Pandey's (2018) recommendation. At random, 20 grams of each treatment's feed pellets were grinded. Each ground sample was weighed and then later dried at 105 ^{0} C for four hours and weighed again. The number of replicates for each sample was 4. The difference in final weight of feed sample after drying to initial weight before drying in the oven gives the dry matter percentage.

4.4.7. Colorimetric Analysis

Using a chroma meter (CR-400, Konica Minolta Sensing Inc., Osaka, Japan) capable of measuring L*, a*, and b* color space, the color of feed pellets was assessed. The L*, a*, and b* system allows for the representation of color in three separate coordinates (dimensions), with the following color components referred as L* for lightness, a* for red/green, and b* for yellow/blue. The number of replicates per each feed treatment were 20.

4.5. Deformity Analysis

X-rays were taken utilizing semi digital X-ray system. For deformity analysis, categorization method (Figure 5) developed by Witten et al . (2009) were used to establish the sorts of deformities, and deformed fish were considered as those with at least one twisted vertebra. Altogether 20 different fish were used deformity analysis. The number of vertebrae in each individual fish was counted, and if two or more fused vertebrae were present, they were recorded.

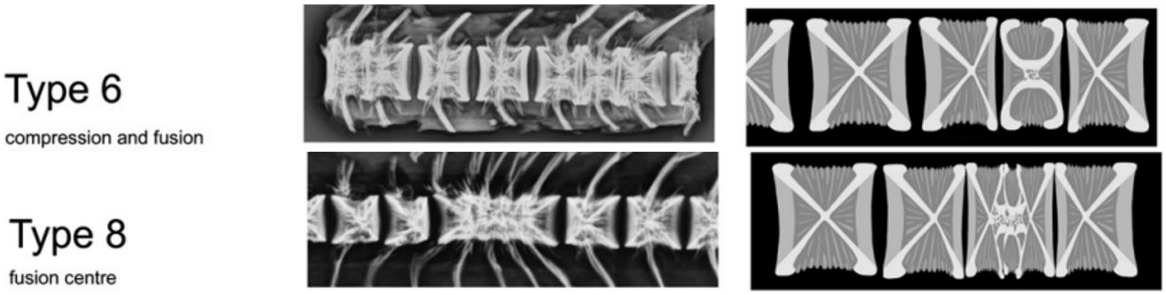


Figure 5. Compressions and fusions are examples of type 6 injuries (involving the Anterior-posterior compression, reduced intervertebral space, also the mineralization of intervertebral spaces) Type 8 : Multiple vertebral body fusions (Adapted from Witten et al., 2009))

5. Statistical analysis

R studio software and Microsoft-Excel were used for statistical analysis. Two-way variance analysis (ANOVA) was used for significance tests. Tukey’s HSD test was used to determine the difference among treatments at a 95 % significance level.

6. Results

6.1. PDI %

The PDI (%) level was similar for MP/MO and MP/VO and significantly higher compared with the other diets. The PDI (%) of the commercial diet was intermediate, while the experimental diets with vegetable protein had lower PDI (%). The diet with significantly lowest PDI (%) was VP/VO was significantly lowest for the VP/VO diet (Figure . 6).

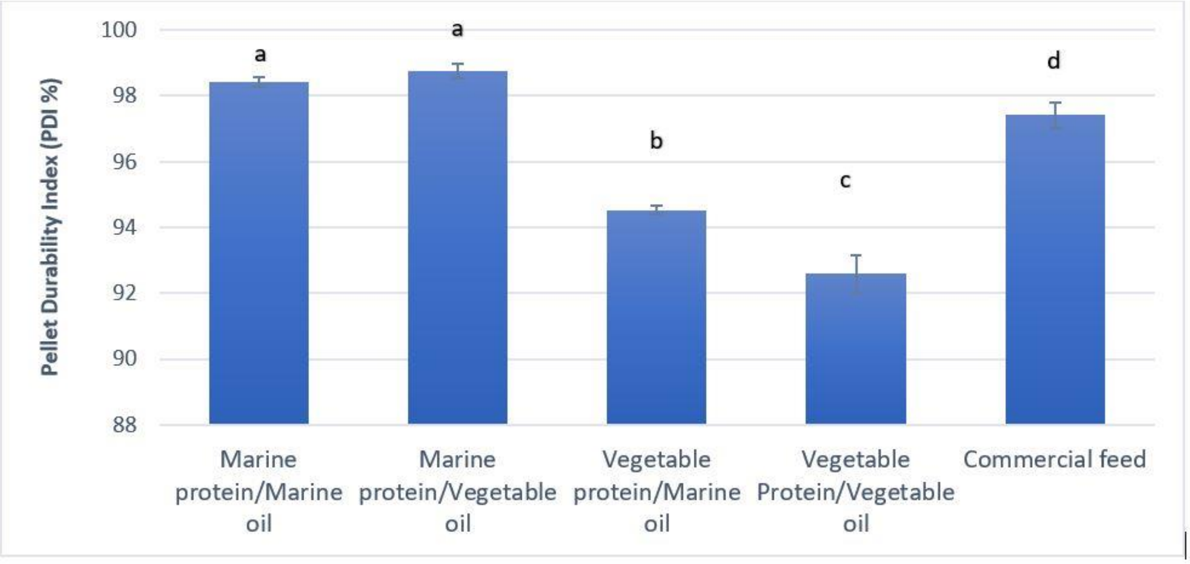


Figure 6. Pellet Durability Index (PDI) of five different feed pellet used in the experiment. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤0.05 level, using Tukey's HSD test

6.2. Water Stability

Overall, the water stability test after 30 minutes of incubation showed a significantly higher stability index for MP/MO (94.7 %), compared with the commercial feed (92.0 %). The water stability index for MP/VO, VP/MO was intermediate, with no significant difference to MP/MO. However, VP/VO had lowest water stability index among this treatment excluding commercial feed (Figure.7).

This also means that the water stability test of MP/VO or VP/VO is nearly identical to that of the MP/MO combination. In terms of feed sample water stability, the usage of VP/MO, VP/VO, and VP/VO combinations is competent for MP/MO samples.

The water stability test after 60 minutes of incubation showed significant differences between all treatments except VP/MO- MP/MO, VP/VO-MP/MO, and VP/VO-VP/MO (Figure.7). The water stability of those plant-based samples is comparable to MP/MO, implying that strong water stability and does have the potential to minimize the use of marine protein and marine oil sources in the salmon diet.

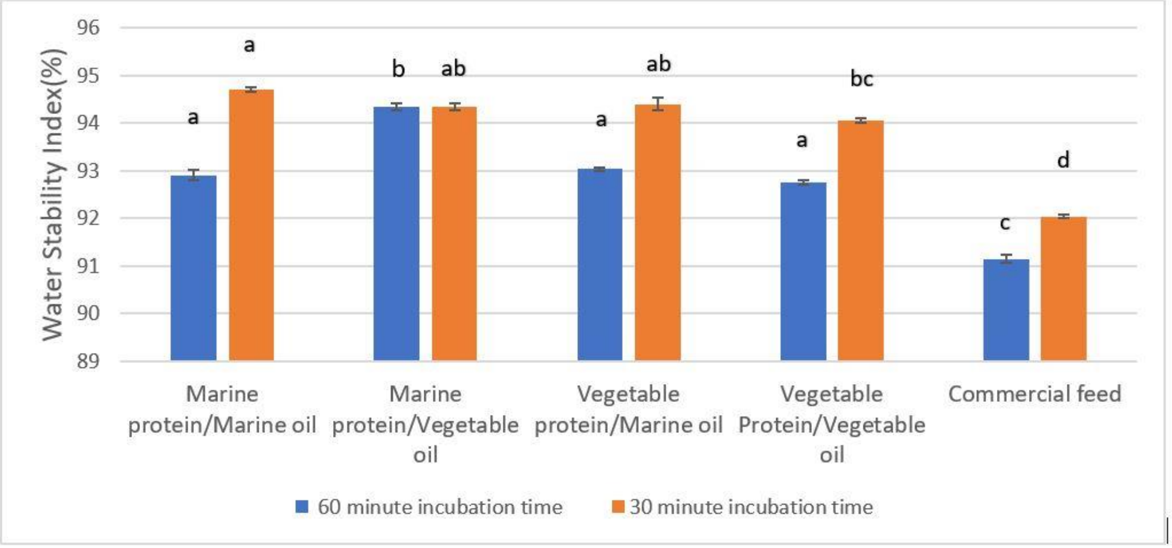


Figure 7. Water stability index measured of feed samples at 30 minute and 60 minute incubation time. Results are shown as means ± standard deviation (sd). Different letter above the sd bars indicate significant differences within incubation time at the P ≤0.05 level, using Tukey's HSD test

6.3. Dry Matter (%)

The dry matter content was significantly highest for the commercial feed (95%), while the dry matter comments was similar for the experimental diets produced in the study (range 92-93.5%) (Figure 8).

