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Black soldier fly larvae (acid conserved or dry meal) in extruded salmon diets – effects on feed processing, pellet quality, growth, and nutrient digestibility

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Master of Science in Feed Manufacturing Technology

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To summarize the thesis and its work

"The Fourth Golden Rule of Extrusion:

Some is good, more isn't necessarily better!" (Forte & Young, 2016)

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Abstract

The study was designed to investigate the effect of full fat black soldier fly larvae (BSFL) (*Hermetia illucens L.*) in diets for Atlantic salmon (*Salmo salar*) with emphasis on extrusion processing parameters, pellet quality, digestibility and growth. BSFL was substituted on a protein level in commercial like salmon diet except wheat flour and vitamin/minerals to maintain similar relationship between protein and energy, and protein and starch. BSFL was added as a dried insect meal or a formic acid conserved insect paste.

The control diet, IM_C, served as the control for increasing inclusion of insect meal (6.25%, 12.5 and 25%) and, IP_C, as the control diet including formic acid for the increasing inclusion of insect paste diets (3,7% and 6,7%).

Challenges during extrusion were observed with the increasing insect inclusions, due to increased lipid content in the mash. This resulted in changes in extrusion processing shown as, decreased specific mechanical energy (SME), temperature, torque, die pressure and residence time. This provided decreased cooking of the product which led to decrease pellet quality measurement i.e. hardness, durability, expansion and water stability.

Formic acid addition in IP_C reduced palatability of the diet, which reduced feed intake, thus reduced growth. Digestibility of nutrients were however, elevated compared to the other diets.

Growth stagnated with the highest inclusion of insect meal and paste as a result of significant reduction of protein and lipid digestibility with increasing insect product inclusion. This was thought to be a function of chitin, hindering availability of nutrient in the gastro intestinal tract for the Atlantic salmon. Reduced protein digestibility could also be influenced by the nitrogen content in chitin, which has a low digestibility in Atlantic salmon. The digestibility of starch was, however, improved with increasing inclusion of insect meal. This was believed to be a function of the balance in water holding capacity (WHC) of the ingredients resulting in increased availability in the Atlantic salmon.

Keywords: Insects meal; Insect paste; Extrusion processing; high lipid mash; Water holding capacity; Nutrient digestibility; Growth; Feed intake; Atlantic salmon (*salmo salar*)

Sammendrag

Studien ble gjennomført for å undersøke effekten av fettrike svarte soldatfluelarver (BSFL) (*Hermetia illucens L.*) i dietter for atlantisk laks (*Salmo salar*) med vekt på prosesseringsparametere via ekstrudering, pellets kvalitet, fordøyelighet og vekst. BSFL ble erstattet på proteinnivå med en kommersiell lignende laksediett, med unntak av hvetemel og vitamin/mineraler for å opprettholde et likt forhold mellom protein og energi, og protein og stivelse. BSFL ble tilsatt som et tørket insektmel eller som en maursyre-konservert insektpasta.

Kontrolldiett, IM_C, fungerte som kontroll for økende inkludering av insektmel (6,25%, 12,5 og 25%) og, IP_C, som kontrolldiett med maursyre for økende inkludering av insektpasta (3,7% og 6,7%).

Utfordringer under ekstrudering ble observert med de økende insektinneslutningene, på grunn av økt lipidinnhold i fôrmiks. Under ekstruderingsprosesseringen resulterte dette i redusert spesifikk mekanisk energi (SME), temperatur, dreiemoment, dysepress og oppholdstid. Dette ga redusert tilberedning av produktet som førte til reduserte målinger av pellets kvalitet, dvs. hardhet, holdbarhet, ekspansjon og vannstabilitet.

Maursyretilsetning i IP_C reduserte smakbarheten til dietten, noe som reduserte fôropptaket, og dermed reduserte veksten. Fordøyeligheten av næringsstoffer ble imidlertid forhøyet sammenlignet med de andre diettene.

Veksten stagnerte ved høyest tilsetning av insektmel og pasta som et resultat av betydelig reduksjon av protein og lipid fordøyelighet med økende inkludering av insektprodukter. Dette ble antatt å være en funksjon av kitin, noe som hindrer tilgjengeligheten av næringsstoffer i mage-tarmkanalen for atlantisk laks. Redusert protein fordøyelighet kan også påvirkes av nitrogeninnholdet i kitin, som har lav fordøyings grad hos atlantisk laks. Fordøyeligheten av stivelse ble imidlertid forbedret med økende inkludering av insektmel. Dette antas å være en funksjon av balansen i vannbindingsevnen (WHC) til ingrediensene, noe som resulterte i økt tilgjengelighet av stivelse i atlantisk laks.

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Abbreviations

- RO-water Reverse Osmosis - water
- AD Apparent Digestibility
- DM Dry Matter
- SME Specific Mechanical Energy
- FCR Feed Conversion Ratio
- SPC Soy Protein Concentrate
- CGM Corn Gluten Meal
- FM Fish Meal
- BSF Black Soldier Fly
- BSFL Black Soldier Fly Larvae
- EPA EicosaPentaenoic Acid
- DHA DokosaHeksaenoik Acid
- NSP Non-Starch Polysaccharide
- ANOVA Analysis of Variance
- AA Amino Acid
- EAA Essential Amino Acid
- WHC Water Holding Capacity
- SFA Saturated Fatty acid
- MUFA Mono Unsaturated Fatty Acid
- PUFA Poly Unsaturated Fatty Acid

1 Introduction

The continuous growth of the global population and higher demands from consumers to increase sustainable farmed fish production urges the need for exploring other protein and oils (EPA and DHA) ingredients (Sørensen et al., 2011). Norway as a fish producing nation have difficulties producing enough protein for its own aquaculture feed production. Even though the aquaculture production has decreased since 2015 (aquaculture quantity (q): 1 381 thousand tonnes) to 2016 (q: 1 326 thousand tonnes) (FAO, 2018), and is still dependant on import of soy protein and fish meal (FM) to meet the requirements. Especially when good quality products such as FM and fish oil is expensive, limited and in high demand. As well as the supply of soybeans are dependent on import to Norway. Therefore, there is an urgent need to find suitable and sustainable (socially, economically and environmentally) raw ingredients for fish feed. One suitable candidate can be insects, which can be produced anywhere in the world. They can recirculate organic waste as well as recover nutrients. As explained by, Diener et al. (2011) black soldier fly larvae (BSFL) (*Hermetia illucens L.*) are efficient in growing on different waste e.g. manure, sludge and other organic waste products from households. The BSFL generally has a beneficial nutrient composition based on their feed, but are usually high in protein and lipid (40% and 30%) (Newton et al., 2005). Insects are however considered "farmed animal" according to EC (2009). Due to this labelling, substrates such as manure, catering wastes and substrates containing meat or fish are prohibited as feed for insects. EC (2017) approved the use of seven types of insects as a feed ingredient for aquaculture production, thereof the BSFL.

Lock et al. (2016) showed promising results in Atlantic salmon fed diets replacing FM with insect meal. 50% replacement did not show any significant negative effect on growth. The same study also showed promise with 100% replacement, as growth and feed conversion ratio (FCR) for Atlantic salmon were similar as well as no difficulties during feed production.

Extrusion is a production system that bases itself on mechanical energy through friction and steam/water as thermal energy to form/shape the product. Physicochemical changes in the ingredients are facilitated by the addition of energy through extrusion in the presence of water. Increased inclusions of lipid and water in the mash would function as a lubricant during processing, decreasing friction which could worsen pellet quality and digestibility i.e. denaturation and gelatinization (Plattner, 2007). As seen in Hansen (2011), the effects of

replacing FM with a high lipid ingredient (krill meal) decreased pellet quality corresponding to the replacement values.

High moisture containing products such as fish and insects, can obtain storage stability by conserving it with acid, instead of using energy and perhaps changing the product with a drying process. Wet ingredients in feed production, however, may be problematic to use in high inclusions for practical reasons. Either as problems occurred during mixing (formation of lumps), transportation (clogging) or processing due to unwanted moisture increase. Usually, ingredients are therefore dried for easier handling and storage stability. A drying process can change nutrient availability, functional properties and perhaps palatability of specific ingredients as noted for insect meal (Nogales- Mérida et al., 2018). Drying would also be a quite energy demanding procedure and therefore costly.

This study focused on fish feed production through twin-screw extrusion process and how extrusion parameters and pellet quality are affected by ingredient replacement, i.e. insect meal or insect paste preserved with formic acid. The BSF paste contained approximately 77% moisture and 34% of lipid in dry matter (DM) basis, whereas BSF meal contained around 29% lipid on DM basis. More specifically, the experiment assessed the effects on production parameters when replacing 6,25%, 12,5% and 25% of the proteins in a commercial comparable diet with insect meal or 3,7% and 6,7% with insect paste. Furthermore, it was studied how production parameters can reflect and influence pellets quality, growth, feed intake and nutrient digestibility in Atlantic salmon (*Salmo salar*).

2 Literature background

This literature background mainly focuses on a general fish feed production line, digestive system, nutrient requirement and some factors affecting digestibility for salmonids as well as general information on BSFs and nutrient composition. Fish feed production line includes particle size reduction with hammer mill, mixing, conditioning, co-rotating intermeshed twin-screw extruder, drying, vacuum coating and pellet quality assessment.

2.1 Fish feed process line

2.1.1 Pre- extrusion

The particle size of materials used for feed should generally be reduced for further processes being done. This reduction would decrease segregation in general but also ensure better homogeneity during mixing and facilitate further processes such as extrusion (Behnke, 1996). Fine grinding is essential to enhance and facilitate interactions, between particles and the particles/polymers themselves. For instance, increasing the surface area would enhance hydration time (Hemmingsen et al., 2008). Particle size can also influence digestibility and performance in animals e.g. Atlantic salmon (Hemmingsen et al., 2008; Sveier et al., 1999). Some particle size reduction may still occur during extrusion processing (Sveier et al., 1999).

Mixing is an essential process during feed production. Obtaining homogeneity of the material mix is important to ensure an even distribution of each material in the mix. Lumping or segregation of material due to poor mixing could be detrimental for animals eating a product with too high level of a given material, such as mineral and/or vitamins. A material mix that is not fully homogenised can also decrease pellet quality due to a lack of necessary ingredient/polymers and would reduce binding capabilities. The mixing process could be performed either continuous or batch-wise.

A conditioner functions as a high-speed mixer and feeder for the next process. In this case an extruder. The conditioning process involves adding steam/water or other liquids to a closed high-speed paddle mixer. Hydrating and suppling thermal energy to the material and starting

certain processes such as gelatinization and denaturation with the help of residence time occurs within the conditioner. The process of hydrating takes optimally 60-90 seconds (Forte & Young, 2016). This process is beneficial for enhancing the effects of the extruder, and therefore commonly used in the industry. Pre-heated and hydrated product reduce the needed residence time to facilitate chemical processes as well as a hindering "two face flow" which can produce non-uniform pellets with a hard, dry and unchanged core, this is highly dependent on particle size and time exposed to water (Forte & Young, 2016).

2.1.2 Extrusion

A twin-screw extruder is made up of a barrel hosing two rotating screws threaded with different screw elements with an inlet and outlet. The outlet is an endplate or dies. This process bases itself on friction while conveying the material from inlet to outlet. The screw configuration can be divided into three zones within an extruder: feed zone (by the inlet, mainly consisting of large conveying screw elements), kneading zone (mainly consisting of screw elements with smaller pitch which enhances mixing and kneading, increasing shear forces and pressure) and final cooking zone (usually consisting of smaller pitch conveying screw elements or reverse screw elements, pressure is increased due to resistance flow created by the outlet, this is where the main developments of the materials occur) (Plattner, 2007). Friction or shear forces are being created between material and screw, material and barrel wall and particle to particle collision within the barrel. These shear forces are reported as specific mechanical energy (SME) (Sørensen, 2012). Extrusion is considered a high temperature short time process, meaning the material in an extrusion process is exposed to high heat (120-130°C), high pressure (20-30bar) and shear forces for a short time (10-40seconds (Forte & Young, 2016)) which form the material into a "melt" (Sørensen, 2012). For instance, parameters of a twin-screw extruder with: screw speed: 397 rpm, barrel temperature: 130°C for a broiler diet had an approximate residence time of 35 seconds (Edwards Jr et al., 1999). This residence time would depend on temperature, screw speed, screw configuration, diet formulation, moisture and throughput. Steam or water is used to add thermal energy to the mix. The addition of moisture (steam, water) and lipids functions as a lubricant and reduces shear forces and residence time (Forte & Young, 2016; Plattner, 2007; Sørensen et al., 2002). The moisture will also facilitate chemical processes to occur such as gelatinization of starch which would also function as a partly digestible binder in fish feed production (Krogdahl et al., 2005). With high lipid levels in the feed mash (>12%),

there is an increasing difficulty of producing necessary amounts of heat through mechanical energy for chemical changes to occur such as starch gelatinization (Plattner, 2007). The presence of lipids in the material can also lead to a coating of feed components. This could interfere with moisture available for components such as starch due to the lipids' hydrophobic properties, preventing/reducing gelatinization (Zimonja et al., 2007). However, Sørensen et al. (2002) reported that temperature alone does not affect the apparent digestibility (AD) of protein or energy in rainbow trout (*Oncorhynchus mykiss*). Which underbuilds that the extrusion process is a dynamic system affected by several variables. Table 2-1 shows a general overview of how processing variables affect moisture, mechanical,- thermal energy, retention time and protein digestibility during extrusion.

