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The effect of black soldier fly (*Hermetia illucens*) larvae fractions in diets for Atlantic salmon (*Salmo salar*) on extruder parameters, pellet quality, growth performance and nutrient utilization

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Master of Science in Feed Manufacturing Technology

Abstract

This study focused on the effects of dietary inclusion of black soldier fly larvae (*Hermetia illucens*) fractions on extruder parameters, pellet quality, growth performance and nutrient digestibility and utilization in Atlantic salmon (*Salmo salar*). The different types of diets used were full-fat, defatted, dechitinized, BSFL oil and exoskeleton diets. For the diets containing BSFL meals, 15% of the dietary protein was replaced with insect protein. BSFL inclusion of full-fat and dechitinized BSFL in the diet led to low energy inputs during extrusion due to high lipid content in the insect, which resulted in lower durability, hardness, expansion and water stability of the pellets. In the fish experiment, 900 Atlantic salmon of 28 g initial weight were distributed in 18 tanks and fed with one of the diets for 62 days. The final body weight, body weight gain and specific growth rate did not differ in fish fed defatted, BSFL oil and exoskeleton diets compared to the control diet, whereas, they were higher in full-fat and dechitinized diets. Feed intake was similar among different BSFL diets and control diet, except higher for fish fed full-fat BSFL diet than other diets. However, feed conversion ratio was similar among fish fed BSFL diets and control diet. Apparent digestibility coefficient of dry matter, ash, starch and lipid were not affected by the dietary treatments whereas protein digestibility was lower in fish fed with defatted, dechitinized and exoskeleton diets compared to control diet. The apparent protein retention was not affected by dietary treatment, whereas the fish fed defatted BSFL obtained higher protein efficiency ratio compared to the control fed fish. In contrast, all BSFL diets had lower lipid efficiency ratio and apparent lipid retention than control diet, whereas lipid retention of fish fed dechitinized and control diet were similar. Phosphorus retention were similar among the diets except for defatted BSFL diet, which had higher retention of phosphorus compared to the control diet.

Overall, growth performance of BSFL diets in Atlantic salmon was either better or similar to control diets whereas, lipid efficiency and lipid retention was reduced, except for dechitinized diet which showed similar lipid retention to control diet. Protein digestibility, on the other hand, was reduced by defatted, dechitinized and exoskeleton diets whereas, phosphorus retention was improved by defatted diet.

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Abbreviations

AA	=	Amino Acid
ADC	=	Apparent Digestibility Coefficient
ADF	=	Acid Detergent Fiber
ANF	=	Anti-nutritional Factor
BSF	=	Black Soldier Fly
BSFL	=	Black Soldier Fly Larvae
BWG	=	Body Weight Gain
DI	=	Distal Intestine
DM	=	Dry Matter
EAA	=	Essential Amino Acid
ES	=	Esophagus
FA	=	Fatty Acid
FBW	=	Final Body Weight
FCR	=	Feed Conversion Ratio
FI	=	Feed Intake
FM	=	Fish meal
GIT	=	Gastrointestinal Tract
LER	=	Lipid Efficiency Ratio
MCP	=	Monocalcium Phoshphate
MI	=	Mid Intestine
N	=	Nitrogen
NSP	=	Non-soluble Polysaccharides
P	=	Phosphorus
PER	=	Protein Efficiency Ratio
PI	=	Proximal Intestine
SBM	=	Soybean Meal
SFA	=	Saturated Fatty Acid
SGR	=	Specific Growth Rate
SME	=	Specific Mechanical Energy
SPC	=	Soy Protein Concentrate
ST	=	Stomach

Chapter 1

Introduction

Fish is one of the most consumed food worldwide and its consumption continues to increase. The world population is expected to increase by 2 billion by 2050 and fish is a healthy and a sustainable protein choice to meet this demand (ISFA, 2018). The global fish production was approximately 177.8 million metric tons in 2019 (Shahbandeh, 2020). Along with the increased demand, the production is also expected to increase gradually in the coming years, as a consequent, there is also a higher demand for fish. According to a global feed survey carried out by Alltech (Norrie, 2019), fish feed industries showed an annual growth of 4%.