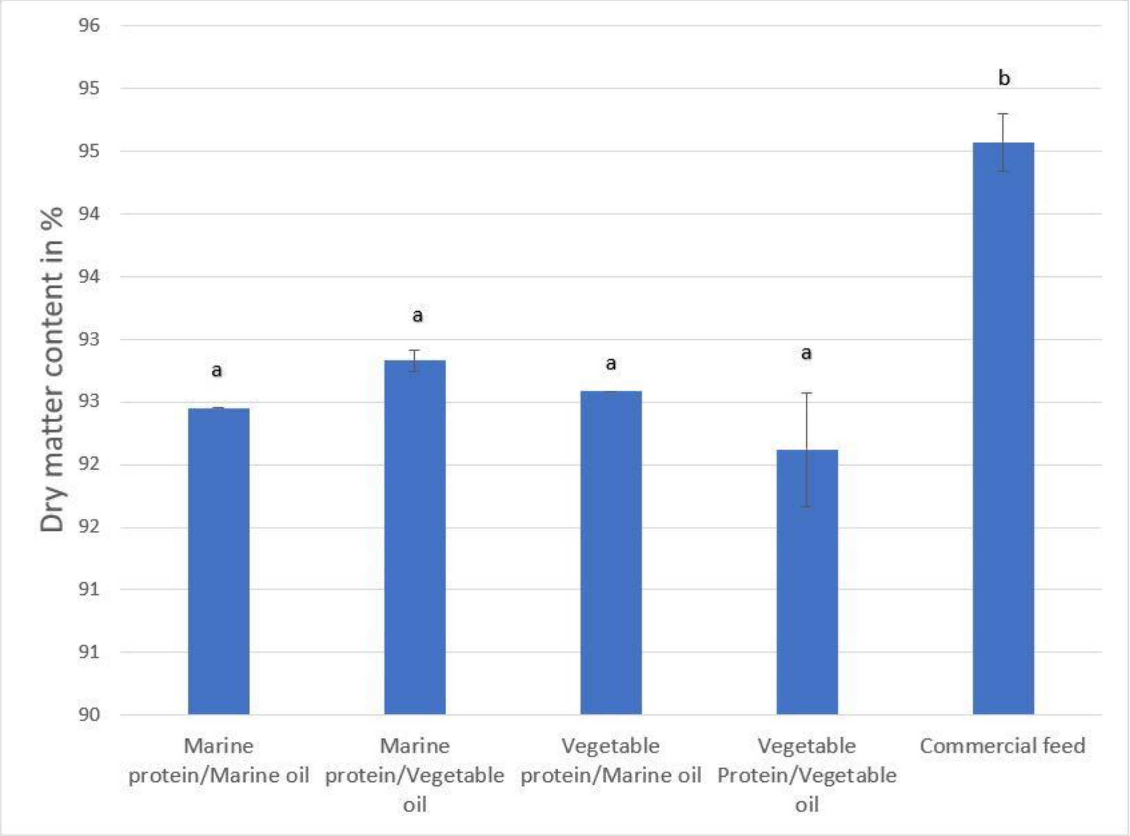


Figure 8. Dry matter % of feed samples measured. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤ 0.05 level, using Tukey's HSD test

6.4. Bulk Density

Bulk density showed no significant differences among the feeds (range 609-716 gm/liter) (Figure. 9).

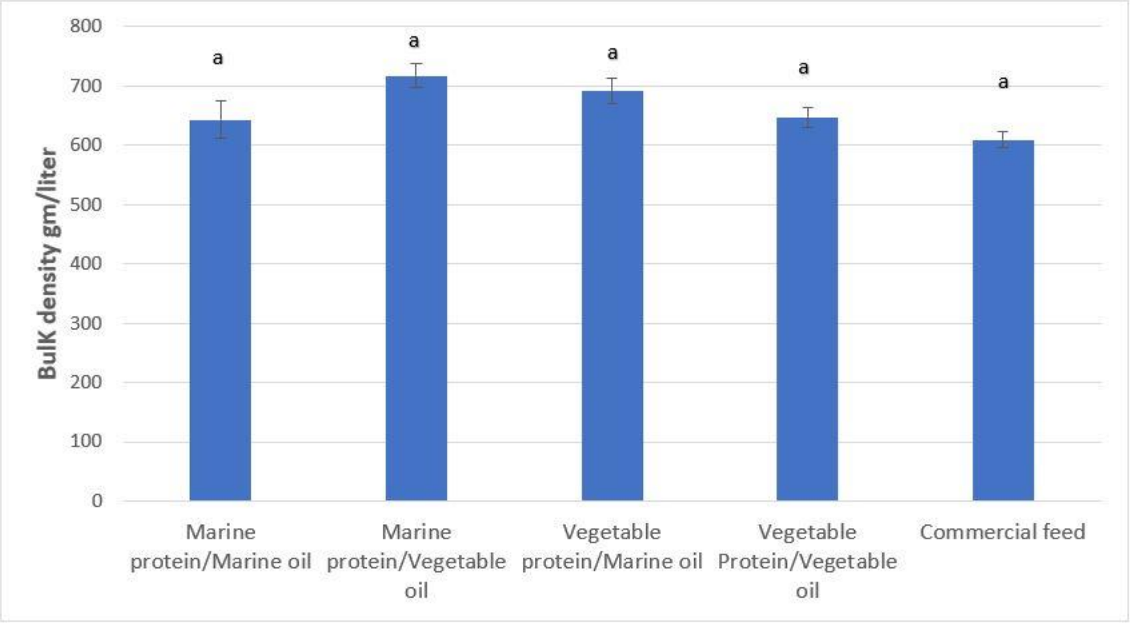


Figure 9. Bulk density of feed samples. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤0.05 level, using Tukey's HSD test

6.5. Texture Analysis

6.5.1. Area (N*sec)

The mean value of area ranged from 27.1 N*s for the commercial feed to 88.8 N*s for the VP/MO diet. The VP/MO and VP/VO diets (74.3 N*s) did not differ significantly. The area of MP/MO and MP/VO were similar (49.4-58 N*s), and significantly different from VP/MO. There was an overall significant difference in area between plant and marine based diet (Figure 10).

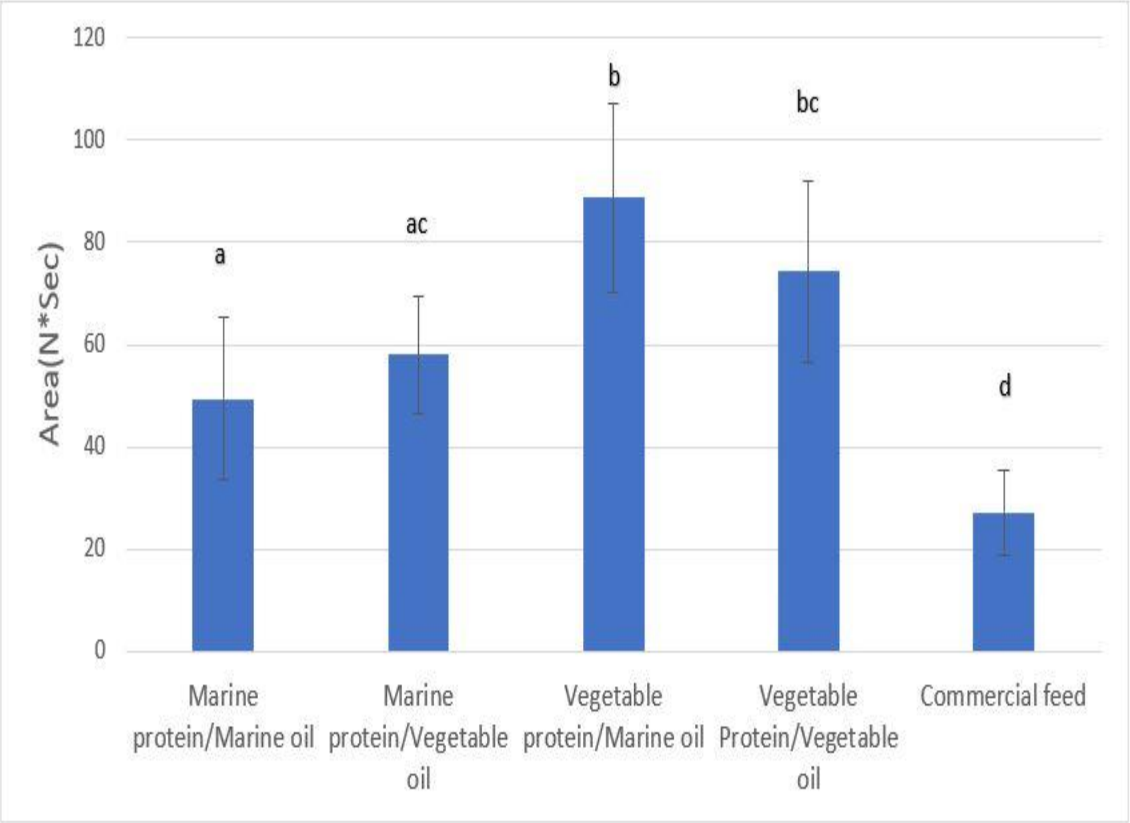


*Figure 10. Total work (area , N*sec) of the feeds, analyzed instrumentally by Texture analyzer. Results are presented as means ± standard deviation (sd). Different letter above the sd indicate significant difference between the feeds (P< 0.05, Tukey's HSD test).*

6.5.2. Maximum compression force , Force (N)

The compression force required to break the pellet was significantly higher for the VP/MO (106.7N) compared with the MP/MO feed (77.2N). The compression force was significantly lowest for the commercial feed (43.0 N) (Figure 11).