Table 2-1 - General interactions chart between parameters and variable (Plattner, 2007).

Independent Processing Variables	Moisture	Mechanical Energy	Thermal energy	Retention time	Protein digestibility*
Increase Feed rate	↓	↑↑	↓	↓	↑
Increase lipid addition	↔	↓	↔	↑	
Increase water	↑↑↑	↓↓↓	↑	↑↑	
Increase steam energy	↑	↓↓	↑↑↑	↑	
Increase extruder speed	↔	↑	↓	↓	↑
Increase barrel temperature	↔	↓	↑	↑	↑
Increase extruder flow resistance	↔	↑↑	↔	↑	
Increase die restriction	↔	↑	↔	↑	
Key:	↔ = Neutral, ↑ or ↓ = Minimal impact		↑↑ or ↓↓ = moderate impact ↑↑↑ or ↓↓↓ = Significant impact		

*: Digestibility measurements were done for rats (Singh et al., 2007). For food sources: corn gluten-whey blend, fish and wheat flour and fish-wheat blends.

2.1.3 Post- extrusion

Drying is usually a necessary process after extruding feed. The extrusion system most commonly require a certain amount of water (approximately 25 – 30%) for the process to be successful. There is some water evaporation occurring after extrusion, however drying is still usually needed to ensure that the product is storage stable, meaning no microbial growth. This would usually mean a moisture level below 13% or/and a relative vapour pressure below 0,60 (p/p^0) which is a function of the water activity (a_w) of the material divided by the a_w of pure water in a stable environment (Reid & Fennema, 2007). Packaging, storage temperature, humidity, and the facility would influence the shelf life of the product. Typically around 65% of the total energy used for fish feed production is drying (Draganovic, 2013).

Production of a high energy fish feed (grower diet) would consist of adding 30-40 % lipid. Due to the negative impacts of oil in the extruder (Plattner, 2007), most of the lipid is therefore added after extrusion and drying with the help of a vacuum coating process. The Vacuum coating process is where dry pellets and lipid is added to closed environment and pressure is created and released during agitation. Addition of 30-40% oil is possible due to expansion and the vacuum coating process. Expansion is evaporation of water inside the material due to environmental changes, high temperature (>100℃) and pressure (e.g. 20bar) inside the extruder to room temperature and atmospheric pressure. This would increase mainly the diameter and length of the pellets as the evaporation creates pours/cavities within. The porous structure is what facilitates the absorptions and stability of oil within the pellets (Sørensen et al., 2010). The porous structure created by expansion is however dependent on ingredients (mainly starch and its source), processing parameters, screw configuration, moisture and oil content (Aarseth et al., 2006b; Sørensen et al., 2010). Controlling the expansion is of importance, as stability of oil, sinking velocity and pellet quality (hardness and durability) is affected by it (Aarseth et al., 2006b; Kraugerud & Svihus, 2011; Øverland et al., 2007). The sinking velocity of pellets is important to attract and encourage feeding in fish. A slow sinking velocity of pellets is desirable when feeding the Atlantic salmon (Øverland et al., 2007).

Measuring of pellet quality of fish feed compared to a land-living animal would be handled differently. For example, breakage or accumulation of dust during feeding of fish will be a loss of material as it would absolve in the water or get stuck in the transport pipe. However, feed for land-living animals with some breakage or dust the animal could potentially still ingest it. The transport system from storage bins to net pens are usually done by pneumatic conveying.

Attrition of pellets would be effected by bens, air velocity and length of the transport pipe which could be from a few hundred up to 1400 meters long (Aarseth, 2004; Draganovic, 2013). This rough handling and the importance of pellets being resistant to abrasive and impact stresses is therefore important. Production of fines and breakage would not be eaten by fish. This would be an economic loss and would also worsen the quality of the water for the local marine life or clogging the pipe. Therefore requirements of water stability, durability and hardness are set to ensure minimal loss in any form possible. An interesting solution could be, transport through pipes using water as a medium instead of air. This would ease much of the stresses, but the pellets' water resistance becomes even more important (Aarseth et al., 2006a).

2.2 The Atlantic Salmon

2.2.1 The digestive system

The gastrointestinal tract of the Atlantic salmon is about 0,8 of the length of the fish, not counting the unfolded pyloric caeca. It is a carnivore fish, meaning its diet mainly consists of a high level of protein and lipid. The gastrointestinal tract for the salmon is built up of a mouth area (teeth, gill gut, throat), oesophagus, stomach, proximal intestine attached to blind appendages, mid intestine and distal intestine. For the Atlantic salmon, the mouth cavity is arranged with serrated teeth, to be used as a gripping organ. Chemical sensors (taste buds) are situated around the body but more so around and inside of the mouth area most dense around the teeth in salmonids (Kasumyan & Døving, 2003). These sensors act as their tasting and smelling organs. The fishes' perception of something edible would be what chemical composition of the supposed feed leaks into the water. If the feed is acceptable it can be swallowed whole, ripped/nibbled to extract more compounds or rejected. Chemical compounds that are found to stimulate ingestion seems to be a variety of amino acids (AA), some better than others, as well as organic acids (Mearns et al., 1987). The Atlantic salmon seems to prefer a slightly acidic feed and avoid sourness and bitterness (Kasumyan & Døving, 2003).

Oesophagus is a tube with longitude foldings. It is able to stretch in diameter which transports food ingested with contractions to the stomach. Food and water entering stimulates the mucosa (the innermost layer of cells in the stomach) which in turn mainly secretes water, mucus (protecting the cell wall from enzymes and acid), hydrochloric acids and inactive proteases and

a minority of other enzymes like lipases, softening the food. The acid facilitates hydrolysis (mainly protein hydrolysis) by activating enzymes (pepsinogen to pepsin and lipase) as well as reducing pH to about 4-5 (Krogdahl, 2001; Nordrum et al., 2000). Contractions of the stomach mixes everything and forms a chyme. Emptying of the stomach via the pylorus is stimulated by distension of the antral wall and the presence of hydrolysed liquid chyme.

Proximal intestine which is connected with blind appendages called pyloric caeca (largely increases the surface area) follows after the pylorus. When the acidic chyme enters the area it is flushed with mucus, bicarbonate (from the intestinal mucosa) and bile (conjugated with taurine from the liver via the gallbladder) providing a buffer to the mix, raising the pH to about 8 (Nordrum et al., 2000). Pancreatic juices contain enzymes such as trypsin, chymotrypsin, lipase, and α-amylase are secreted into the digestive tract, from endocrine and exocrine pancreatic tissues surrounding the pyloric caeca and proximal intestine via ducts (Sahlmann, 2013). Most of the intestinal wall consists of enterocytes, cylindrical cells with microvilli facing the lumen (intestinal content) increasing the surface area (Sahlmann, 2013). The digestion and absorption of nutrients mainly occur through the proximal intestine and the pyloric caeca. The transition from the proximal intestine to the mid intestine is shown by the lack of pyloric caeca. Mid intestine to distal intestine is characterized by increased diameter. The distal intestine is able to digest and absorb large molecules such as intact proteins, or simply transport them out (Krogdahl, 2001). Food intake and osmoregulation are also regulated by chemical signalling through the gastrointestinal tract and are an important factor of the digestive system.

2.2.2 Nutrient requirements

Carnivore fish does not have a requirement for carbohydrates but also reduced capacity for digesting it, with relative low α-amylase and α-glucosidase activity (Hemre, 2001). Glucose is however sufficiently and efficiently synthesised through the gluconeogenesis to meet the needs, mainly through AAs (Hardy et al., 2011). Low inclusion levels of easily digestible starch such as gelatinized wheat starch in the diet could enhance or better utilize lipids and AAs present (Hardy et al., 2011).

Salmon and trout have a requirement of 1-2% n-3 fatty acids in the diet to avoid deficiency (Castell et al., 1972; Ruyter et al., 2000). However, a supply of very long and highly unsaturated n-3 fatty acids (C22:5 or C20:5, and C22:6) instead of or together with C18:3 in the diet have seen to increase growth rates (Lovell, 2003; Ruyter et al., 2000). Dietary inclusion of 5% of fish oil would usually be adequate (Lovell, 2003).

A requirement of essential amino acid (EAA) would be determined by the weight of the fish. In Table 2-2 a digestible AA requirement can be seen for different weight class of Atlantic salmon. The Atlantic salmon are able to synthesize taurine from methionine and cysteine efficiently (Nordrum et al., 2000). Taurine is conjugated with bile to help with lipid digestion.

Table 2-2 - Digestible essential amino acid (EAA) requirements of Atlantic salmon (Hardy et al., 2011).

	Weight class			
	0,2-20 g	20 – 500 g	500-1500 g	>1500 g
AA	% diet DM			
Arginine	1,79	1,82	1,70	1,46
Histidine	0,80[a]	0,80[a]	0,75[a]	0,64[a]
Isoleucine	1,32	1,32	1,22	1,04
Leucine	2,31	2,31	2,14	1,82
Lysine	2,55	2,54	2,35	2,00
Met + Cys	1,28	1,30	1,21	1,03
Phe + Tyr	2,71	2,68	2,46	2,09
Threonine	1,55	1,60	1,51	1,30
Tryptophan	0,35	0,37	0,35	0,30
Valine	1,75	1,79	1,67	1,44

[a]histidine levels adequate to support optimal growth but not optimal ocular health

2.2.3 Some factors affecting digestibility

Changes of the material within the extruder are mostly determined by temperature, moisture, residence time, shear forces and pH as well as the amounts and structure of the macro components (protein, carbohydrates, lipids, and water) (Sørensen et al., 2002). These changes influence nutrient digestibility via chemical or physicochemical changes in the raw materials themselves due to thermal heating and mechanical shear forces provided by the extruder process (Camire, 2001). Lack of water (<18%) and too high temperatures (>150°C) within the system may lead to damages for the material such as dextrinization of starch, destruction or inactivation of heat-labile vitamins, antioxidants, enzymes (Camire, 2001) and amylose-lipid formation (mainly monoglycerides) (Mercier et al., 1980). Formation of disulphide bridges (S-S) within or between proteins, and heat oxidation of cysteine and methionine in presence of e.g. pro-oxidants lipid would lower protein and AA digestibility under these conditions (Opstvedt et al., 1984). Maillard is also a reaction that reduces protein and AA digestibility under these conditions (Singh et al., 2007). This is a reaction between carbonyl group in reducing sugar and a free amino group, usually lysine due to it having two free amino groups (Singh et al., 2007). This reaction is highly influenced by temperature, moisture, residence time and acidic pH in extrusion (Bates et al., 1994). On the other hand extrusion process with moderate heating and moisture (120 – 130°C and 25 – 30% moisture) could contribute to a positive increase in protein digestibility due to easier access of active sites for digestive enzymes on native protein (Camire, 2001). As well as a somewhat stable S-H and S-S (Aslaksen et al., 2006), and destruction or inactivation of antinutritional factors (ANF), such as trypsin inhibitor in soybean meal and lectins (Aslaksen et al., 2007; Barrows et al., 2007; Singh et al., 2007). Non-starch polysaccharides (NSP) would also be considered an ANF, and are not as easily destroyed by the extrusion process (Aslaksen et al., 2007), and can provide varying effects during digestion in fish.

Soluble NSP and insoluble NSP can both affect the digestion negatively in Atlantic salmon. Soluble NSP binds to water in the digestive tract, especially the water near the mucosa. This increases viscosity and may hinder some absorption of water-soluble nutrients. As well as lowering the activity of enzymes such as trypsin, pepsin and α-amylase which in turn reduces the digestion of protein and starch (Hemre, 2001). Furthermore, NSPs are believed to disrupt micelle formation, hence reducing digestion of lipids in fish (Øverland et al., 2009). Dalsgaard et al. (2012) showed a significant difference in AP of organic matter when supplementing β-

glucanase in salmonid diets containing plant ingredients such as soybean meal, rapeseed meal and sunflower meal. Enhancing that NSPs have a negative effect when in their non hydrolysed state. Insoluble NSPs, however, reduces the retention time of the digesta, providing reduced time for enzymes and absorption of the nutrients (Hemre, 2001). Despite this, Hansen and Storebakken (2007) showed to a dietary inclusion of 15% cellulose without negative effect of AD of protein, starch or lipids for rainbow trout.

Increased levels of starch has seen to decrease nutrient digestibility in the diets significantly for Atlantic salmon (Krogdahl et al., 2004). Digestibility of starch with dietary inclusion of 7% (83% was digested) and 23% (56% was digested) (Krogdahl et al., 2004).

Acid hydrolysis is a function of an acid able to catalyze one or more protons (H^+) in access of water, changing structures of e.g. starch granules. Making the granules more susceptible to enzymes and further changes. These changes could increase digestibility, water-solubility, reduced inherent viscosity when heated (Wang & Copeland, 2015).

Even though pellet quality is usually affiliated with physical strain, there could be effects on a nutritional level as well. Baeverfjord et al. (2006) showed a tendency of lower feed intake with increased water stability of the feed. However, low water stability and soft pellets may also cause trouble during digestion in rainbow trout. Oil belching being the issue as indicated in, Aas et al. (2011). The hardness of the pellets would usually tell us if pellets are able to withstand storage without breaking. By overfeeding with hard pellets, swelling and rupturing of the stomach may occur in Rainbow trout (Pillay & Kutty, 2005).