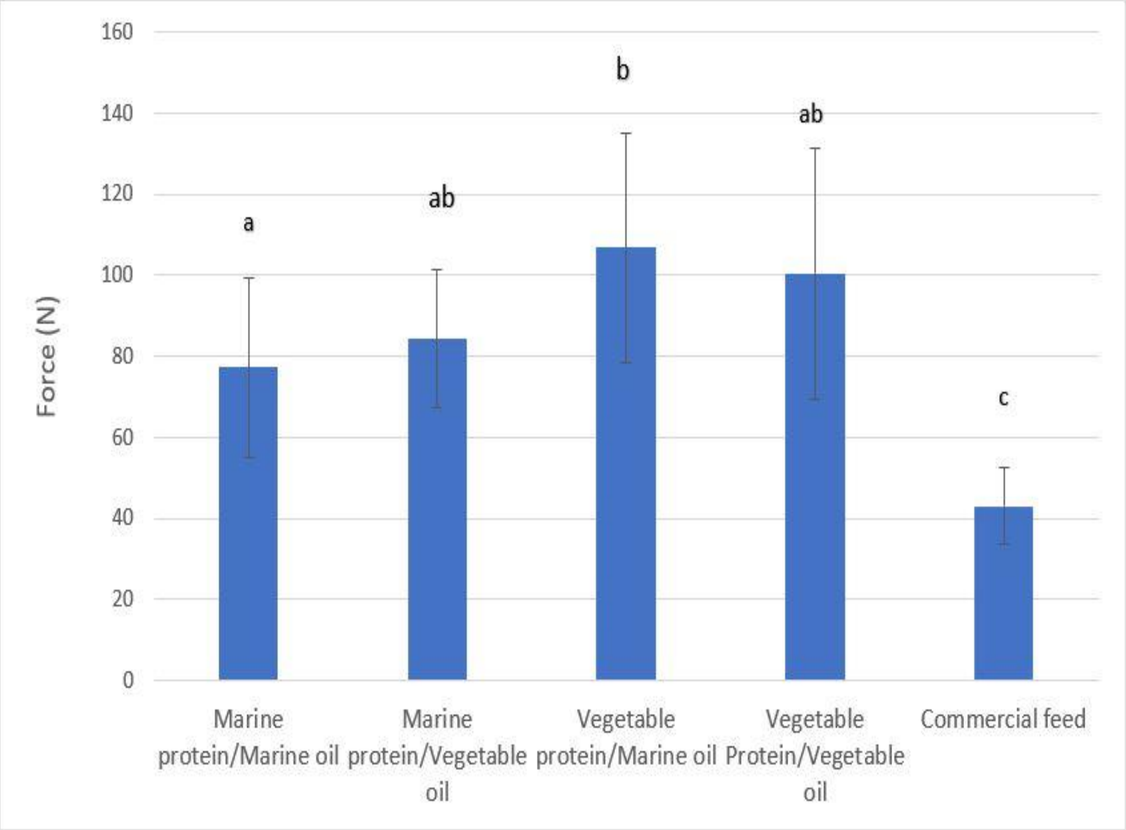


Figure 11. Maximum compression force required to compress the feed pellet samples analyzed by texture analyser. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤0.05 level, using Tukey's HSD test

6.5.3. Pellet Thickness

The pellet thickness of the MP/VO was similar to the MP/MO, but was significantly lower compared with all other feeds (Figure.12).

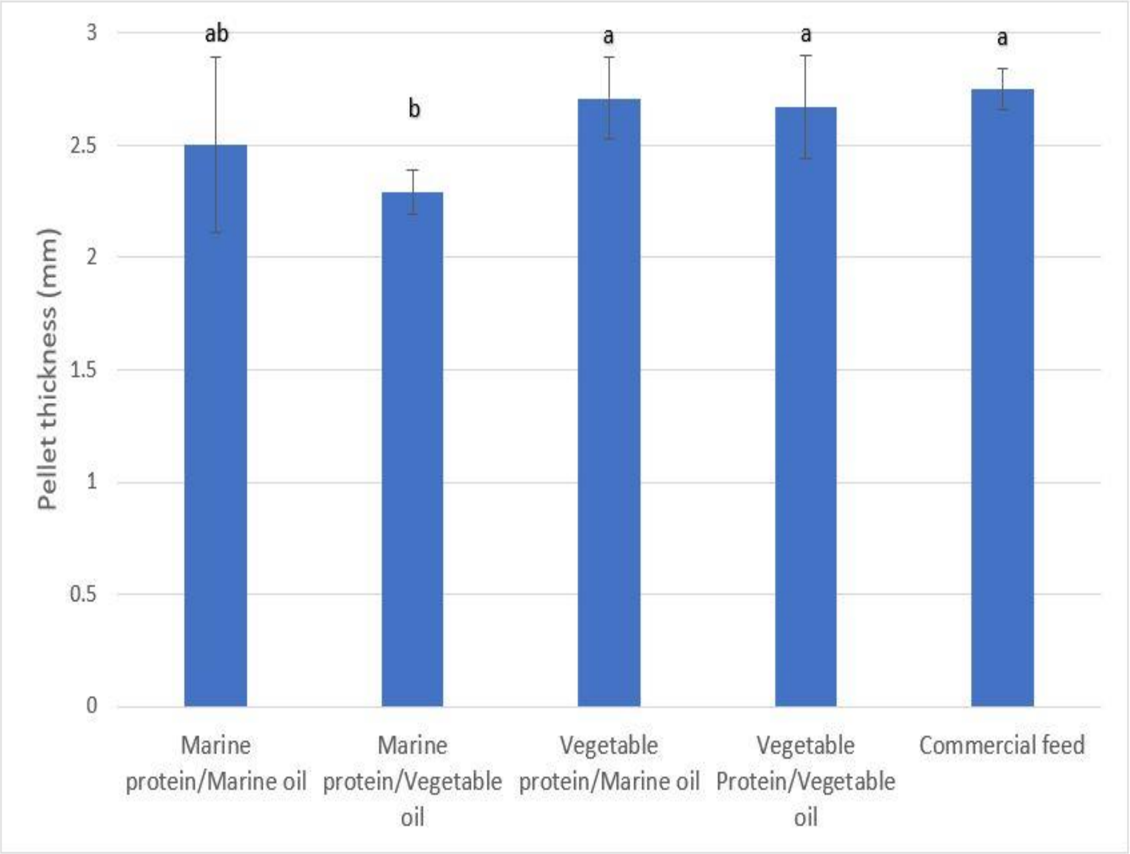


Figure 12. Pellet thickness of the feed samples measured by texture analyzer. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤0.05 level, using Tukey's HSD test

6.5.4. Fat Leakage

The percentage of fat leakage from the pellets varied significantly between the plant-based and marine-based diets. However, the fat leakage of the VP/MO diet was not significantly different from the fat leakage of the plant-only diet. The fat leakage percentage of VP/VO was numerically lowest and similar with the commercial diet (Figure. 13).

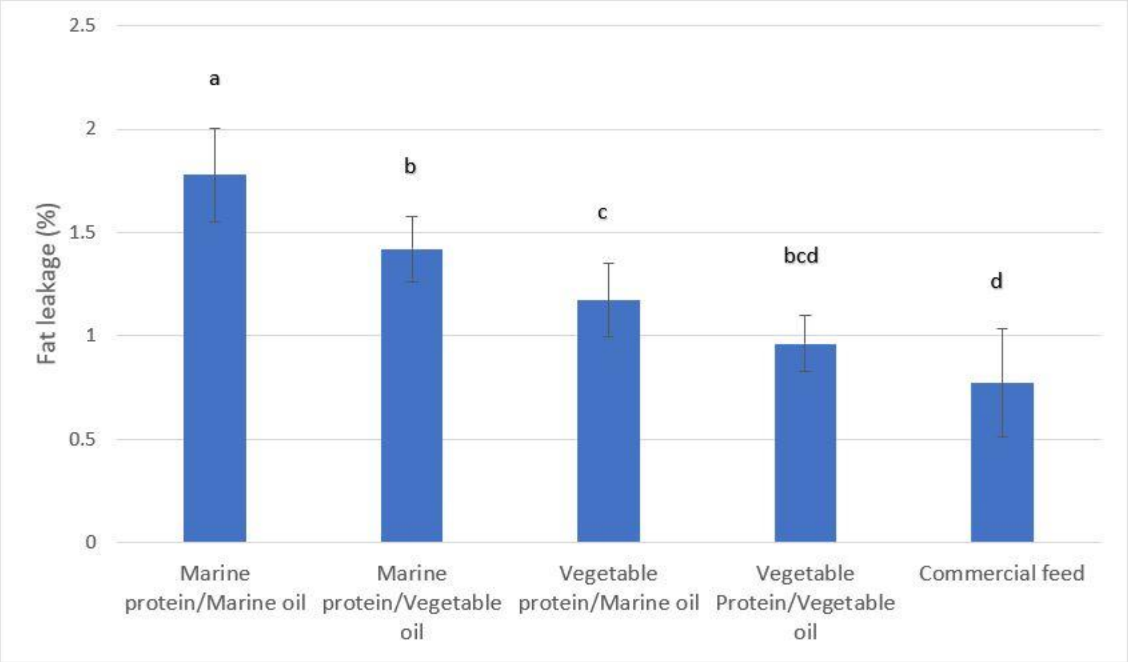


Figure 13. Fat leakage analyses of feed sample analyzed by texture analyzer. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤0.05 level, using Tukey's HSD test

6.6. Colorimetric analysis

The L-value did not differ significantly between the feeds (Figure 14**)**.