Water holding capacity (WHC) is a term that defines a materials ability to hold and entrap water (due to gravitational forces). The WHC of a material varies depending on the materials composition (protein, lipids, carbohydrates, salt composition) and their state as well as temperature and pH level (Reid & Fennema, 2007). The amount of time needed for wetting of material is reduced with increased WHC (Figure 2-1) (Nguyen et al., 2015), and can in turn perhaps enhance the effects of extrusion.

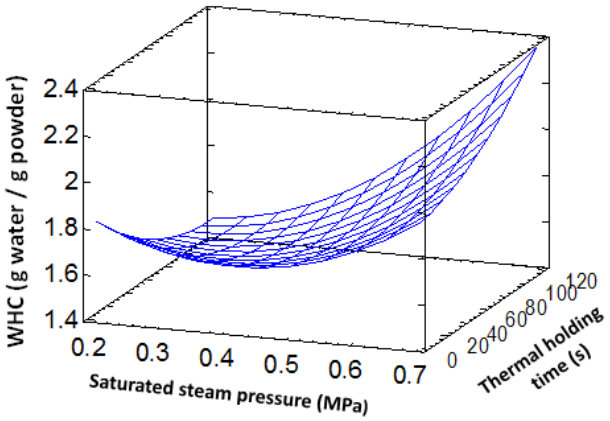

Figure 2-1 - Effect of WHC to thermal residence time with different saturated steam pressure (Nguyen et al., 2015).

2.3 Black soldier fly larvae (BSFL)

These insects could also help utilize and efficiently manage waste like, fish offal and other allowed waste products as described in the Introduction. In aquaculture this could be exploited and used as a way of recirculating and recover important nutrient such as very long chains of n-3 fatty acids and reduce waste (St- Hilaire et al., 2007; Sørensen et al., 2011), making the BSFL interesting as a protein and/or lipid source for the feed industry.

The production of BSFL is efficient, approximately 12 days under optimal condition from egg to the end of the larvae stage called prepupae (Hardy et al., 2015). The BSF only feeds during its larvae stage and therefore having an optimal nutrient composition at the end of the feeding. The insects are easy to handle as the larvae will seek dry land for their pupae stage, collecting themselves into a bin (Diener et al., 2011). These larvae are also highly resilient to environmental change, however, they rear most efficiently in warm (28-30℃) and humid climate (60% relative humidity) (Diener et al., 2011; Hardy et al., 2015). The processing method of the insect can be seen in Figure 2-2.

2.3.1 Insect as a feed ingredient in fish feed

For fish, an inclusion of insect meal might be limited due to its AA composition. Sulphur containing AAs are relatively low as well as arginine and taurine have not been detected (Finke, 2013; Nogales- Mérida et al., 2018). The fatty acid composition usually contains a high level of saturated fatty acid (SFA), most abundantly is lauric acid C12:0 (42 - 60%). While adequate levels of monounsaturated fatty acid (MUFA) and n-6 polyunsaturated fatty acid (PUFA) C18:2 n-6, but relatively low level of n-3 PUFA such as C18:3 n-3 (Finke, 2013; Spranghers et al., 2017). However, the nutrient composition of AAs and fatty acids profile can be changed according to productions by changing the nourishment (Nogales- Mérida et al., 2018; Spranghers et al., 2017). The EAA from BSFL is considered highly digestible in Atlantic salmon (Belghit et al., 2018; Lock et al., 2016) and European seabass (*Dicentrarchus labrax*) (Magalhães et al., 2017), as well as its fatty acid profile in Atlantic salmon, especially lauric acid C12:0 (Lock et al., 2016). However, the BSFLs exoskeleton contain a certain amount of Chitin, approximately 6% on of the whole body on DM (Spranghers et al., 2017) that is known to reduce the digestibility of nutrients (Karlsen et al., 2017).

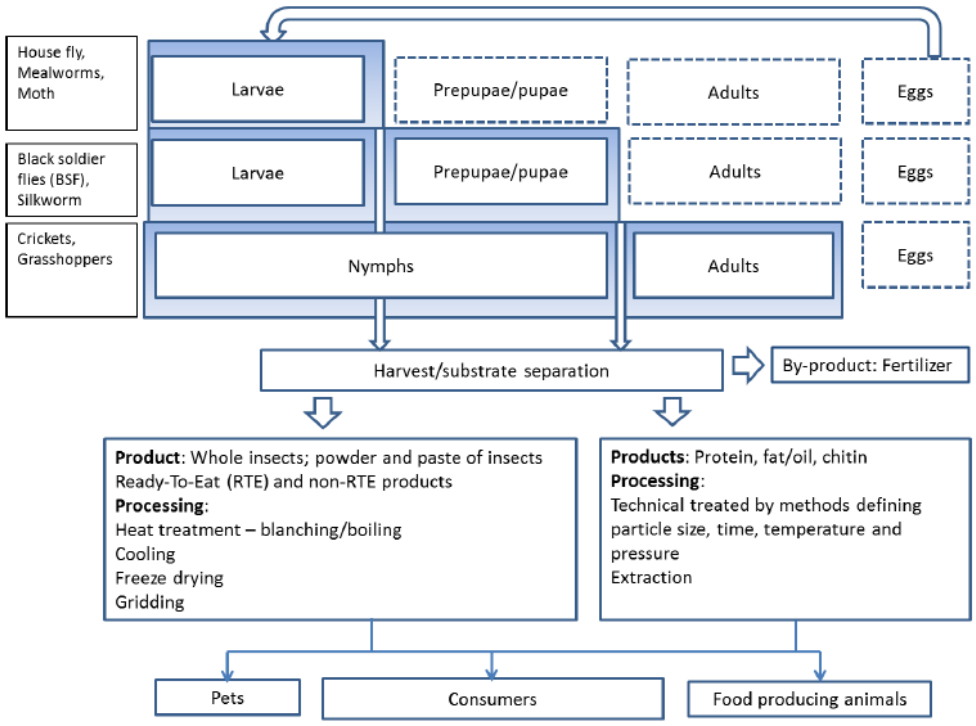

Figure 2-2 - General overview of insect production (Hardy et al., 2015).

3 Materials and Methods

Feed production was conducted at the Centre for feed technology – Fôrtek-NMBU

The fish trail was conducted at NMBUs fish lab on a Recirculating Aquaculture System (RAS) with an average recirculation of 97,2%.

IM_C is the control diet for insect meal diets: $IM_{6,25.12,5.25}$. IP_C is the control diet for insect paste diets: $IP_{3,7.6,7}$. Insect meal is a ground and drum dried product of BSFL. Insect paste (preserved with formic acid), a moist product of BSFL (approximately 23% DM), which was boiled and ground.

3.1 Raw materials and diet production

The insect paste required further grinding prior to feed production. This was done in frozen condition with a meat grinder (Tripas-Wexiö, RK-82, Sweden). The mesh size of the die was 3mm. The ground insect paste was left to thaw overnight at room temperature (approximately 20°C). Then the ground insect paste was placed into a large basket (60liter). Water was added to dilute the paste, 1kg warm water (52°C for every 6kg paste. Further grinding of the paste was done with a grinder pump (Pedrollo TR 1.1, San Bonifacio (VR), Italy). The grinder pump had some difficulty pumping the material due to its high DM and low temperature. 48kg paste and 8kg hot spring water (52°C) was added to the basket and ground with the grinder pump in 15 min intervals until 1 hour and 45 minutes were reached. The grinder pump was set in a cold-water bath between every second interval to cool down.

The macro ingredients including soy protein concentrate (SPC), fishmeal (FM), corn gluten meal (CGM), wheat bran, faba beans, wheat flour, and insect meal were manually weighed and then mixed with an ISDECA mixer (60-liter paddle-mixer, prototype, Fôrtek, Forberg, Norway) for 2,5 minutes. The material mix was ground in a small Hammer mill (Bill bliss, horizontal, 18,5kW, USA) with a 1mm sieve. Feeding of the hammer mill was done with a small K-Tron at 20Hz on all diets except for the diet with the highest inclusion of insect meal. This diet blocked the sieve twice and the speed was therefore reduced to 15Hz. Two batches consisting of 35 kg of dry materials were made for each diet, except three for $IP_{6,7}$. After grinding, each

batch was again mixed for 2,5 minutes together with all the micro-ingredients (yttrium oxide (Y_2O_3), choline chloride, L-methionine, and a Vit/mineral mix) and insect paste for $IP_{3,7}$ and $IP_{6,7}$ (see the ingredient list for all diets in Table 3-1). Formic acid was sprayed on the material in IP_C while mixing in the ISDECA mixer with the help of a pressurized tank and a spray nozzle (nozzle type: 11004). The formic acid was diluted with 1 part acid and 2 part water to distribute the acid more evenly during mixing. The diets with the addition of insect paste required more mixing time. The mixing order was: 2,5 minutes mixing of the macro ingredient with vitamin/mineral followed by 2,5 minutes of mixing time with half of the paste, 10 minutes after the rest of the paste was added and another 2,5 minutes after scraping the paddles clean with a brush, as some of the paste stuck to the paddles. Speed of the pin mill was 10 out of 10 (settings (RPM)) and paddles mixing speed was on 6 out of 10 (settings (RPM)) through all mixing processes.

Table 3-1 - Recipe of g/kg inclusion for all diet as is. Dietary inclusion of insect meal and insect paste were done on protein replacement.

	Insect meal				Insect paste		
Protein replacement %	0	6,25	12,5	25	0	3,7	6,7
g/kg	IM_C	$IM_{6,25}$	$IM_{12,5}$	IM_{25}	IP_C	$IP_{3,7}$	$IP_{6,7}$
SPC[a]	354	335	310	256	354	291	239
Fish meal[b]	250	232	215	177	250	203	166
Wheat flour[c]	137	136	136	136	137	119	106
Corn gluten meal[d]	40,0	37,2	34,4	26,0	40,0	32,4	26,6
Wheat bran[e]	40,0	24,8	12,9	0	31,2	21,6	10,0
Faba beans[f]	18,5	17,2	15,9	10,3	18,5	15,0	12,3
Vit/min mix[g]	6,50	6,50	6,50	6,50	6,50	5,70	5,0
Methionine[h]	2,0	2,0	2,0	2,0	2,0	1,70	1,50
Choline chloride[i]	1,50	1,50	1,50	1,50	1,50	1,30	1,20
Yttrium oxide[j]	0,10	0,10	0,10	0,10	0,10	0,10	0,10
Formic acid[k]	0	0	0	0	8,80	0	0
Insect meal[l]	0	80,7	161	323	0	0	0
Insect paste[m]	0	0	0	0	0	198	351
Fish oil[n]	150	127	105	61,9	150	111	81,3

[a] soy protein concentrate AX3®, Triple A AS, Hornsyld, Denmark. [b] LT fishmeal, Norsildmel AS, Bergen, Norway. [c] Wheat flour 78%, batch number: 5093060546, Norgesmøllene, Bergen, Norway. [d] Corn gluten meal, Baolingbao Biology, Shangdong Yucheng, China. [e] Wheat bran, Norgesmøllene, Bergen, Norway. [f] Faba beans, Norgesfôr, Oslo, Norway. [g] Trouw Nutrition Netherlands, Putten, Netherlands. [h] L-methionine, Bestamino™ Cj Cheiljedang, Seoul, Korea. [i] Choline chloride 70%, $C_5H_{14}ClNO$, 139,6g/mol, Vilomix, Hønefoss, Norway. [j] Yttrium oxide (Y_2O_3) Metal Rare Earth Limited, Shenzhen, China. [k] Formic acid, Ensil Maursyre 85%, Felleskjøpet, Norway. [l] HiPromeal, Hipromine, Robakowo, Poland. [m] HiProPulp, Hipromine, Robakowo, Poland. [n] Fish oil, batch number: 4010105201800110/294, Norsildmel AS, Bergen, Norway.

IM_C: Control diet for insect meal

$IM_{6,25.12,5.25}$: percent protein replacement with insect meal

IP_C: Control diet for insect paste

$IP_{3,7.6,7}$: percent protein replacement with insect paste

The extrusion process was done with reduced capacity, meaning the conditioner was not used. Therefore, a small K-tron feeder was used to feed the material directly into the first chamber of the extruder. The speed of the feeder was set to 6 Hz(40,3kg/h) for diet IM_C, $IM_{6,25}$, $IM_{12,5}$, IP_C,

and $IP_{3,7}$. For IM_{25} the rate was set to 5,5 Hz (39,2kg/h) and 8 Hz (43kg/h) for $IP_{6,7}$ (See Table 4-1).

The extruder used was a five-section Bülher co-rotating twin-screw extruder (BCTG 62/60 D, Uzwil, Switzerland) with a length to diameter ratio of 20:1, at Center for Feed Technology-Fôrtek. The screw configuration used for all diets, see Figure 3-1.

																	Inlet		
Outlet	40	40	60	60	60	80	80	20	20	120	100	80	60	40	60	100	80	80	80
	R	R	R	R	R	R	R	L	L	R	R	R	R	R	R	R	R	R	R
										P					P		UC	UC	

Figure 3-1 - Screw configurations

20, 40, 60, 80, 100, 120 : length in cm of each screw element

R (right), L (left): Flow direction of each screw element

P: Polygon

UC: Undercut conveying screw element (larger channel depth than the other conveying screw elements).

↓ : 5mm spacer ring and 90º offset between the screw elements.

Extrusion was done in the order of IM_C, $IM_{6,25}$ and then $IM_{12,5}$ on 07.02.19. and on 08.02.19 the order was IP_C, $IP_{3,7}$, $IP_{6,7}$ and then IM_{25}. In an estimated order of extrusion difficulty, as high levels of lipid and water would generally cause difficulties during the shaping of the product in an extrusion process (see 2.1.2).

During extrusion, steam was added to the insulation chamber of the extruder barrel to heat the extruder up in section 2, 3 and 4. This heating was used during extrusion for all diets. Cooling (cold water in the insolation chamber) in section 5 of the extruder barrel was used during all diets.

Extrusion parameters were recorded twice, once at the start of sampling production (when parameters were stable) and second at the middle/end. The parameters shown in Table 4-1 are an average of the two.

After extrusion and shaping of pellets, drying was done in batch driers (see Figure 3-2). Fan heaters (15KW, Inelco heaters, Dania-heater 15kW, Fjerritslev, Denmark) were used for approximately 1 hour for each diet until 8-12% moisture was obtained. An IR machine (MB25 moisture analyser, Ohaus, Nänikon, Switzerland) was used to measure when these levels were met. Each batch dryer was filled with approximately 30kg wet feed.

Figure 3-2 - From left to right, Picture of the batch drier with an outward blowing fan lid, Fan heater in front blowing hot air (15KW) into the batch drier (operating position).

Dust and broken off pieces of pellets were removed using sieving (mesh size: 1,6mm) for each diet. The amount of dust and particles was then weighed and calculated to the percentage of loss (<1,6mm).

For the vacuum coating process, fish oil was manually weighed and heated to 60-70℃ except for IM_C which was heated up to 80℃ to preheat the metal in the vacuum coater, as the metal was cold and IM_C was the first diet to be coated. The heated fish oil was transferred into a small pressurized tank (30 liters) with a hose and nozzle (nozzle type: 6508). Each diet had 25kg pellets added to the vacuum coater used (Gentle Vacuum Coater (GVC) – 80 prototype, Fôrtek, Amandus-Kahl). Spraying of the oil was done while the vacuum coater was turning (18 rpm). A vacuum was created to approximately 0,15 bar. Thereafter turned off and a small opening in a valve was opened releasing the pressure. Reaching atmospheric pressure within 2 – 3 minutes.

3.2 Physical and chemical analysis

Pellet quality measurements: water stability, durability, hardness, expansion, and sinking velocity were compared to a commercial produced diet (Skretting AS, Bruhagen N-6530 Averoy, Norway, Product: Nutra RC 3, size: 3mm, batch number: 6225592). This diet will be noted as S.

3.2.1 Water stability

The water stability of pellets was measured according to, Baeverfjord et al. (2006), with a few exceptions; the sample size was closer to 20grams. Reverse Osmosis (RO)-water was used in the beakers and shakings was set to 120rpm. Measurements were carried out in triplicates with three incubation times, i.e. 30 min, 60 min and 120 minutes. After the incubation time, the replicates were dried for about 20 hours at 100-104°C. When DM was retained constant water stability index was calculated based on percent pellets remaining in the baskets.

3.2.2 Durability

The durability of the pellets was measured in duplicates on a Doris pellet tester (AKVAsmart, Bryne, Norway). Approximately 350g sample was used for each replicate. Samples were sieved on a (Retsch, Germany) with a 2 mm screen (whole pellets), 1 mm screen (broken or small pellets) and a bottom pan (dust) stacked on top of each other. Settings for the sieving set to 1,2 amplitude for 60 seconds.

3.2.3 Hardness

Hardness was measured with a Texture analyzer (Tinius Olsen, H5KT, Salfords, England). Length and diameter for 30 randomly selected pellets per diet were taken. These numbers were also used to determine the expansion ratio of our diets. 15 Pellets with the average diameter and length (±0,2mm) were handpicked to be analyzed. The analyzer measured the amount of force

(measured in Newton) needed to break/crack the pellet at the length of the pellet. The measurements recorded were for the first breakpoint.

3.2.4 Sinking speed

To measure the sinking speed of the pellets a 1,2-meter long plastic tube, vertically placed (12cm in diameter) filled with drinkable tap water (17℃ ± 2℃) were used. The tube had markings for 1 meter. One pellet at a time was dropped into the tube, ten pellets per diet. Time in seconds was measured for how long the pellets needed to travel 1 meter.

3.2.5 Water holding capacity (WHC)

The WHC of feed ingredients was measured according to, Nguyen et al. (2015), with a few exceptions, such as 15 ml plastic tubes and RO-water was used. Measurements were carried out in triplicates for each ingredient. None of the ingredients underwent any pre-treatment, except mixing for the diets. The centrifuge used was a Thermo Scientific™, Heraeus™ Multifuge™ X1R Centrifuge, Waltham, Massachusetts, USA. Settings of the centrifuge was 1200g, 2574rpm.

3.2.6 Chemical analysis

Ash content was measured in a furnace (Nabertherm) at 550℃ between 4 and 20 hours.

The determination of energy in our samples were performed by bomb calorimetry (PARR 6400 Bomb calorimeter). Samples are burned under a closed environment and the amount of released/absorbed heat is measured.

Method to determine crude lipid was done by accelerated solvent extraction, ASE (ASE® 350 Accelerated Solvent Extractor, Nerliens Mezanski) (Schäfer, 1998).

Total starch analysis (pre-treated with acetone to remove lipids) was performed by adding α – amylase to break up the starch and thereafter addition of amyloglukosidase as to ensure that the

starch is fully broken down to glucose units. The amount of glucose is determined by colour spectrometer (RX4041 Randox Daytona+, England).

The amount of nitrogen was measured by Dumas method, burning (1150℃) the material in an enclosed oxygen-rich environment. Products (CO_2, H_2O, N_2, N_xO_Y and SO_2) are thereafter transferred through a reduction tube (850℃), the tube is filled with copper which removes excess oxygen and reduces N_xO_Y to N_2. The gasses are separated and detected with the help of a heat thread detector. The process is done through, Vario el Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Thereafter crude protein was calculated based on the nitrogen level obtained (nitrogen * 6,25).

Analysis of the degree of gelatinization of starch was performed by NOFIMA. Differential scanning calorimetry (DSC 823e Module Mettler-Toledo, Switzerland) as described by, Kraugerud and Svihus (2011) was the method used. The analysis was done in duplicates with a none heat-treated diet as a reference.

Measurements of nutrient digestibility were done with the help of an indigestible marker (Yttrium oxide). Measuring concentration in feed to the concentration in faeces. The method for measuring the amount of yttrium is determined by spectrophotometric (Microwave Plasma Atomic Emission Spectrometer 4200, Agilent Technologies, Santa Clara, United States).

3.3 Fish trail

The fish trail consisted of a total of 1260 fish distributed among 21 circular tanks (approximately 230 liters), 60 fish in each tank. Before the start of the trail, the biomass for each tank was evened out, ending up with 2,04kg biomass in each tank with the average initial body weight for each fish to be approximately 34 grams (see Table 4-3). AQUI-S® aquatic anaesthetic was used during the initial weighing and separation of the fishes. All fishes were starved for 48 hours before the feeding trial commenced.

The fish were fed with experimental diets for 7 weeks. An automatic feeding belt system sat on top of each tank was used (see Figure 3-3). The belt ran twice a day, feeding the fish for 1 hour and 15 minutes in the morning (8:30 – 9:45) and 1 hour and 5 minutes in the afternoon (14:30 – 15:35). The nutrient composition of the feed can be seen in Table 3-2, and AA composition in Table 3-3. The fish were fed to appetite with approximately 10% excess. To determine the

excess, uneaten pellets were collected after being separated from the faeces with the help of a wire screen. Thereafter, the uneaten feed was weighed and stored at -20°C.

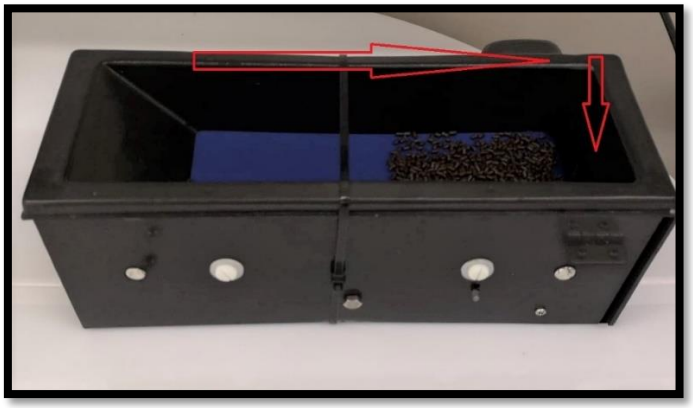

Figure 3-3 - Automatic belt feeder, red arrows indicating the direction of flow.

Table 3-2 - Nutrient composition of finished diets

	IM_C	$IM_{6,25}$	$IM_{12,5}$	IM_{25}	IP_C	$IP_{3,7}$	$IP_{6,7}$
Dry Matter (g/kg)	929	925	916	908	911	919	919
Crude Protein (g/kg DM)	468	474	464	457	458	472	479
Crude Lipid (g/kg DM)	146	155	172	159	162	130	133
Starch (g/kg DM)	123	124	115	115	116	120	128
Ash (g/kg DM)	55,2	58,8	61,7	68,3	53,0	56,7	60,8
Yttrium oxide (g/kg)	0,08	0,08	0,08	0,07	0,07	0,08	0,10
Ca (g/kg)	9,29	10,2	11,0	12,1	10,1	10,6	10,5
Na (g/kg)	4,59	4,49	4,77	3,88	4,58	4,39	3,98
Mg (g/kg)	1,03	1,25	1,23	1,50	1,05	1,07	1,17
K (g/kg)	5,18	6,23	6,62	6,64	4,63	5,81	6,07
Total-P (g/kg)	9,09	8,94	8,70	8,84	9,14	9,02	8,91
Formic acid (g/kg)	0,00	0,00	0,00	0,00	7,20	5,80	11,0
Energy (MJ/kg)	21,9	21,7	21,7	21,5	21,6	21,4	21,1
Protein: Starch (g/MJ)	3,80	3,82	4,03	3,97	3,96	3,94	3,74
Protein: Lipid (g/MJ)	3,19	3,07	2,70	2,87	2,82	3,63	3,59
Protein: Energy (g/MJ)	21,3	21,8	21,4	21,2	21,2	22,1	22,7

Table 3-3 - Calculated essential amino acid (EAA) composition (DM basis) g/kg based on amino acids (AA) in ingredients. AA content in Fish meal (FM) and Insect meal (IM).

	IM_C	$IM_{6,25}$	$IM_{12,5}$	IM_{25}	IP_C	$IP_{3,7}$	$IP_{6,7}$	FM	IM
Methionine	10,7	10,8	10,8	10,8	10,8	10,7	10,7	16,5	6,98
Threonine	19,1	19,1	18,9	18,4	19,2	19,0	18,8	24,3	12,8
Valine	21,7	21,8	21,8	21,5	21,9	21,7	21,6	27,8	16,2
Isoleucine	20,6	20,6	20,4	19,7	20,8	20,5	20,2	24,5	13,2
Leucine	36,6	36,4	35,8	34,2	36,7	36,2	35,6	43,2	21,8
Phenylalanine	21,5	21,6	21,6	21,2	21,7	21,5	21,4	23,3	15,6
Histidine	10,6	10,8	11,0	11,2	10,7	10,8	10,8	11,8	9,74
Lysine	30,5	30,8	31,0	30,9	30,7	30,6	30,6	46,8	24,8
Arginine	31,8	31,2	30,3	28,0	32,1	31,1	30,2	38,9	13,8
Tryptophan	5,42	5,54	5,61	5,68	5,47	5,50	5,53	6,46	4,84

The temperature in the tanks were set to approximately 15 ℃. Mortality of fishes were checked once a day after afternoon feeding. Oxygen saturation (%), oxygen amount (mg/l), temperature, and water flow were checked for in the morning of each day after feeding. Water flow was increased according to the saturation level (Min. 80%).

3.3.1 Sampling

At the end of the feeding period, six randomly selected fish from each tank which was sedated with 1,5g AQUI-S® in their original tank (150 liters) was then put into a bucket with FINQUEL® vet. (lethal dose). First, mucus sample was taken, the mucus was scraped off with a rubber scraper on one side of the fish. Weight and length of the fish were measured, thereafter the fish was stunned/killed with a hit to the head. Blood samples were taken first, then fish was gutted and all other necessary samples were taken.

The rest of the fish in the tank were sedated with AQUI-S® (1,5g/150liter) and lightly anesthetize with FINQUEL® vet. (3g/40 liters), weighted and stripped for a faeces sample see Figure 3-4, and put back into its original tank. The same procedure was repeated one week later and once more the week after to accumulate enough faeces for analysis.