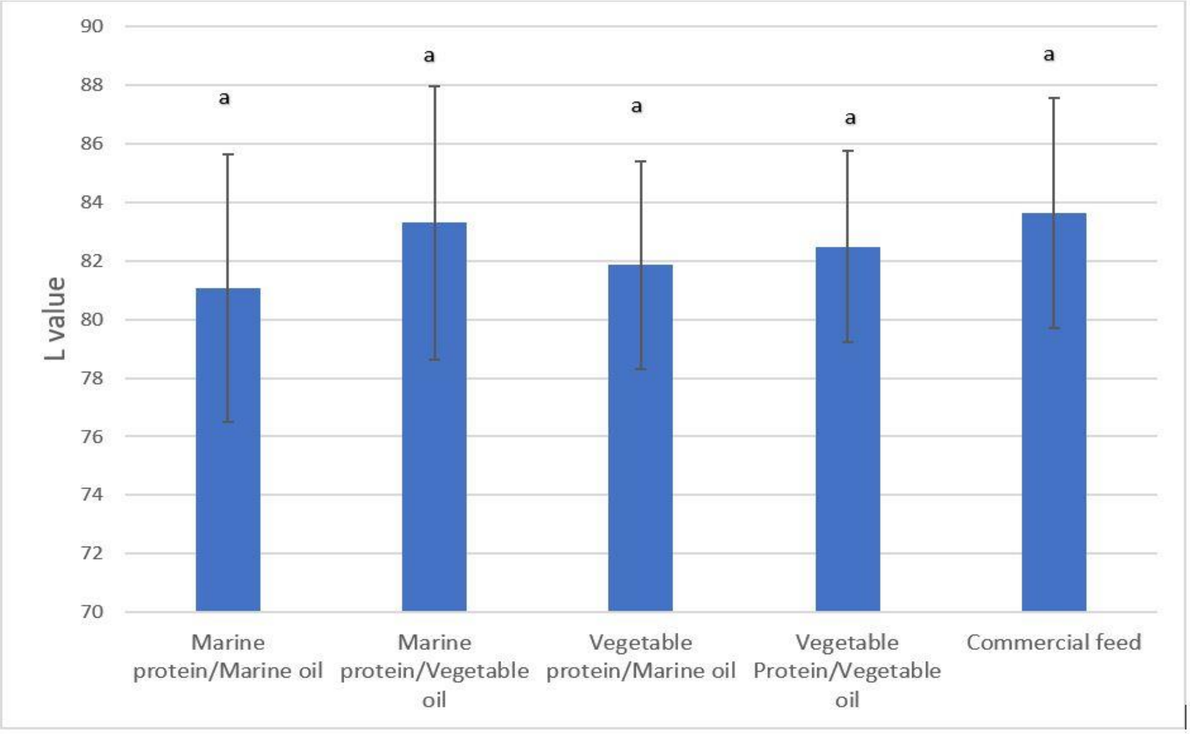


Figure 14. L value of feed sample. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the $P \leq 0.05$ level, using Tukey's HSD test

But the a-value differed significantly between the VP/MO and MP/MO. In addition, there was also a significant difference in the a-value among the plant based and marine based source diets. However, there is no significant difference among the MP/VO to MP/MO (Figure 15). The use of a plant-based oil source combined with a marine-based protein source does not result in a significant variation in a-value.

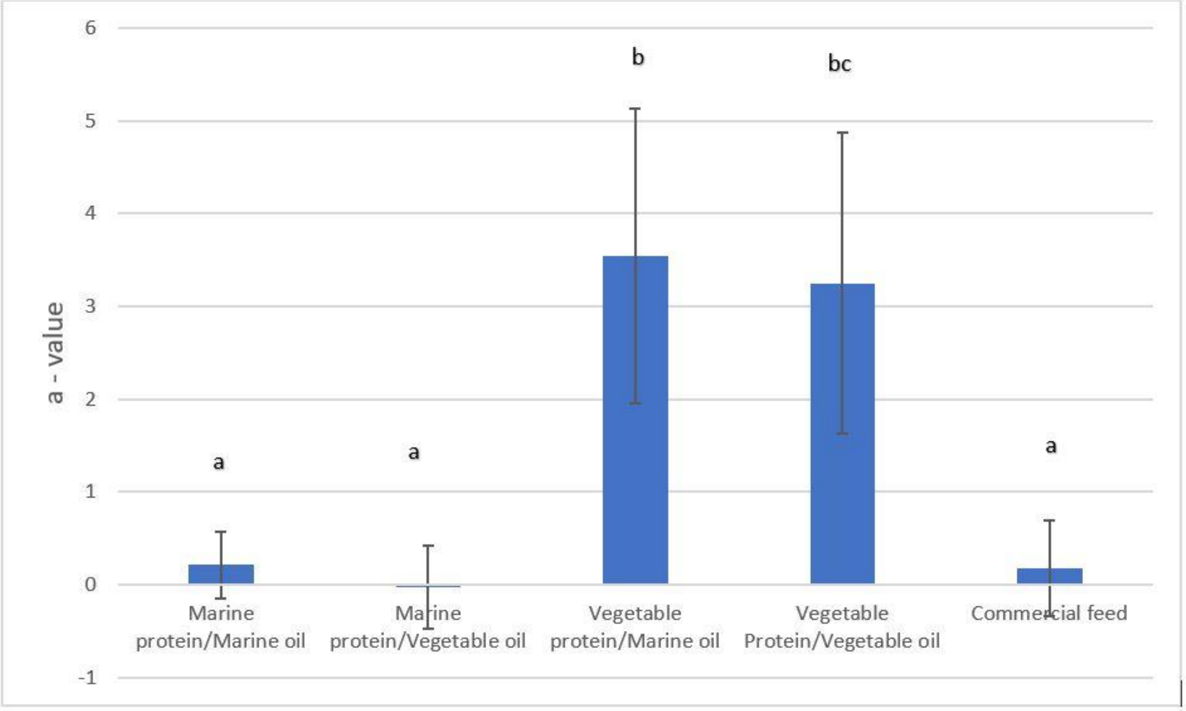


Figure 15. a- value of feed samples. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤0.05 level, using Tukey's HSD test

The b -value yields a similar result. The b value of the MP/VO and MP/MO differed significantly. Furthermore, the b value also differed significantly between plant-based and marine-based diets (Figure 16).

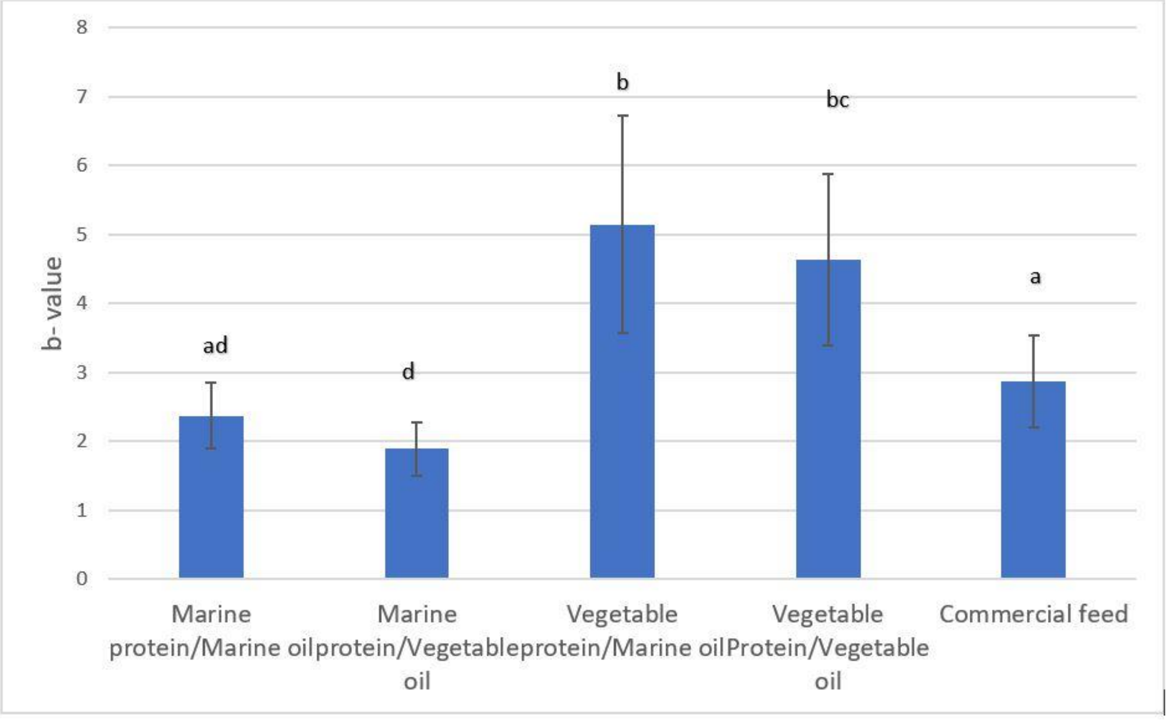


Figure 16. b- value of feed samples. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤0.05 level, using Tukey's HSD test

6.7. Sinking velocity in fresh water

The average velocity of feed pellets in fresh water was significantly highest for the MP/VO feed and lowest for the commercial feed. The sinking velocity of the MP/MO, VP/MO and VP/VO was intermediate and non-significant between the feeds (Figure. 17).