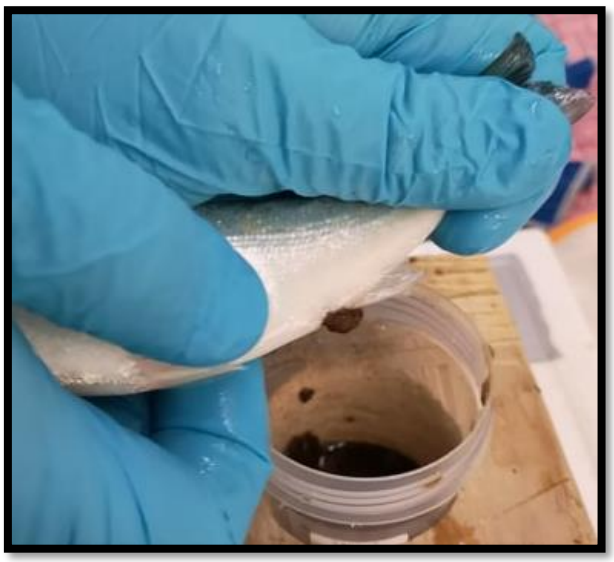

Figure 3-4 - Stripping of fish

3.4 Calculations and statistical analysis

$$WHC = \frac{Total\ water\ mass}{Total\ Dry\ mass(as\ is)}$$

$$Expansion\ \% = \left((Pellet\ width - die\ diameter\ ) * die\ diameter^{-1}\right) * 100$$

$$SME = (2 * \pi * 60^{-1}) * (S_{rpm} * Tk_{nm} * T_{t/h}^{-1})$$

Where S_{rpm} is screw speed, T_{knm} is Torque and $T_{t/h}$ is throughput (Hansen, 2011).

$$\%\ gelatinized\ starch = \ 100 - \left(\frac{DSC_H}{DSC_N} * 100\right)$$

Were DSC_H value is joule/gram starch for heat-treated feed sample and DSC_N value is joule/gram not heat-treated feed sample.

$$Feed\ intake\ per\ tank = (Total\ feed * Feed\ DM) - \left(\frac{Uneaten\ feed * DM\ of\ uneaten\ pellets}{Recovery\ value}\right)$$

$$Feed\ intake\ per\ fish = \frac{Feed\ intake\ per\ tank}{number\ of\ fish\ in\ the\ tank}$$

$$FCR = \frac{Feed\ intake\ per\ fish}{(Final\ BW - initial\ BW)}$$

Were Initial BW is the average body weight in grams for one fish in one tank. And the Final BW is the average body weight per fish for the same tank.

$$Geometric\ mean = \sqrt{Final\ BW * Initial\ BW}$$

$$Conditioning\ factor = \frac{Final\ BW\ (g)}{\left(Length\ (cm)\right)^3}$$

$$Spesific\ Growth\ Rate = 100 * \frac{\ln(Final\ BW) - \ln(Start\ BW)}{\Delta t(experimental\ days)}$$

$$Relative\ Feeding\ Rate = \frac{Feed\ intake\ per\ fish}{\frac{\Delta t(experimental\ days)}{Geometric\ mean\ BW}}$$

$$ADC = (1 - \left(\frac{D_i}{F_i}\right) * \left(\frac{F_n}{D_n}\right)) * 100$$

Were D_i and F_i represent the concentration of the marker in diet and faeces. D_n and F_n represent the concentration of nutrients in the diet and faeces.

Growth performance and nutrient digestibility were analysed by one-way analysis of variance (ANOVA).

Tukey's multiple comparison test to determine the difference between group on a significance value ($P < 0,05$). P-value seen in between 0,05 and 0,1 will be considered as trends.

Linear regression model ($Y = \beta_0 + \beta_1 x + \varepsilon$) was used to observe the effect of increasing inclusion of insect meal or paste on apparent protein digestibility.

4 Results

4.1 Feed production parameters

The energy input (SME, drive power and most temperatures) for the ingredient mixture of IM_C was highest among all the diets as shown in Table 4-1. These energy inputs were decreasing with increasing inclusions of insects irrespective of meal or paste. With increasing levels of insect meal most extrusion parameters were decreased, most notably: bar pressure, torque, drive power and SME which were clearly observed for IM_{25}. An increase in screw speed corresponding to the insect meal was practiced to balance the parameters and to a small increase in extruder water for IM_{25}. A similar effect and balancing can be seen for increasing levels of insect paste, however not as large. A decrease in throughput was observed with the increasing insect paste and a decrease of water to extruder and increase of feeding rate for $IP_{6,7}$. The screw speeds for $IP_{3,7}$ and $IP_{6,7}$ were equal. All changes to screw speed, throughput, and water to extruder was done to try and achieve the best possible result on pellet quality.

Table 4-1 - Extruder parameters.

Extrusion Parameters							
	IM_C	$IM_{6,25}$	$IM_{12,5}$	IM_{25}	IP_C	$IP_{3,7}$	$IP_{6,7}$
Die size (mm)	2,5	2,5	2,5	2,5	2,5	2,5	2,5
Number of dies	4	4	4	4	4	4	4
Calibration (Hz)	6	6	6	5,5	6	6	8
Feeder (kg/h)	40,3*	40,3	40,3*	39,2	40,3*	40,3*	43,0
Throughput (kg/h)	54,3	54,3	54,3	54,7	54,3	46,8	43,0
Barrel 1 (°C)	38,9	31,4	29,9	34,3	40,3	35,0	34,2
Barrel 2 (°C)[a]	61,2	47,1	47,9	68,9	63,6	62,3	56,5
Barrel 3 (°C)[a]	108	96,6	92,5	96,1	97,2	112	109
Barrel 4 (°C)[a]	117	118	115	104	112	114	112
Barrel 5 (°C)[b]	75,1	64,2	58,7	53,8	79,9	84,7	85,2
Die Temp. (°C)	96,5	92,5	90,5	78,5	92,0	92,5	94,0
Die pressure (Bar)	29,5	20,2	16,2	1,35	22,8	6,80	3,95
Screw speed (rpm)	270	290	345	475	275	400	400
Torque (Nm)	339	270	226	103	277	180	156
Torque (%)	78,0	62,5	52,0	23,5	63,0	41,0	36,0
Drive power (kW)	9,30	8,25	8,10	5,10	7,75	7,50	6,50
SME (Wh/kg^{-1})	177	151	150	93,6	147	161	152
Extr. Water (kg/h)	14,0	14,0	14,0	15,5	14,0	6,50	0
Knife speed (rpm)	2980	2980	2980	2970	2950	2950	2750
Number of knives	3	3	3	3	3	3	3
Water (%)[c]	30,7	30,9	31,0	33,5	30,7	31,4	31,5
Lipid (%)[d]	2,67	5,04	7,64	12,6	2,67	4,32	6,14

[a] heating around these sections of the extruder barrel.

[b] cooling around this section of the extruder barrel.

[c] percentage of water in the material during extrusion.

[d] calculated amounts in the mash during extrusion, on DM.

*: feeding rate was set to the same speed as $IM_{6,25}$, as differences in lipid and water were minimal between IM_C, $IM_{6,25}$, $IM_{12,5}$, IP_C and $IP_{3,7}$ .And was therefore estimated to have similar kg/h as we observed similar filling rate in the extruder.

4.2 Pellet quality

When comparing physical pellet quality, $IM_{12,5}$ had several fractures during expansion which resulted in accumulation of fractures and dust (<1,6mm) as seen in Table 4-2 under, bulk loss. Durability was also poor for these pellets compared to other diets which scored 98,6% and higher. Hardness was numerically quite similar for most diets except IP_C, which had the highest value of 46,4N and diet S with the lowest 28,6N. Gelatinization largely varies within the two duplicates. But there is still a numerically trend for $IM_{6,25}$, IM_{25}, $IP_{3,7}$ and $IP_{6,7}$ to be lower than the rest, especially $IP_{3,7}$ and $IP_{6,7}$. $IM_{6,25}$ had the highest numerical value (95,2%) as shown in Table 4-2. Analysis of gelatinization with only two replicates gave high variance within each group (SEM value: 14), the data might therefore not be very secure.

IM_C and IP_C obtained similar pellet quality measurements as the commercial diet, S, shown in Table 4-2 and Figure 4-1. It was measured that IM_{25} and $IP_{6,7}$ had a smaller diameter than the die diameter. The pellets for IM_{25} were observed and measured to have oval shapes, there was a distinction of two sides being shorter than the other two around the circumference.

Table 4-2 - Physical pellet quality measurements as well as bulk density and gelatinization % of the diets.

	IM_C	$IM_{6,25}$	$IM_{12,5}$	IM_{25}	IP_C	$IP_{3,7}$	$IP_{6,7}$	S*
Bulk loss[a] (%)	0,25	1,31	4,08	1,93	0,25	0,62	0,73	
Durability (%)	99,1	98,9	96,3	98,8	99,6	99,0	98,6	98,9
Hardness (N)	35,5	37,0	35,5	31,3	46,4	32,8	34,4	28,6
Expansion (%)	13,8	6,88	4,80	-8,99	12,2	3,59	-2,85	14,8
Sinking (Sec)	10,9	10,6	12,6	16,7	11,3	15,4	14,1	18,3
Bulk density (g/l)[b]	548	554	538	607	540	524	563	
Gelatinization[c] (%)	72,7	95,2	75,8	84,2	83,0	54,4	67,9	

[a]: Dry and uncoated pellets were sieved (mesh size: 1,6mm). Percent bulk loss was then measured.

[b]: Values were recorded of the pellets while in a warm, wet and uncoated condition.

[c]: percent gelatinized starch in the diet

*: Skretting diet, 3mm

All produced diets had lower numerical levels on the water stability index compared to the commercial diet, S. However, the control diets: IM_C and IP_C showed water stability closer to

the commercial diet, at least for 30 and 60 minutes in the water bath. IM_{25} had the numerically lowest water stability index for all three durations (Figure 4-1). With increasing level of insect meal and insect paste in the diet, there was a decrease in water stability.

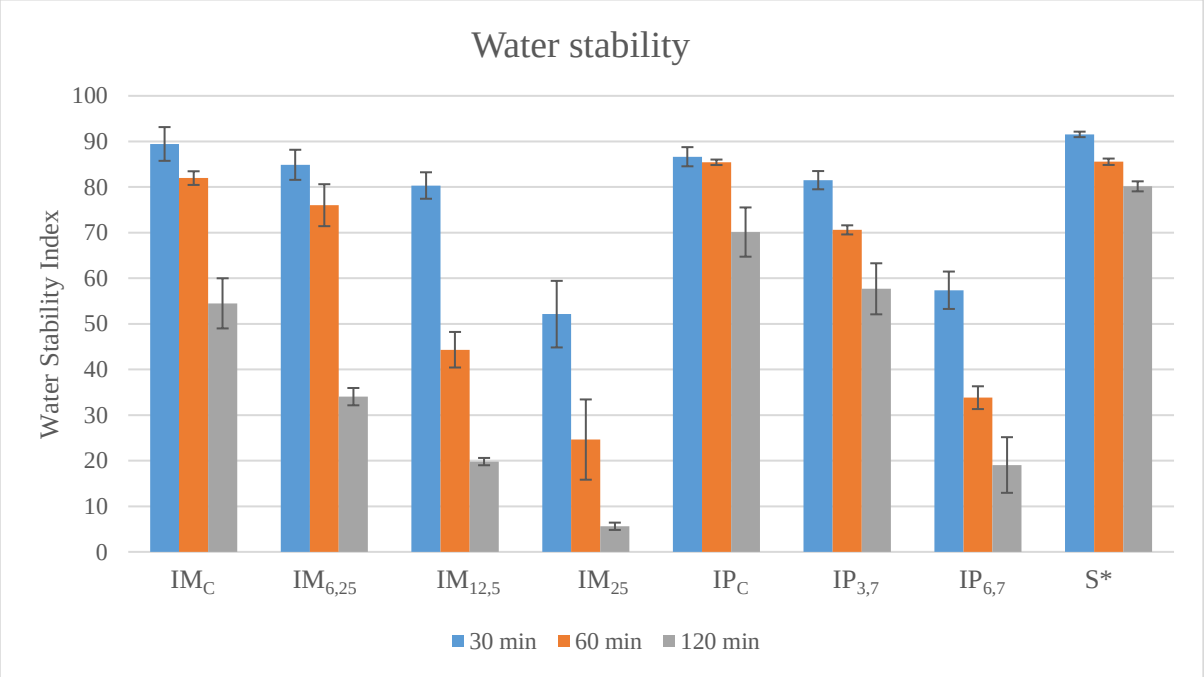

Figure 4-1 - Water stability index based on percentage loss due to time in the water bath..

*: skretting 3mm

4.3 Water holding capacity (WHC)

The WHC of SPC and wheat bran were higher than the other raw materials, as can been numerically shown in Figure 4-2 and Figure 4-3, whereas wheat flour had the lowest numerically value for WHC. For the material mixes, $IP_{3,7}$ and $IP_{6,7}$ show a decrease in values according to insect paste inclusion (Figure 4-4). IM_{25} shows a slight numerically decrease.

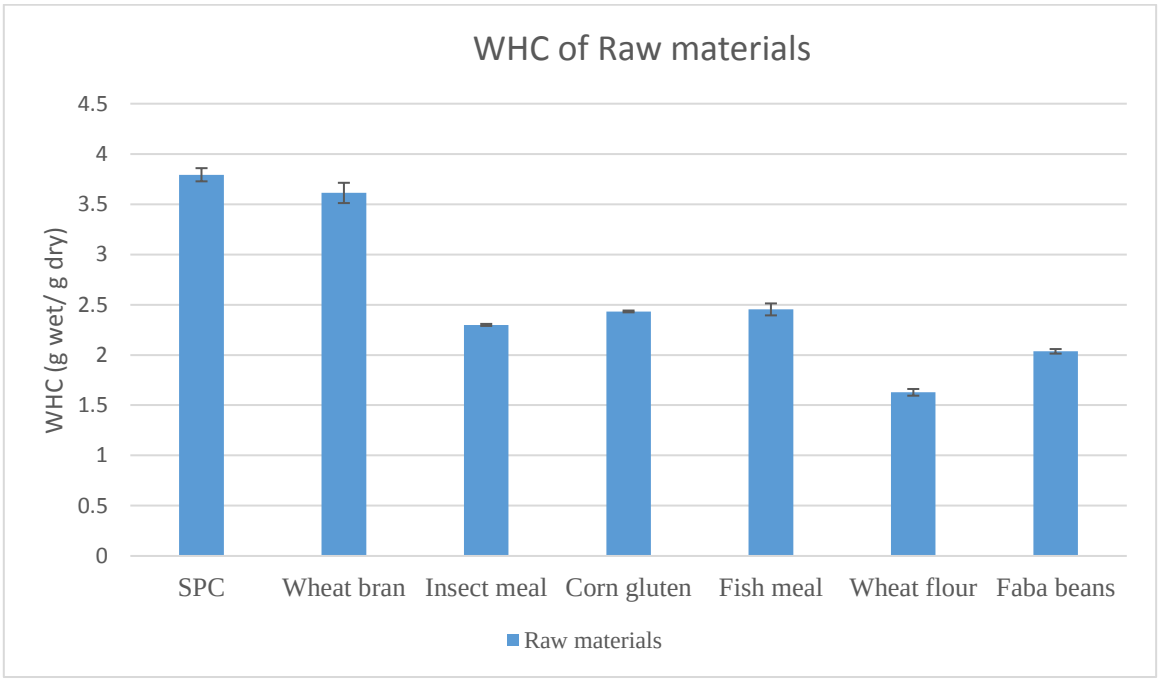

Figure 4-2 - WHC of the main raw ingredients used for diet formulation, except for insect paste.

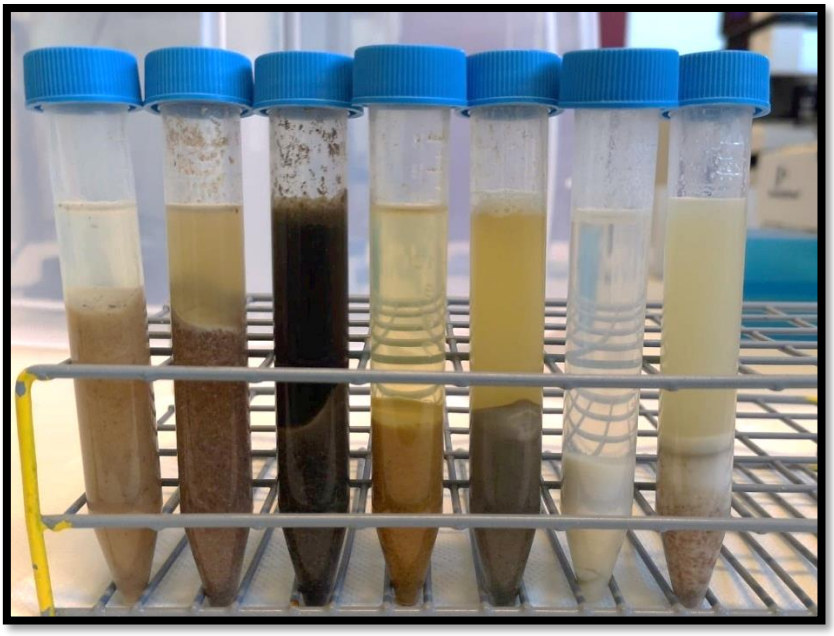

Figure 4-3 - Results for WHC of raw materials where we can visually see to a certain extent amount of water absorbed. From left to right: SPC, Wheat bran, Insect meal, corn gluten, fish meal, wheat flour, faba beans.

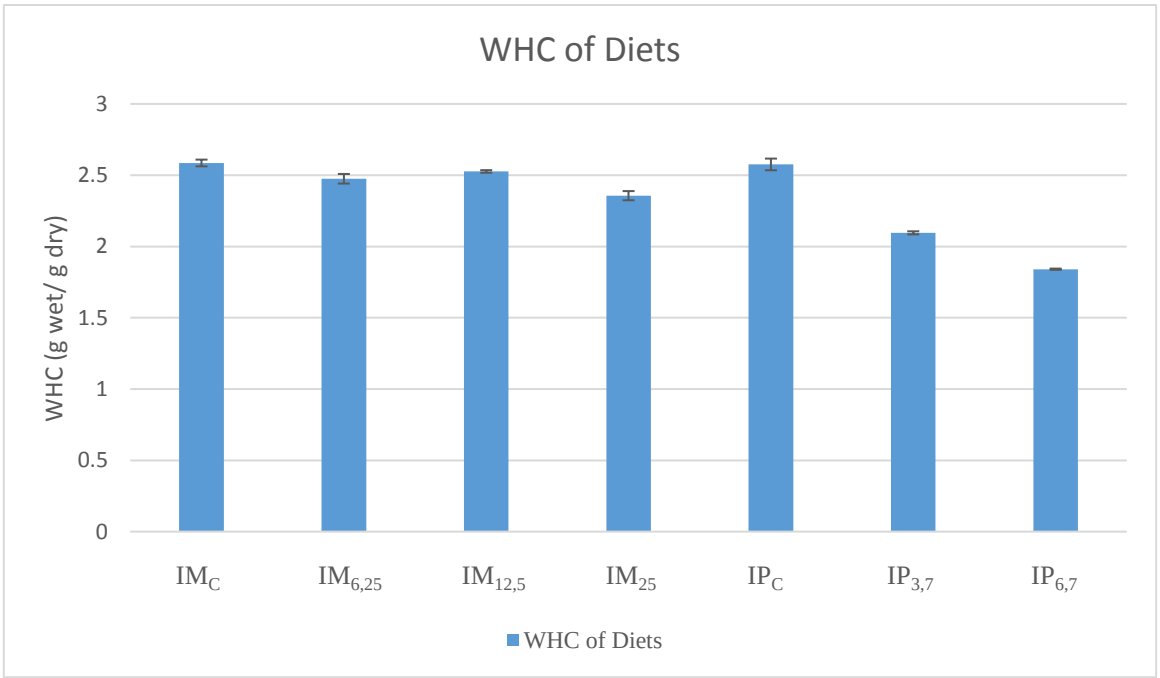

Figure 4-4 - WHC of the readily mixed diets as is.

4.4 Fish performance

4.4.1 Growth

The growth performance of fish fed experimental diets for 7 weeks are shown in Table 4-3. There was no significant difference observed among the dietary groups for any of the growth performance data, except for relative feeding rate. The relative feeding rate was significant (P<0,05) different between only IM_C and IP_C. A trend was observed showing the highest growth performance values in IM_C and $IP_{3,7}$, however, growth performance in IP_C was observed to be lower than the other diets especially on feed intake (P-value: 0,060). Increased FCR with increasing inclusion of either insect products was observed as a trend and shown in Table 4-3.

Table 4-3 - Growth performance in Atlantic Salmon fed increased levels of insect meal (IM) and insect paste (IP).

	IM_C	$IM_{6,25}$	$IM_{12,5}$	IM_{25}	IP_C	$IP_{3,7}$	$IP_{6,7}$	SEM	P-Value
Initial BW (g)	34,4	34,3	34,3	34,3	34,3	34,4	34,3	0,03	0,933
Final BW (g)	94,4	92,4	93,9	89,1	85,2	94,8	89,3	1,13	0,184
SGR	2,24	2,20	2,24	2,12	2,01	2,26	2,12	0,03	0,252
BW gain (g/fish)	60,0	58,1	59,6	54,8	50,9	60,5	55,0	1,13	0,199
CF (g/cm^3)	0,016	0,015	0,014	0,014	0,015	0,014	0,015	0,00	0,240
Feed intake	46,4	45,4	45,5	44,3	37,5	46,1	44,1	0,87	0,060
RFR	$1,81^{a}$	$1,79^{ab}$	$1,78^{ab}$	$1,78^{ab}$	$1,54^{b}$	$1,79^{ab}$	$1,77^{ab}$	0,03	0,042
FCR (g/g^{-1})	0,77	0,78	0,76	0,81	0,74	0,76	0,81	0,01	0,085
Mortality	0,56	0,00	0,00	0,00	0,00	0,00	0,00	0,08	0,463

SGR: specific growth rate (% body weight increase per day on average)

Feed intake: g feed/fish

RFR: Relative feeding rate (% body weight per day)

FCR: Feed conversion ratio (g/g^{-1})

SEM: pooled Standard error mean

CF: Condition factor

[ab] different groups explains significant difference (P<0,05).

4.4.2 Nutrient digestibility

The nutrient digestibility in fish fed experimental diets are shown in Table 4-4. The AD DM and energy was stable with increasing insect meal. Increasing levels of insect paste in diets are showing a trend in decreased AD DM and energy. The increasing level of insect meal shows a decrease in AD protein and AD lipid, but an increase in AD of starch. IP_C and $IP_{3,7}$ can be seen to have the highest AD values through all the nutrients in Table 4-4.

The decrease in apparent protein digestibility with increasing insect meal and insect paste is shown in Figure 4-5 and Figure 4-6 respectively.

Table 4-4 - Apparent nutrient digestibility in Atlantic salmon fed increasing levels of insect meal (IM) and insect paste (IP).

	IM_C	$IM_{6,25}$	$IM_{12,5}$	IM_{25}	IP_C	$IP_{3,7}$	$IP_{6,7}$	SEM	P-Value
AD of DM	66,6	67,1	66,3	66,3	70,9	70,4	67,4	0,56	0,068
AD of Energy	77,1	77,1	75.8	75,4	80,0	79,6	77,1	0,51	0,080
AD of Protein	$87,2^{abc}$	$86,9^{abc}$	$84,7^{bc}$	$83,3^{c}$	$89,6^{a}$	$88,9^{ab}$	$86,7^{abc}$	0,55	0,006
AD of Lipid	$95,2^{a}$	$94,6^{a}$	$91,3^{b}$	$88,0^{c}$	$96,0^{a}$	$95,3^{a}$	$94,5^{a}$	0,62	0,000
AD of Starch	$64,3^{b}$	$65,1^{b}$	$66,5^{ab}$	$70,4^{a}$	$63,2^{b}$	$66,3^{ab}$	$62,7^{b}$	0,60	0,000

^{abc}Different letter explains significance between groups on a significance value of $P<0,05$

AD: Apparent digestibility

SEM: pooled standard error mean

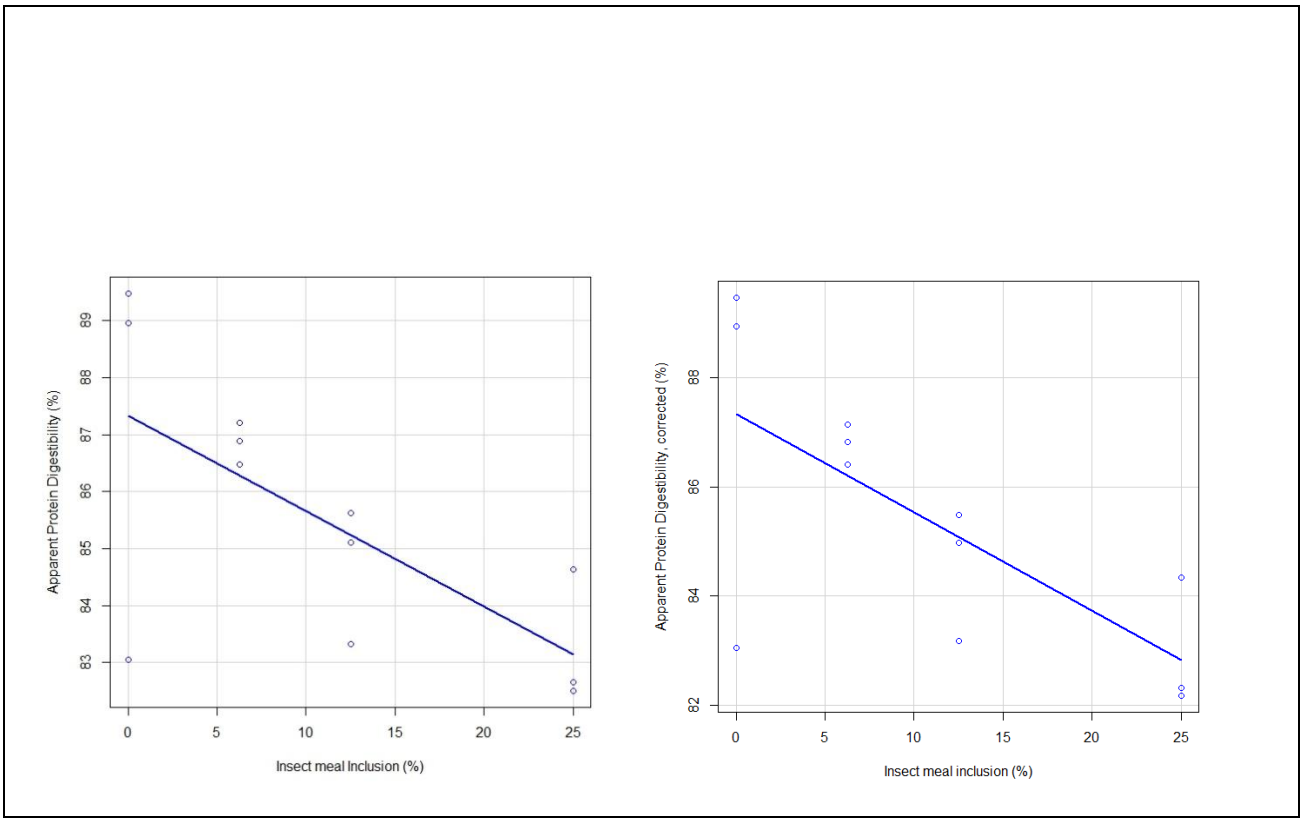

Figure 4-5 - to the left: Scatterplot with least square line of Apparent protein digestibility to Insect meal inclusion. P-value:0,015, R2: 0,46. to the right: Apparent protein digestibility corrected for the nitrogen in chitin, assuming nitrogen content in chitin is 7% and chitin content of BSFL is 6% P-value: 0,010, R^2: 0,50