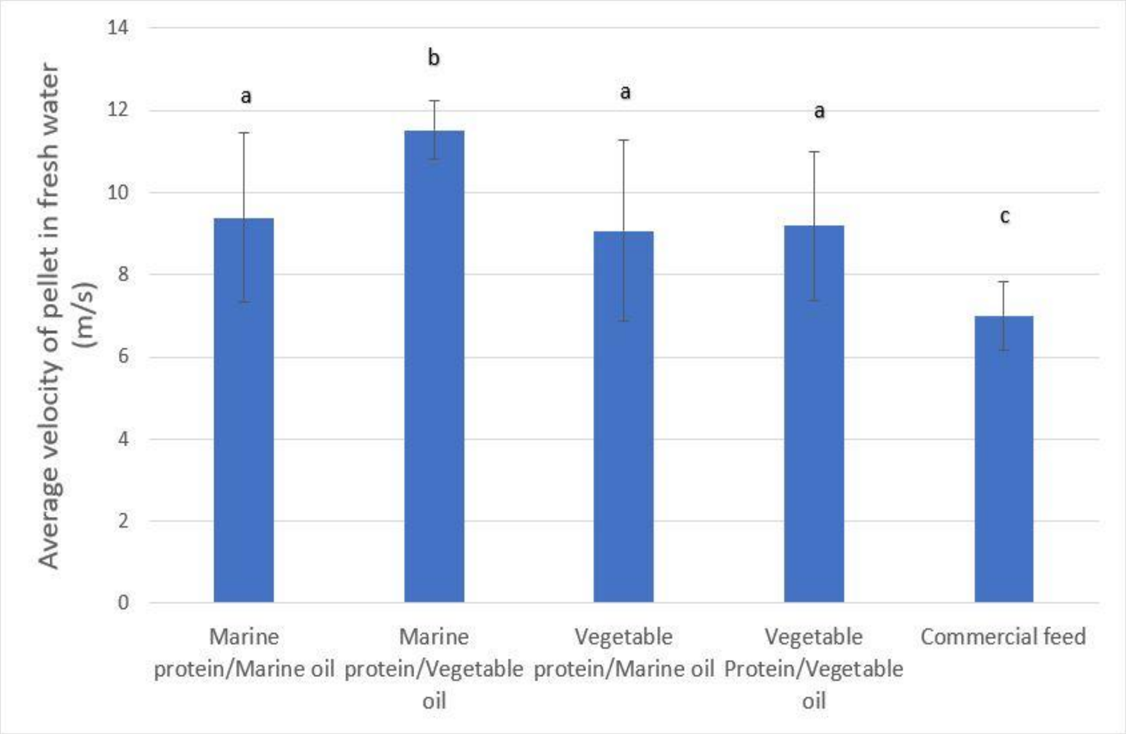


Figure 17. Average velocity of feed pellets in fresh water. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the $P \leq 0.05$ *level, using Tukey's HSD test*

6.8. Sinking velocity in saline water

Feeds with marine protein had higher sinking velocity than feeds with vegetable proteins. However, the difference was not significant between MP/MO and VP/VO. The sinking velocity was significantly lowest for the commercial diet (Figure. 18). There were no significant differences in the average velocity of falling pellets in saline water between plant-based and marine-based sources.

However, feed pellets derived from plant-based protein sources combined with fish oil, as compared to marine-based protein and lipid sources, may have a considerable difference in the average velocity of pellet dropping in saline water.

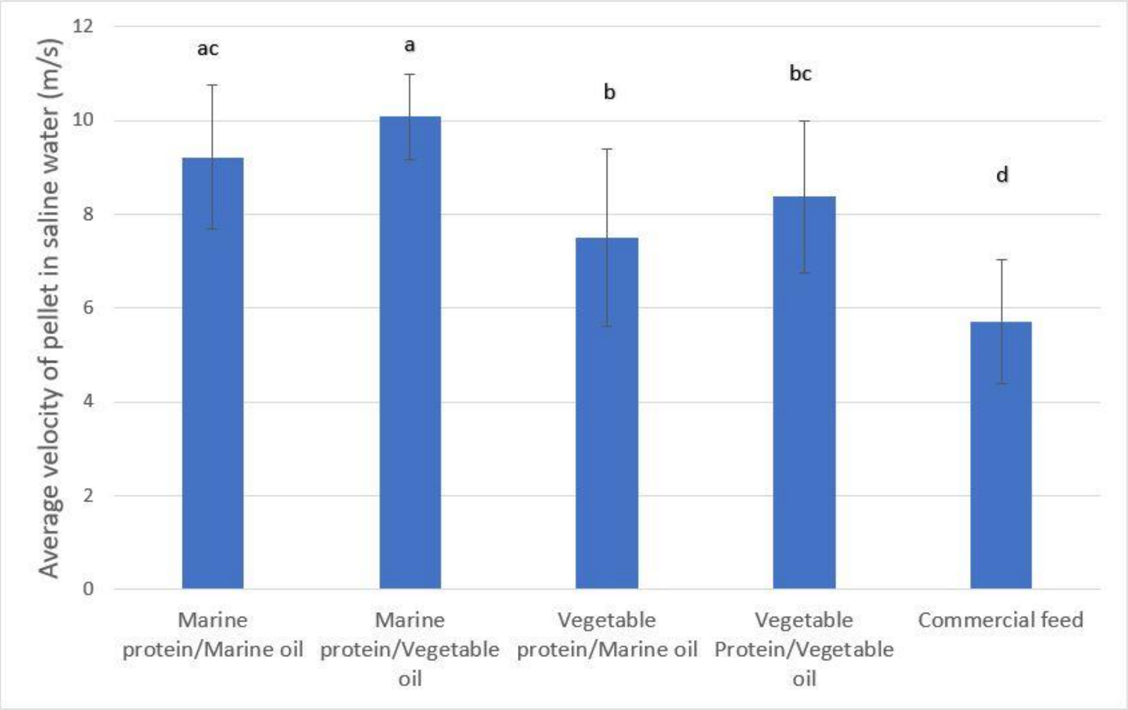


Figure 18. Average velocity of feed pellets in saline water. Result values are means ± standard deviation (sd). Different letter above the sd bars indicate significant differences at the P ≤ 0.05 level, using Tukey's HSD test

6.9. Deformities

6.9.1. Compression and Fusion (Type 6)/ Fusion Centre(Type 8)

No significant difference was detected between the dietary treatment groups. Among the fish analyzed, only two fish were recognized with abnormality issues and as per the categorization of vertebral body deformation established by Witten et al., 2009, only type 6 and type 8 deformity were found to examined (Figure 19; Figure 20).

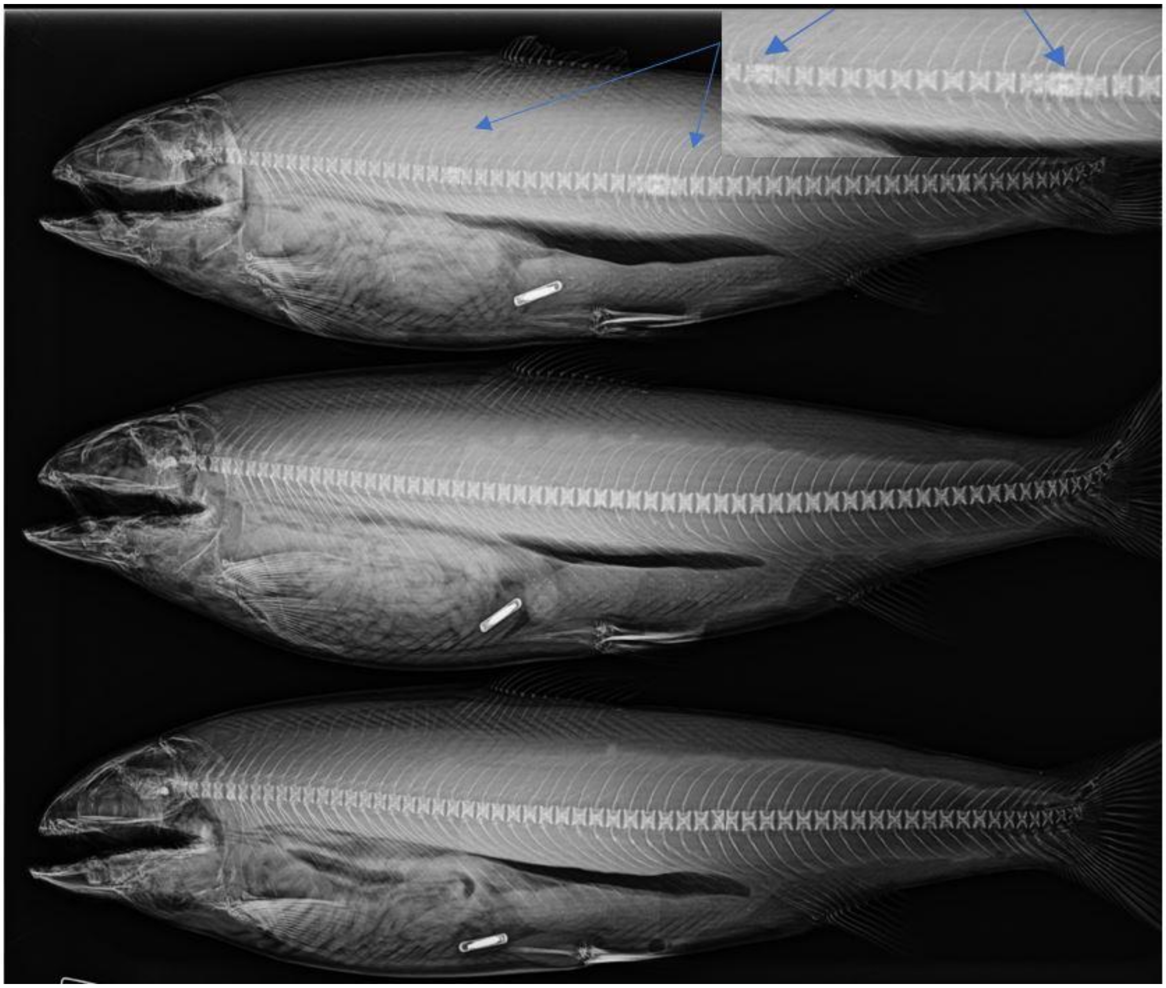


Figure 19. Compression and fusion (Type 6 injuries according to Witten et al. 2009). No significant difference was detected between the dietary treatment groups.

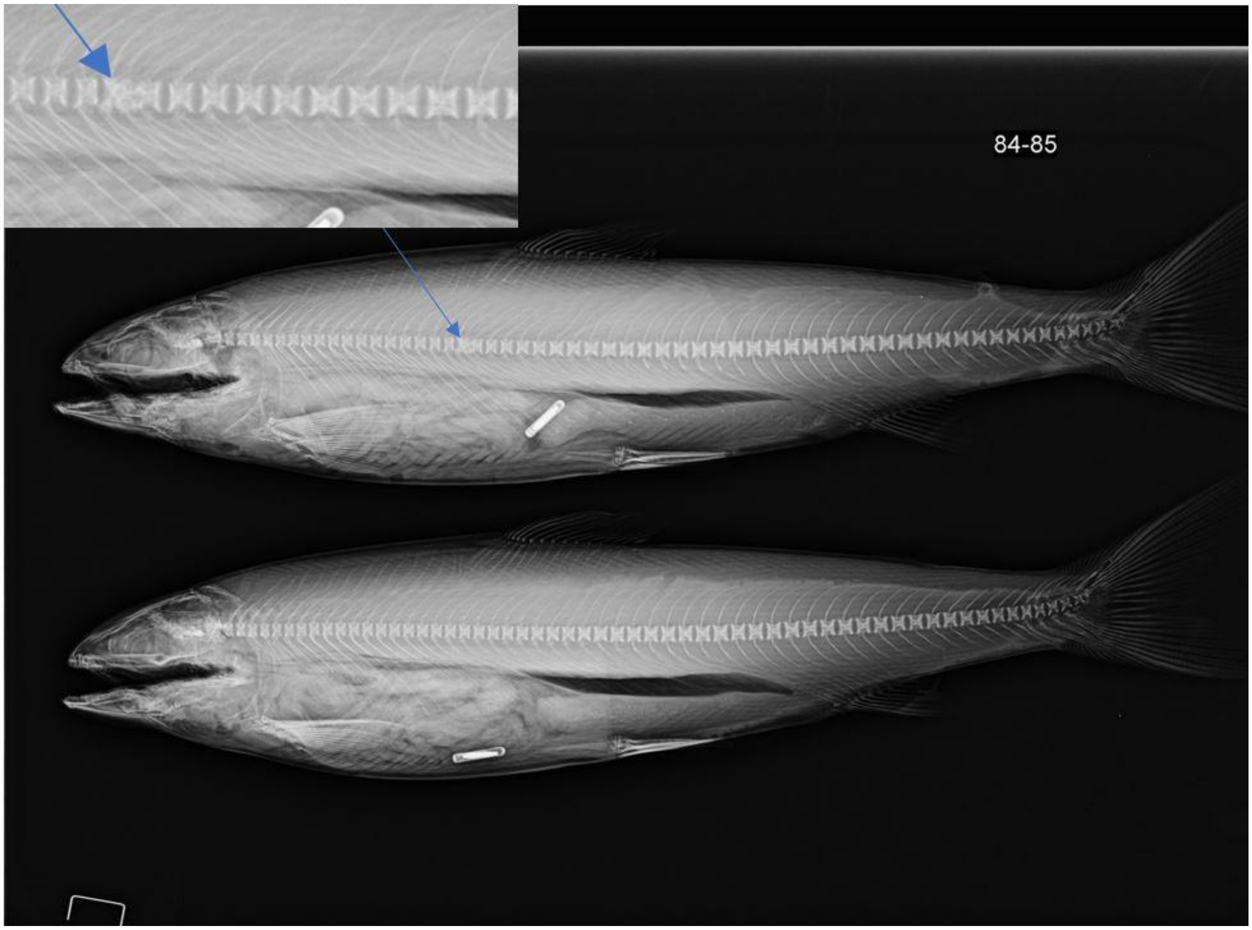


Figure 20. Type 8 injuries (Witten et al. 2009) considered to have multiple vertebral body fusions as shown in the fish. No significant difference was detected between the dietary treatment groups.

7. Discussion

Pellets of high quality can survive repeated handling, such as during bagging, transportation, storage, and movement on feed lines, without breaking down or producing fine particles. The pellet durability index is a common metric for describing the quality of pellets (PDI). Pellets with a PDI of less than 85 % tend to shatter during crumbling, resulting in more fines, and lower output at the end (Engineering, 2022). Wheat and other starch-rich raw materials contribute to high-quality pellets. A study done by Borquez in 2007 showed that PDI or quality of pellet can be increased by the vacuum infusion of fats to pellet, and its higher inclusion can lead to good quality through improvement in the elasticity of the pellet. Borquez further claims that pellets coated at a lower temperature have a greater PDI value. Even though using a plant-based diet in VP/VO could result in a PDI of more than 85%, indicating good pellet quality, completely replacing fish oil with rapeseed oil also signifies the potential to have comparable PDI (between MP/MO and MP/VO,), demonstrating the enormous potential use in aquafeeds.

Fat leakage was highest in MP/MO, then in MP/VO or VP/MO (which are significantly different), then in VP/VO or commercial feed (which are not significantly different either). Oil leaks might wreak havoc on feeding machinery and packing materials too. As a result, using vegetable rapeseed oil to avoid oil leaks might be beneficial. Moreover, even adding a, even a small amount of coconut oil in addition to rapeseed oil can drastically minimize fat leakage (Pandey, 2018).

Durability test reveals that MP/VO has the maximum durability, followed by MP/MO, commercial feed, VP/MO, and VP/VO. In terms of the ideal feeding system, high pellet durability and low fat leakage are necessary (MP/VO or MP/MO with better durability, and VP/VO or commercial feed appears to be most preferred).

There wasn't a significant difference between the force needed to break VP/VO pellets and VP/MO pellets. However, substituting vegetable oil (rapeseed oil) for fish oil in the Marine based or in combination with plant based protein diet could soften the pellets (Pandey, 2018). The same study also suggests in the use of plant-based oil in higher amounts in order to see greater results.

The toughness or hardness of the VP/VO-MP/MO was almost identical or not significantly different i.e., the softness of plant based diet and marine based diets was almost similar.
During the prolonged incubation time for stability test, the commercial diet had the lowest water stability, followed by VP/MO, MP/MO, and VP/VO (no significant differences), while MP/VO had the highest water stability. According to a study conducted by oehme et al., 2014, a reduced water stability diet may result in greater feed consumption in Atlantic salmon. In our current study, resulted with lower water stability in the diet, VP/VO, greatest durability in VP/VO, and lower leakage in VP/VO; there is a potential that feed intake of this plant-based feeds shall be greater in salmon, demonstrating that increasing pellet durability does not necessarily diminish feed intake rate. Usually for the bottom feeder fish which eats the feed at slower rate, the feed need to be stable so that it doesn't lead to any leaching of the fats and other nutrients. Therefore, the standardization of the stability of the feed in water is very important **(**Sørensen, 2012). An example of oil belching has been found from the lower water stable feed leading to osmoregulatory stress in rainbow trout (*Oncorhynchus mykiss*) (Baeverfjord et al., 2006). Water stability of feed has found to significantly affecting the intake of feed. In addition, a 20% difference in feed intake of Rainbow trout was experienced in the study conducted by Aas et al. 2011b. Moreover, the same study and Bell et al, 2003 also found that Atlantic salmon outperformed in terms of development and fatty acid composition despite being raised on a combination of vegetable oil without any fish oil diet during the full sea water period, and performed better.

In another study, Torstensen et al., 2005 found that using 100 percent vegetable oil (a combination of rapeseed, palm, and linseed in extruded diet) outperformed control fish oil over the winter sea water phase time. Furthermore, the same study reveals that using vegetable oil results in less rancid with marine properties, making it a superior choice for flesh when compared to other dietary groups. The study conducted by Espe et al., 2006; de Francesco et al., 2004 showed the possibility of using maize gluten, wheat gluten, and soymeal for optimal fish development. The same study showed no detrimental impacts on growth, nutrient utilization, or digestibility with a 40% replacement of plant protein for marine protein and a 70% replacement of vegetable oil for marine fish oil. However, reduction in Protein productive value (ppv) might be seen when totally substituting with plant-based oil. Supplementing the amino acid needed throughout the life stage, on the other hand, may improve and give the best results in the freshwater and seawater stages.

Furthermore, favorable environmental variables such as lower water temperature (2-6^{o}C) might contribute to improved development, boosting PPV, and enhancing the digestibility of dietary lipid and protein due to the proven protein sparing effect of plant oil (Bendiksen et al., 2003). Thus, in the same study, 80 percent of the fish meal with substituted by plant proteins and 70 percent of the fish oil with a plant based oil mix resulted in a 9 percent drop in growth during the production phase in saltwater. The optimal growth temperature for post-smolt Atlantic salmon is believed to be 12.8°C for fish weighing 70 to 150 g, and it is also explained that temperature differences may affect growth results due to their effects on protein and lipid content in the body (Sissener et al., 2021).

In addition, study by Madaro et al., 2018 demonstrated that plasma cortisol synthesis is temperature-dependent and increases as the temperature rises. In regard to this, in comparison to our study, the fresh water stage had an average temperature of 10.1 °C, and if we tried to maintain a similar temperature (not a significant temperature difference compared to fresh water) during the sea water transition phase, it would be able to achieve a zero to negligible mortality rate in the salt water stage due to acquiring lower acute stress level during the transfer.