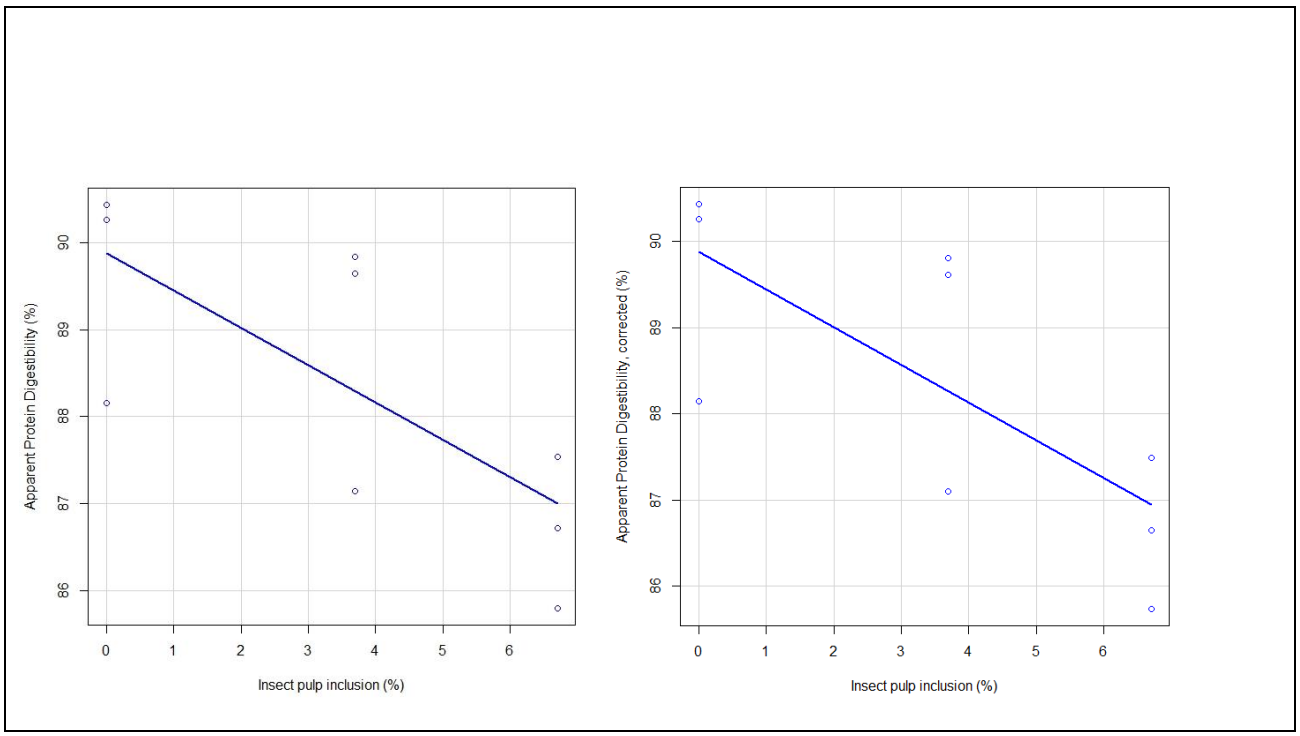

Figure 4-6 - to the left - Scatterplot with least square regression line on Apparent protein digestilibty to insect paste inclusion. P-value:0,02, R^2 : 0,54. To the right: Apparent protein digestibility corrected for nitrogen in chitin, assuming nitrogen content in chitin is 7% and chitin content of BSFL is 6%. P-value: 0,02, R^2: 0,54

5 Discussion

The present study focused to obtain the best possible result regarding pellet quality. Therefore, several parameters were changed according to observed physical dough and pellet quality/texture during production. Diet differences together with processing changes could make pinpointing causes and effects difficult.

Effect of feed ingredients on extruder parameters

High lipid level in insect meal increased level of lipid in mash prior to extrusion. This led to increased lubrification during extrusion and is in line with Hansen et al. (2011) and Lin et al. (1997). In the present experiment, the increased lubrification was compensated by increasing screw speed, mainly to obtain similar physical pellet quality between diets. Despite the increased screw speed, an increasing inclusion level of insect meal or insect paste decreased torque, temperature, die pressure and SME. In contrast to the present experiment, Hansen et al. (2011) compensated increased lipid levels in mash with decreased water level addition during extrusion. With this they obtained fairly similar extrusion parameters between diets, but changes were seen in pellet quality.

The effect of higher lipid addition was evident by inclusions of insect meal. Increasing inclusion levels of insect meal or insect paste showed to decrease torque, temperature, die pressure and SME. This is in line with, Aslaksen et al. (2007), that observed increased SME by ingredient replacement, due to reduced lipid content in the mash.

Flowability (Viscosity and lubrification), screw speed and throughput would affect residence time and potentially cooking time (Plattner, 2007). Increased screw speed would reduce residence time by increasing the conveying capabilities of the screws (Puaux et al., 2000). Throughput would be affected by resistance to flow, resistance provided by die, screw or mash composition and binding (viscosity and lubrification), thereafter the amount of backfilling in the extruder barrel. The extruder functions as a multi-functional pump. If the resistance at the die is high, the material would have to backfill to create high enough pressure in order to pass through the die, directly relating die pressure with backfilling (Forte & Young, 2016). With increasing level of lipid within the mash, there will also be an increasing level of lubrication. Lubrification, decreases torque which is a function of flowability (friction), barrel filling rate

(backfilling) and viscosity (Forte & Young, 2016). This would result in reduced backfilling (reduced residence time and cooking) as could be measured by die pressure and torque.

SME calculation does not directly take residence time into consideration, only through torque. Cooking of the material by mechanical energy through this calculation might not be accurate in high lipid diets as the residence time would be highly influenced. Increasing screw speed increases the SME linearly (Plattner, 2007), however, residence time of material with increased screw speed would be reduced. High lipid mash during extrusion processing would be less affected by mechanical energy (shear forces) as less friction would occur. This would reduce cooking as well as reduce residence time the material receives during processing. Diet $IM_{6,25}$ and $IM_{12,5}$ have similar SME, however, it could be argued that cooking time and residence time is reduced in $IM_{12,5}$ compared to $IM_{6,25}$. As could be seen by the reduction in die pressure, torque and increase in screw speed, all providing and leading to reduced residence time, with the same SME. Pellet quality measurements such as durability, hardness, expansion and water stability for $IM_{12,5}$ compared to $IM_{6,25}$ are lower and the temperature is slightly reduced. However, the slight reduction in temperature in between diets would not explain the large differences in pellet quality alone.

Torque as a function of viscosity which would also be affected by temperature, screw speed and mash composition due to the mash usually behaving as a shear-thinning liquid when entering a melt stage (Forte & Young, 2016). This could also affect the torque percent observed as well as the increased level of lipid in mash during extrusion. This is in line with Lin et al. (1997) which showed increased screw speed with increased lipid content (25, 50 and 75g/kg). This further decreased residence time and product temperature as a consequence of reduced conduction from the barrel wall and friction. However, Lin et al. (1997) observed a reduction in starch gelatinization with increased lipid content and moisture content, which are conflicting results from the present study. The main reason might be that Lin et al. (1997) performed a pet food extrusion with low water content (160-200g/kg) and high carbohydrate content (570-600g/kg). Therefore, major part of the gelatinization was based on mechanical breakdown (dextrinization) of the starch granules. Further addition of water may have functioned more as a lubricant than facilitating gelatinizing resulting in reduced gelatinization with increasing lipid and water level. The mash in the present study obtained on average approximately 31 percent water and approximately 12 percent starch during processing, which may explain that the gelatinization was less dependent on mechanical breakdown as supposed to, Lin et al. (1997). Lund and Lorenz (1984) reported that the ratio of water to starch needs to be 1.5:1 to achieve

complete gelatinization of starch. Furthermore, Hansen et al., (2010; 2011) also observed reduction in starch gelatinization due to increased lipid levels in the mash during fish feed production. Gelatinization of starch is highly related to digestibility of starch in carnivore fish (Krogdahl et al., 2005; Peres & Oliva-Teles, 2002).

However, the gelatinization observed in the present study did not seem to correspond with ingredient changes to the diet nor extrusion parameters. The odd pattern and large SEM value suggest that there were faults during the preparation and/or analysing of samples. Furthermore, the two diets with inclusion of insect paste had a similarly lower gelatinization when compared to all the other diets. However, $IP_{3,7}$ had the third highest AD starch and the lowest gelatinization. Further indicating that the gelatinization numbers are not very secure.

Effect of extrusion parameters on pellet quality

Different ingredients will highly influence extrusion parameters and physical pellet quality (Draganovic, 2013). Pellet quality measurements of both control diets in the present experiment (IM_C and IP_C) were similar to the commercial diet (S). However, the other diets that contained insect meal and paste showed different results.

Solubility of proteins in the mash can influence interactions during extrusion process in the melt stage. High soluble protein of mash can be less resistant to water and reduce water stability test in pellets. In the present study, water stability measurements were observed to be reduced with inclusion of insect products. This may be a function of low water solubility (approximately 20%) for the insects, especially in low pH (4-5) (Bußler et al., 2016), or as a function of high lipid levels which could reduce internal interactions.

Temperature in section 5 was cooled down during extrusion for all diets. For diet IM_{25} the temperature was purposefully reduced even more to lower expansion, due to breakage observed for $IM_{12,5}$, meaning IM_{25} would not be able to handle expansion with the temperatures similar to that of $IM_{12,5}$. The integrity of the pellets in diet IM_{25} reduced further and this might be due to short residence time in the extruder barrel, as the pressure at the die was only 1,35 Bar, 21% torque and a screw speed at 475 rpm. This produced pellets with reduced structural integrity since the pellets were slightly oval-shaped. This suggests reduced physicochemical changes of the material due to reduced cooking with increased insect replacement. This led to reductions

in pellet quality measurements such as durability, hardness, expansion and water stability with increasing insect replacements.

Garber et al. (1997) and Sørensen et al. (2009) showed an inverse relationship between expansion and hardness, and expansion and durability. This is in line with the results in the present experiment. However, if increased expansion was needed or desired, it would have affected the hardness, durability and bulk loss more severely as can be seen for $IM_{12,5}$. Morken et al. (2012) showed an increase in hardness and durability of fish feed pellets when adding potassium diformate. An increase in hardness and durability with addition of formic acid was observed in the present study, however only in the control diet with formic acid, IP_C. Furthermore, reduction in water stability of pellets were associated with an increase in hardness and durability with increased lipid level in the mash by inclusion of a partly deshelled krill meal and pea protein consecrate mixture (Hansen et al., 2011). This relationship was in accordance with the present study. The latter study speculated that the decrease in water stability can be associated with the increased protein solubility for krill meal compared to FM. Inclusion of full-fat soybeans in diets in Morken et al. (2012), showed no change in durability and hardness, but a decrease in water stability with increased temperature. The present study observed high variance within the diets for water stability measurements. This may be due to some differences within the diets, e.g. instability during extrusion thereof providing pulsation and reduced cooking for some of the pellets.

In the present study, the conditioning process was not used due to limited amount of test ingredients available as well as predicted problems during transport of mash with high water content. Loss of prewetting for 90 seconds and agitation provided through conditioning would possibly create a larger gap of material components access to water. In the present studies result of WHC, showed higher levels of binding for SPC and wheat bran compared to that of insect meal and wheat flour. Increased levels of NSP in the mash can deter starch gelatinization and reduce pellet expansion (Hansen & Storebakken, 2007). The latter article speculated that the main function of reduced expansion was due to lowered dietary starch, in diets with increased cellulose inclusion. Insect replacement in the present experiment was done by changing out all the other ingredients except for wheat flour and vitamin/mineral, as to maintain the starch level and fulfilling all nutrient requirements. This would shift the balance of WHC so that the starch in wheat flour might have access to more water and in turn could increase gelatinization (Samuelsen et al., 2013). The numerical decrease in WHC for IM_{25} compared to IM_C might be due to the reasons stated above or the difference in lipid content in mash. During processing of

diet IM_{25}, the moisture content was increased which in turn could further enhance the theory above. The material mash for $IP_{3,7}$ and $IP_{6,7}$ contained more water compared to the other diets. This was probably the reason of the differences for WHC in material mash. There is also a trend of increased gelatinization with inclusion of insect meal. However, the increased amount of included lipid during processing may have lowered the gelatinization and thereafter AD of starch. Lipid in mash would be able to coat and create a hydrophobic layer preventing annealing and gelatinization of the starch granules as well as reduce friction (the reduced SME observed) (Lin et al., 1997).