Diets for Atlantic salmon were adjusted for crude protein and calories, as well as balanced amino acids, by using wheat gluten, maize gluten, and soy concentrate as raw materials and results was promising (Espe et al. 2006). As a result, it is crucial that our experimental diets be examined in seawater-stage salmon.

In saline water, it takes longer for feed pellets to sink to the bottom than in fresh water. Sinking rate reduces in saline water diet compared to fresh water, with the lowest being the commercial diet (5.72 m/sec), which was statistically different from other comparable diets as well in fresh water. In our study, it was observed that the addition of plant-based oil increased the average velocity of pellets in both fresh and salt water, indicating an increase in sinking rate reducing the floating pellets (Obirikorang et al., 2015; Pandey, 2018).

Regarding deformity, only the two listed categories of spinal anomalies (Figures 5, figure 19, figure 20) were examined in the vertebral body deformity category. Salmon and other farmed teleost species are selectively bred for great muscle mass and rapid growth. Inadequate vertebral body mineralization in rapidly growing animals (Aunsmo et al., 2008; Fjelldal et al., 2006) and mechanical overload, exerted by an increase in muscle mass (muscle size / strength) may make the vertebral bodies susceptible to distortion, resulting in compression, heterotopic cartilage development occupying bone marrow spaces, and fusion. In relation to this issue, compression, fusion, and under mineralisation of vertebral bodies are observed more frequently in animals bred for rapid development (Fjelldal et al., 2006) which can also be seen in our study (Figure 19; Figure 20). Dietary phosphorus supplementation is crucial for preventing spinal deformities (Baeverfjord et al., 1998). The optimal amount of phosphorus in a salmon's diet varies according to its stage of development. As indicated for Atlantic salmon weighing 1 to 5 grams, 7.9–9.8 g/kg of available phosphorus is necessary (Åsgård and Shearer, 1997) , followed by 5.6 g/kg of available phosphorus for juveniles weighing 15 to 40 grams (Vielma and Lall, 1998) during early freshwater stages. However, a dietary supplement of 5.1-7.4 g/kg of available phosphorus is necessary for four months during the early transition phase in saltwater for proper bone health (Fraser et al., 2019). Even though only two fishes were observed with deformities in early stage, it is vital to consider supplementing the fish with the optimal amount of dietary phosphorus required for improved bone health, and the trial should also be undertaken in sea water until marketing.

If spinal anomalies are investigated at an earlier time, it may be possible to locate a treatment that prevents faults from occurring at a later level as these deformities expect to increase with the life stages (De Clercq et al., 2018). It may also be useful to remove deformed fish from the population at an early age in order to prevent future economic losses linked with the deformity of the fish

With all the aforementioned characteristics assessed, it is feasible to make feed with a variety of physical pellet qualities. However, higher replacement of both protein and fat components by plant-based sources may result in lower feed intake and growth during the early feeding period; however, growth can be exceeded throughout the time with the lengthier adaption.

The growth retardation which can be caused by a decrease in feed intake during the early feeding phases, which can be remedied by supplementing with amino acids. The supplementation of the diet with vitamins and amino acids can contribute greatly to rapid growth (Xu et al., 2016). During the transition to seawater, deficiencies in methionine, vitamins -b, B6, and B12 could cause phenotypic modifications (Saito et al., 2020). Typically, plant-based components, such as soy protein concentrate, are low in methionine, taurine, threonine, lysine, and vitamins-b; adding these nutrients could increase the development potential following seawater phase transition (Sissener et al., 2021). In addition, the use of vegetable-based oils such as rapeseed oil and linseed oil as contrast to fish oil has resulted in a reduction of plasma cortisol in sea water phase and an improvement in osmoregulation abilities (Tocher et al., 2000).

Overall, pellet quality has can have impact on feed intake and, consequently, growth; increasing pellet quality has the potential to increase feed cost efficiency for farmed salmonids.

There is a shortage of evidence regarding the relationship between physical pellet quality and nutritional responses in fish. In times of poor feed intake, disease outbreaks, or during the transition to sea water, soaking the feed may increase the rate of feed consumption. When evaluating and maintaining the physical quality of feed for intensive production, the pellet's resilience, breaking, feed intake, growth, apparent digestibility, energy and nutrient retention are all major and crucial factors to consider (Aas et al., 2020).

Thus, a greater replacement of both marine protein and fat components by plant-based sources with distinct physical quality parameters such as lower water stability, lower leakage, and higher durability as in our plant-based diet can do result in lower growth during the early feeding period but, can be minimized with amino acid , EPA and DHA supplementation, acquiring adequate growth and development over time with adaptation (to compete with marine based diet). On the other hand, reducing marine fish oil, may drastically reduce the long-chained unsaturated marine fatty acids EPA and DHA in salmon while boosting n-6 fatty acids from vegetable oil substitution. However, combining amino acids with EPA and DHA may be necessary to achieve the dietary requirement for fish, as well as increasing the quantity of omega-3 fatty acids contained in farmed fish, which the human body requires for normal growth and development.

Thus, if the EPA and DHA requirements for salmon are met by alternate sources, it is possible that the reliance on marine oil will be reduced to a minimal level or an insignificant level. Combining amino acids with supplementation of EPA and DHA could be necessary to lessen dependence on marine oil without compromising growth in later life stages. In order to gain a comprehensive understanding of the differences and similarities between marine and plant-based diets, the

research should be carried out over an extended period of time, commencing with the introduction of sea water and continuing to the point until the fish reach the appropriate harvesting size.
The differences in redness and yellowness color value among the plant based and marine based diet can reflect the varying degree of rancidity in the diets which could be topic of interest for further study.

In addition, optimal dietary phosphorus supplementation according to life phases may be essential for preventing from the associated vertebral abnormalities. In order to maximize Atlantic salmon productivity, it is essential to consider all of technical quality aspects while choosing plant-based diet as an appropriate alternative.

As alternatives to fish meals become more popular, feed component interactions will most likely become more complicated. The nature of such component interactions might have far-reaching implications for ingredient research and is further essential for doing more and more experimental trials including testing in seawater in the long run until harvesting. Additionally, while studying the various alternative sources for fish meal and fish oil, the impact of these sources on the sensory and fillet quality should also be majorly investigated.

Lastly explaining the physical quality of feed pellets can vary with dietary treatment and was evaluated for Atlantic salmon using several approaches. During the physical analysis, the particles can be broken and dispersed out of the filter paper, used to collect oil. Also can be more relevant during the texture analysis when the probe applied force during the measurement, which can be one of the major challenges in studying the physical attributes. This test might be enhanced in the future doing the comparison test using different probe types, with different procedures. However, pellets with desirable features, such as reduced water stability in the diet, highest durability, lowest leakage, and toughness of pellets might be developed for improved physical pellet quality.

8. Conclusion:

Here are the main results according to a brief glance

Vegetable protein vs. marine protein

- Lower durability
- Harder pellets
- Lower fat leakage
- Lower sinking rate

Vegetable oil vs. marine oil

- Lower fat leakage

Combination, marine protein and vegetable oil

- Highest water stability
- Highest sinking velocity

Despite the fact that using a plant-based diet (VP/VO) can result in a PDI of over 85%, indicating good pellet quality, completely replacing fish oil with rapeseed oil can also result in a comparable PDI (between MP/MO and MP/VO), demonstrating the enormous potential from plant based oil sources diet in aquafeeds.

In our analysis of fat leakage, only the vacuum-coated (MP/MO) pellet sample showed significant fat leakage, whereas the VP/VO diet had significantly less leakage.

Overall, blending marine protein with plant-based oils may alter pellet velocity significantly. Using plant-based protein and plant-based oil had no effect on the velocity of sinking pellets compared to using marine-based protein and oil sources. Plant-based oil increased average pellet velocity in both fresh and salt water, indicating an increase in sinking rate, lowering floating pellets. Thus, the feeds can have a variety of physical pellet qualities, which might have a big influence on how much Atlantic salmon eat. Pellets with desirable features, such as reduced water stability in the diet, highest durability, lowest leakage, and toughness of pellets can be developed for improved physical pellet quality. To increase Atlantic salmon productivity, all of these technical pellet qualities must be considered when selecting plant-based diets as suitable alternative.

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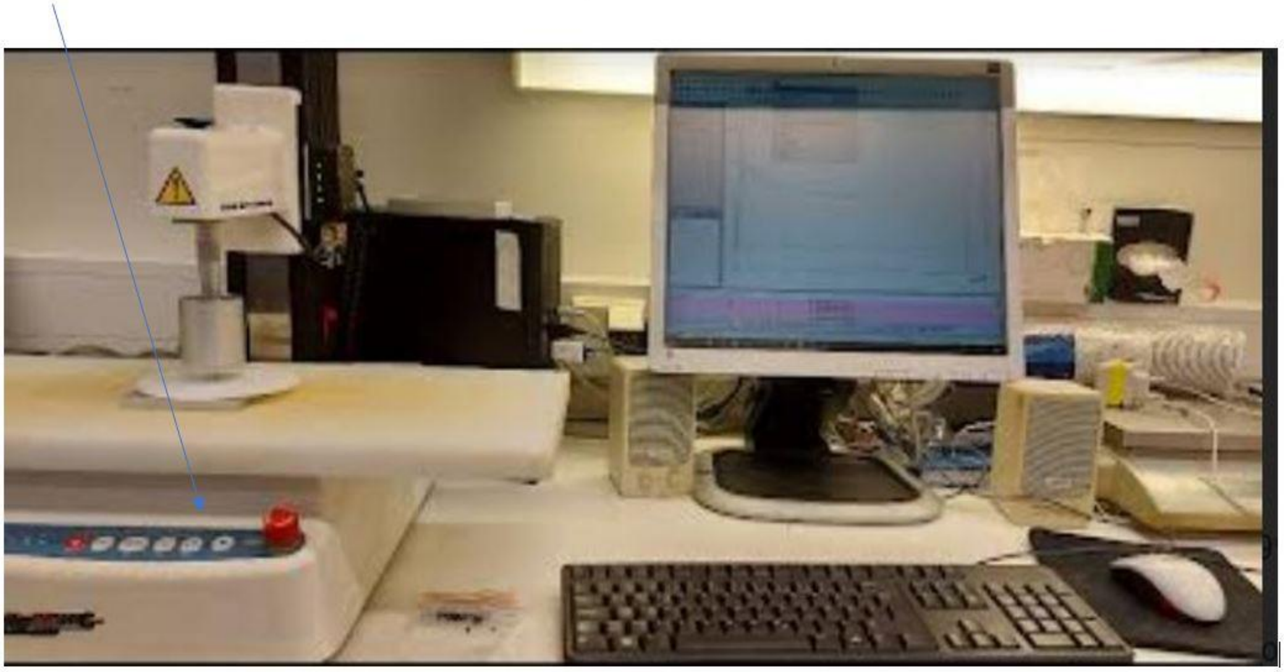
10. Appendix

10.1. Origin of Feed Ingredients

Fish meal	*Norse-LT, Vedde AS, Langevåg, Norway*
SPC	Imcosoy 62 Aqua, Imcopa, Araucarias, Brazil
Wheat gluten	Amytex 100, Tereos Syral, Aalst, Belgium
Corn gluten	Glutalys, Roquette, Lestrem, France
Wheat	Norgesmøllene AS, Bergen, Norway
Fish oil	NorSalmOil, Pelagia, Egersund, Norway
Rapseed oil	Crude rapeseed oil, Emmelev, Otterup, Denmark
MgSO4	Magnesiumsulfat, Delivered by Vilomix, Hønefoss, Norway
K2CO3	Kaliumkarbonat, Delivered by Vilomix, Hønefoss, Norway
Vitamin premix	Nofima Vitmainpremix, Vilomix, Hønefoss, Norway
MSP	Delivered by Vilomix, Hønefoss, Norway
Asta	Lucantin PINK 10% from BASF, delivered by Vilomix, Hønefoss, Norway
Yttrium oxide	VWR, Oslo, Norway
Mineral premix	Nofima Mineralpremix, Vilomix, Hønefoss, Norway

10.2. Fat Leakage Analysis and Pellet hardness examined by Texture Analyser

Texture Analyser and Calibration is seen on display



10.3. Measured P values from the Statistical Analysis

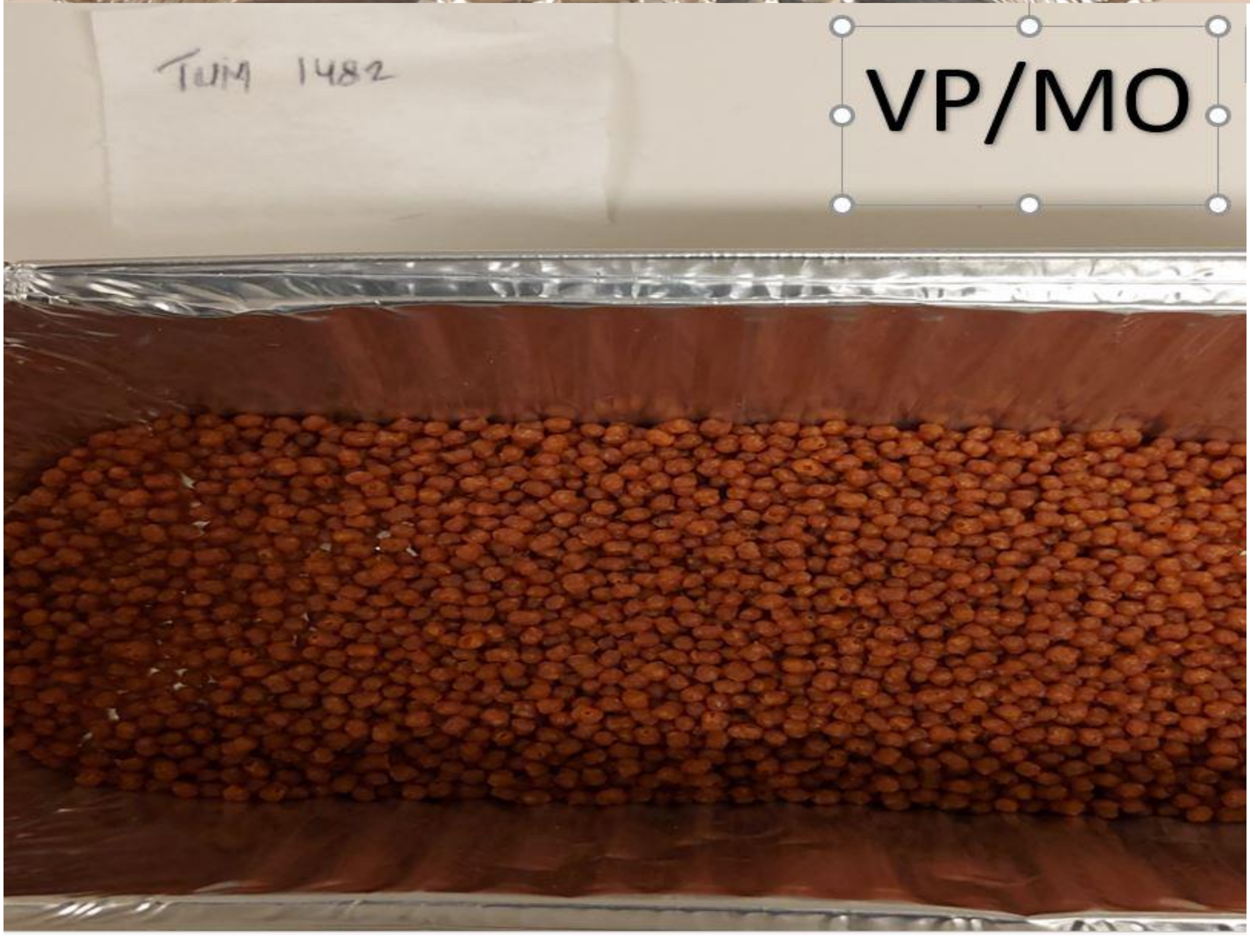
Measurement	Marine protein/Marine oil	Marine protein/Vegetable oil	Vegetable protein/Marine oil	Vegetable Protein/Vegetable oil	Commercial feed	p-value
PDI %	98.4±0.15 a	98.7±0.22 a	94.5±0.13 b	92.5±0.57 c	97.4±0.39 d	0.001232 **
water stability index at 30min incubation time	94.7±0.081 a	94.3±0.125 ab	94.4±0.242 ab	94.0±0.086 bc	92.0±0.052 d	2.244e-09 ***
water stability	92.9±0.179 a	94.3±0.119 b	93.0±0.061 a	92.7±0.070 a	91.1±0.133 c	4.083e-10 ***

index at 60min incubation time						
Dry matter %	92.4±0 a	92.8±0.119 a	92.5±0 a	92.1±0.45 a	94.57±0.33 b	0.001232**
Bulk Density(gm /liter)	643.3±31.02 a	716.8±19.9 a	692.1±21.17 a	646.8±17.44 a	609.07±13.81 a	1
Fat leakage (%)	1.78±0.226 a	1.42±0.157 b	1.17±0.179 c	0.96±0.133 bcd	0.77±0.263 d	2.92 e-14***
Average velocity of pellet in fresh water (m/s)	9.3±2.06 a	11.5±0.71 b	9.0±2.21 a	9.1±1.81 a	7.0±0.84 c	2.2 e-16***
Average velocity of pellet in saline water (m/s)	9.2±1.53 ac	10.0±0.91 a	7.50±1.89 b	8.3±1.62 bc	5.7±1.32 d	2.2 e-16***
Pellet thickness(mm)	2.5±0.39 ab	2.2±0.10 b	2.7±0.18 a	2.6±0.23 a	2.7±0.09 a	0.0001452 ***
Force (N) required to break the pellet	77.2±22.15 a	84.3±17.16 ab	106.7±28.33 b	100.4±31.17 ab	43.04±9.47 c	1.24 e-06 ***

Area(N*Sec)	49.4±15.98 a	58.0±11.44 ac	88.8±18.50 b	74.3±17.82 bc	27.1±8.47 d	6.27 e -11***
L value	81±4.5 a	83.3±4.6a	81.8±3.5a	82.4±3.2a	83.6±3.9 a	0.232
a-value	0.2±0.36a	-0.03±0.44a	3.5±1.6b	3.2±1.6 bc	0.18±0.51 a	2.2 e-16***
b- value	2.3±0.5 ad	1.9±0.4 d	5.1±1.6 b	4.6±1.2 bc	2.8±0.7 a	2.2 e-16***

Values are means ± standard deviation (sd), followed by the different letter in any column are significantly different at the 0.05 level using Tukey's HSD test

10.4. Supplementary figures (Feed samples ; Water stability test)




MP/VO
TUM 1481


MP/MO
TUM 1480


Commercial feed
TUM 1484


Water stability test
Julabo
SW22
11
17
18

Feed sample for stability test

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