Palatability

Taste preference in fish is highly species specific (Kasumyan & Døving, 2003). The Atlantic salmon is a more selective feeder than other fish species such as rainbow trout (Kasumyan & Døving, 2003). The control diet including acid, IP_C showed the lowest feed intake among all diets. Furthermore, there was a trend (P=0,06) of reduced feed intake with increasing inclusion of insect meal or insect paste. In contrast, IP_C showed highest pellet quality measurement except for gelatinization, and higher nutrient digestibilities except for starch. The low feed intake for IP_C is possibly correlated to a low palatability score of the feed, which is strongly believed to be a result of the formic acid addition. This is in line with Morken et al. (2011) which observed varying, but reduced feed intake as a function of potassium diformate addition. However, the reason for not observing a similar reduction in feed intake in fish fed $IP_{3,7}$ and $IP_{6,7}$ could be questioned (lower level of acid, masking taste since it is a part of the mashed paste). Some interactions with the insect may prevent formic acid to reach the taste buds of the Atlantic salmon.

Lock et al. (2016), speculated that 2-thiobarbituric acid-reactive substances (TBARS) (which is a measurement for lipid oxidation) was the reason for the reduced feed intake observed by inclusion of insects to the feed. Insects that are heat-processed twice could have resulted in increased TBARS production. Lin et al. (1998), showed that extrusion with increased moisture and unsaturated fatty acids increased the TBARS during storage. TBARS could thus potentially be a reason for the negative trend of feed intake with inclusion of insect products, which were observed in the present trial. Belghit et al. (2018) did, however, not observe any negative effects on feed intake for Atlantic salmon even with elevated levels of TBARS due to insect meal inclusion.

Growth

Diets were balanced to obtain similar AA content by inclusion of insects for Atlantic salmon. The AA composition in insect is less digestible compared to FM as some AA may be bound to chitin (Finke, 2007). AA in BSFL is affected by the insects' diet, however, the distribution of AA is different to that of FM, and methionine and cysteine content are generally below the requirement for Atlantic salmon (Nogales- Mérida et al., 2018). Therefore, providing difficulties if FM were to be replaced fully by only BSFL. Belghit et al. (2018), showed no significant decrease in growth performance when replacing 85 percent of protein with defatted insect meal from the BSFL for Atlantic salmon. Replacement of FM with BSFL up to 50 percent did not reduce growth in Atlantic salmon (Lock et al., 2016). Replacement exceeding this increased FCR and reduced final weight and feed intake in the latter article. The present study showed a similar trend in FCR as the latter article, which was an increased FCR with increasing inclusion of insect products. Fish performance i.e. weight gain, SGR and final weight were observed to be stable with increasing replacement of insects, except for IM_{25}, IP_C and $IP_{6,7}$ which showed reduced values. IP_C performed poorly on all growth parameters expect conditioning factor compared to all other diets, with RFR being significantly different from IM_C.

Starch content in all the diets were sufficient as to not reduce protein utilization due to lack of glucose.

Reductions in growth for diet IM_{25} and $IP_{6,7}$ are most likely a function of the lowered digestibility of lipids and proteins observed in the present study.

Effect of temperature on AD

The present study received a large variation in barrel temperature in the extruder from diet to diet as a function of ingredients and changes done during extrusion. Sufficient amount of temperature during extrusion would be necessary to facilitate physicochemical processes and enhance digestibility in animals. Morken et al. (2012) showed an increase in barrel temperature from 110°C to 150°C increased AD lipid and protein in Atlantic salmon. However, a temperature of 103-137°C during extrusion did not change AD protein or energy in rainbow trout (Sørensen et al., 2005). This is in line with, Barrows et al. (2007) which showed no effect on AD protein,

lipid, carbohydrate or energy in rainbow trout with changing extrusion temperature (93℃ and 127℃) or residence time (18 seconds and 37 seconds) in the extruder barrel. The present study might have benefitted by increasing the temperature, as the extrusion barrel temperature reached a peak at 118℃, which may have lowered some of the negative effect on protein and lipid digestibility observed. The reason for no effect of residence time in Barrows et al. (2007), can be linked to the use of a conditioning process. AD of protein in fish fed IM_C, $IM_{6,25}$ and $IM_{12,5}$ showed a steady decrease with inclusion of insects, as the temperature was only slightly lower with inclusion.

Effect of insects on digestibility

The major difference when substituting fish oil with insect lipids would be the change in lipid distribution in the diets. Insect lipids are high in SFA such as lauric acid (C12:0) and low in PUFAs (EPA and DHA) compared to fish oil, with higher levels of PUFAs. Ng et al. (2004) showed a decrease in lipid digestibility in Atlantic salmon with lipid replacement (rapeseed oil with crude palm oil which is high in C16:0). A significant reduction was observed when inclusions were increased from 10% to 25%. The present study observed a significant reduction in lipid digestibility with insect replacement. However, Lock et al. (2016), noted that lauric acid was highly digestible in Atlantic salmon. Stadtlander et al. (2017) speculated that high digestibility of lauric acid was the reason for the improved lipid utilization seen when replacing FM with BSF meal in rainbow trout. The diets in Stadtlander et al. (2017) were, however, not optimal in protein:energy ratios or total lipid content (15%). This may have been the main reason for differences in utilization of protein and lipid. This should, however, not affect digestibility in the present study as the amounts of PUFAs (acceptable levels of fish oil) and the protein:energy would have been acceptable.

NSP such as Chitin (β1,4-linked polymer of N-acetyl-D-glucosamine) showed that at even small levels could negatively affect growth and AD lipid in fish as well as decreased faecal DM (Hansen et al., 2010; Olsen et al., 2006). As the Atlantic salmon have poor abilities to digest chitin (13-40%) (Olsen et al., 2006). Karlsen et al. (2017), showed a significant decrease in growth and reduction in AD of protein and lipid, however, it was speculated that the decrease in AD of protein was related to the nitrogen content in chitin providing incorrect negative correlation. The present study considered this by correcting for the amount of nitrogen in chitin in mash. However, chitin has a lower digestibility than protein, therefore, accumulation of chitin

in faeces might occur. The nitrogen was measured by Dumas analysing method and used to calculate crude protein. Higher accumulation of nitrogen from chitin due to the lower digestibility might have provided an incorrect negative reduction in apparent protein digestibility observed in the present study. Decreased faecal DM with increased NSPs from soybean in the diet for fish, can result in increased drinking as a consequence. Perhaps to reduce the viscosity and correcting osmotic balance (Kraugerud et al., 2007; Refstie et al., 1999). In the present study, it was observed that several fish had increased moisture content of the faeces (diarrhea) during stripping, however, distinguishing a trend was difficult. The dietary chitin could, however, have a positive effect on health for the Atlantic salmon by altering the gut microbiota in the distal intestine (Askarian et al., 2012).

Nordrum et al. (2000), showed that an increased inclusion level of sulphur containing ingredients, increased taurine in plasma which increased AP lipid in Atlantic salmon. The decrease in AD lipid with increasing insect product could be associated with a reduction in taurine levels affected by the replacement (Krogdahl et al., 2003). As insects have low levels of sulphur containing AAs and no taurine detected. Which could potentially have reduced AD lipid in the present study. The present study supplemented with methionine to achieve satisfactory levels. The levels of methionine should be above any negative affect to lipid digestion even with reduction of taurine in mash as a result of replacement of FM with insect meal. However, Belghit et al. (2018), speculated that their study did not supply sufficient amount of methionine to compensate for the reduced taurine levels in insect meal, resulting in reduced digestion of lipid. The present study had a similar amount of methionine (approximately 10,8 g/kg) to the latter article (approximately 10-11 g kg^{-1}). The latter article replaced a maximum of 85 percent of the protein (FM and SPC) with insect meal compared to the present study were a maximum of 25 percent of the protein was replaced with insects. Therefore, lack of taurine would be more severe in Belghit et al. (2018) than the present study.

Different parts of BSFL can be fractionated into more manageable part for feed production, i.e. protein-rich fraction, lipid and/or chitin. A protein-rich fraction of the insects are often used in studies when replacing e.g. FM with insect meal (Belghit et al., 2018; Magalhães et al., 2017).

Reduction in digestibility of starch with increasing insect meal in Atlantic salmon was expected. As temperatures, SME and residence time were lowered with insect inclusion, hence lowering energy input into the mash. This was expected to cause lower gelatinization, thereby the digestibility. However, starch digestibility was significantly increased with inclusion of insect meal. The observed increase in apparent starch digestibility of processed high lipid mash is not

in line with the literature (Hansen, 2011; Lin et al., 1997), which showed to decrease with increase lipid in mash. The reason could be related to WHC. The balance of WHC might be affecting the starch more than the lipid inclusion. This pattern is, however, not as easily seen in insect paste inclusion, as IP_C has the lowest apparent starch digestibility when compared to $IP_{3,7}$ and $IP_{6,7}$. It could be a result of the formic acid additions to the diet, as well as mixing. Highly water-soluble acid could have been mixed more homogenous compared to the insect paste addition which formed small lumps during mixing. The formic acid could decrease starch gelatinization by reducing the hydrophilic properties of starch molecules (Morken et al., 2012). This could explain the low gelatinization and AD starch level of the IP diets.

The significant difference of AD starch with increasing inclusion of insect meal could be due to the amount of dietary starch. The Atlantic salmons will have reduced digestibility of starch with increasing levels in the diet. (Krogdahl et al., 2004). The total amount of dietary starch for IM_{25} and $IM_{12,5}$ were equal (115g/kg) compared to that of IM_C (123 g/kg). This did, however, not seem to follow a trend in AD starch as the effects corresponds to other changes such as insect meal inclusion and extrusion parameter rather than dietary starch content.

Effect of formic acid on AD

Formic acid is commonly used as a preservative of moist ingredients, such as fish silage or in the present study, insect paste. Organic acids such as formic acid could lower gastric pH, which in turn may increase the proteolytical activity and inhibit bacterial growth (Morken et al., 2011). Lückstädt (2008) showed to an increase in AD of protein, DM and energy in Atlantic salmon when potassium diformate was added prior to extrusion. The present experiment showed elevated levels of digestibility of energy, DM, protein and lipid in IP_C and $IP_{3,7}$. However, the highest inclusion of formic acid, $IP_{6,7}$, showed lower digestibility levels. $IP_{6,7}$ had similar digestibility as the control diet without formic acid, IM_C.

However, IP_C and $IM_{6,25}$ had similar extrusion parameters and suggest that the difference in AD came from other factors than extrusion parameter, for instance the ingredients. IM_C and IP_C had the same ingredients except formic acid would suggest that differences in AD could be that of formic acid (due to acid hydrolysis) or processing parameters.

6 Conclusion

The increasing lipid content in mash by insect inclusion i.e. insect meal or insect paste, resulted in decreased SME, temperature, torque and die pressure, which in turn decreased residence time and cooking time during extrusion. This lowered the pellet quality measurements i.e. expansion, water stability, and durability. Moreover, the formic acid addition in the IP_C diet increased pellet hardness and durability.

Increasing inclusion of insect meal significantly reduced AD of protein and lipid and significantly increased apparent starch digestibility. The reduction in digestibility is believed to be an effect of chitin content. The increase in starch digestibility is believed to be related to WHC, starch granules having access to increased water during extrusion processing, which in turn provided a higher degree of gelatinization.

However, partial replacement of protein and lipid with full-fat BSFL either as a meal or paste did not affect growth in Atlantic salmon.

7 Concluding remarks and improvements

Replacing ingredients will change extrusion parameter due to different functional properties and nutrient composition. It would, therefore, behave differently when exposed to the environment created by the extruder. This does, however not mean that the ingredient would perform poorly on digestibility or pellet quality, but would require a different processing approach. When doing trail with ingredient replacement as the present experiment it is important to consider this, that new material mix would require a different processing approach. Therefore, to conclude that the ingredient is not suitable for the given species of animal or process, further testing would be necessary.

With this in mind some immediate changes could help optimize the insect meal for fish feed based on the results of the present experiment: The pellets for IM_{25} were observed to be oval-shaped. The reason might be due to excessive water use and therefore soft, or reduced amount of cooking within the extruder reducing the structural integrity of the pellets. Perhaps including a better binder would have solved some of the problems, replacing the corn gluten with wheat gluten and/or some other digestible binder. Wheat gluten might, however, not have been able to form its complex structure due to the low SME and residence time during production with high lipid mash. Reason for excluding wheat gluten in the present study was concerns related to expansion, and oil holding capacity (Draganovic et al., 2013). As the high lipid content in the diets would reduce expansion (Hansen et al., 2011; Morken et al., 2012), wheat gluten was changed with CGM. Other binders or modified wheat gluten such as hydrolysed or devitalized gluten could have been an option. Wheat gluten is sought after due to its high protein digestibility for fish and therefore has two functions: high-quality protein ingredient as well as a structural binder.

An increase in temperature optimally by conditioning and perhaps steam injection into the extruder barrel could enhance the pellet quality in the present study. Increased expansion due to increased temperature might occur, and the cooling in section 5 would perhaps not be enough to limit the expansion. Venting of steam in section 4 could have reduced this expansion and increased temperature. However, this was not possible at the time of production.

Changing the screw configuration in the two highest inclusions of insect meal, by implementing one or two reverse screw elements (20L). As well as removing the 90º offset on the present 20L screw elements could have been an option of improvement. This would significantly increase

resistance to flow of the material, perhaps increasing residence time and kneading the material receives. However, this might be in excess of what is possible and might end up blocking the extruder.

Considering AA profile, fatty acid profile and NSP more closely could have determined if there were any other effects on reduced protein and lipid utilization. As well as determining the chitin in diet and faeces would be beneficial to observe the effect of miscalculation on apparent protein digestibility measurements.

Complete replacement of the protein in diets for fish with insects has potential, however, more than one species of insect may be needed to obtain AA requirements (Nogales- Mérida et al., 2018).

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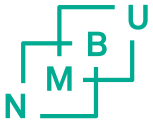

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