Generally, fish feeds are produced through extrusion technology which is a cooking and shaping process in the presence of moisture, high pressure and high temperature. Technical quality of the final feed product does not only depend on the extrusion parameters, but also on the source of feed ingredients used. Feed ingredients determine the input cost of the feed manufacture industry and it generally accounts for more than 50% of the total feed cost (Iversen et al., 2020). Prices of these ingredients are mostly volatile, therefore, choosing a suitable ingredient is very crucial. The shift towards more farmed fish and decreased wild fisheries has decreased the availability of fish meal (FM) and fish oil for fish feed, thus sustainable, nutritional and economical alternatives are needed. Plant ingredients such as soyprotein concentrate (SPC) that are rich in protein are also used as alternatives to FM, however, they contain anti-nutritional factors (ANF) such as phytates, tannins, trypsin inhibitors and oligosaccharides (Adeyemo & Onilude, 2013) and can cause negative effects on fish health such as inflammation in the digestive tract, reduced feed intake and growth rate and reduced nutrient digestibility (Merrifield et al., 2011). The ecological footprint of these plant ingredients and the resources required for their production such as energy, land and water make them less sustainable.

Insects, on the other hand, are considered a potential alternative in aquafeed because they are rich sources of proteins (42-63% crude protein), lipids (upto 36% lipid), vitamins and minerals (Makkar et al., 2014). They are considered sustainable compared to FM and fish oil as they need less land and water for their cultivation and generates less carbon dioxide compared to other animals. EU (2017) allows using processed animal protein from various insects for use in aquaculture feeds, however, this is only limited to the use of plant based substrates to produce insects. Within EU, seven insect species are allowed to be used in aquaculture; common housefly *(Musca domestica)*, yellow mealworm *(Tenebrio molitor)*, lesser mealworm *(Alphitobius diaperinus)*, house cricket *(Acheta domesticus)*, banded cricket *(Gryllodes sigillatus)*, field cricket *(Gryllus assimilis)* and black soldier fly (BSF) *(Hermetia illucens)*. Black soldier fly larvae (BSFL) are promising feed ingredient as they are efficient in converting organic wastes into high quality protein (Belghit et al., 2019a). They are good source of proteins (40-45%) and lipids (30-35%) (Rana et al., 2015) and has a well balanced amino acid (AA) profile (Belghit et al., 2019a).

Numerous studies investigated the effect of inclusion of BSFL as an alternative protein sources in fish diets (Belghit et al., 2019a; Fisher et al., 2020; Kroeckel et al., 2012; Lock et al., 2014; Makkar et al., 2014). Some of the experiments has only replaced upto 30% of the protein content with insect meal (Fisher et al., 2020) whereas some has replaced upto 100% FM (Kroeckel et al., 2012). Previous results have shown that insect proteins are nutritionally suitable proteins for aquatic feeds. In a previous study done by Weththasinghe et al. (2021), different inclusion levels of BSFL meal and paste were evaluated in diets for salmon. Inclusion of 12.5% of insect meal and 6.7% of insect paste resulted in better growth performances compared to a control based on FM and plant protein sources. However, lower protein and lipid digestibility were found with higher BSFL meal and paste level, apparently because of adverse effects of chitin on digestibility. Therefore, in this present study, the focus is to use differently processed BSFL, namely full-fat BSFL, defatted BSFL, dechitinized BSFL, BSFL oil and exoskeleton in diets for Atlantic salmon *(Salmo salar)* to follow up the results of Weththasinghe et al. (2021) and to study how the dietary inclusion of high levels of BSFL fractions can affect growth performance, nutrient digestibility and utilization in salmon. The second aim is to study the effects of these fractions on extruder parameters and pellet quality.

Chapter 2

Background

2.1 Salmon

According to ISFA (2018), 2.5 million tonnes of salmon are produced annually which provides 17.5 billion salmon meals per year, 132,600 jobs and accounts to 15.4 billion USD annual production globally. Salmon is considered one of the energy efficient animals as it releases less amount of greenhouse gases compared to some other animals. Carbon footprint of salmon is similar to that of chicken (2.9 and 2.7 kg,CO_2/kg edible product respectively) but lower than pork and beef (5.9 and 30.0 kg,CO_2/kg edible product respectively). Other factors that make salmon efficient are: they have high fillet yield (60%), their feed production releases lesser greenhouse gases than that of livestock, their conversion of feed into growth is high and they use less amount of fresh water (ISFA, 2018). In 2015, only 5 countries accounted to 95.6% of the total global production, Norway being the largest producer (55.3%). Norway has been the main salmon producer since early 2000 and Norway's production share has been increasing steadily (Iversen et al., 2020).

Atlantic salmon are naturally found in the northern hemisphere. They can be divided into 2 races: West-Atlantic (North American) and European. They are anadromous, meaning they migrate from rivers to sea to feed and then up rivers from the sea to spawn. They require freshwater for spawning and development of early stages (OECD, 2017). Smolts move to sea from rivers mostly during spring. To adjust for sea water, they undergo physiological, behavioral and morphological alterations. For instance, change in body color, increase in ATPase (Adenosine Triphosphatase) production, etc. For spawning, they return to the river and find a suitable site (OECD, 2017). Life cycle of a salmon is shown in Figure 2.1.

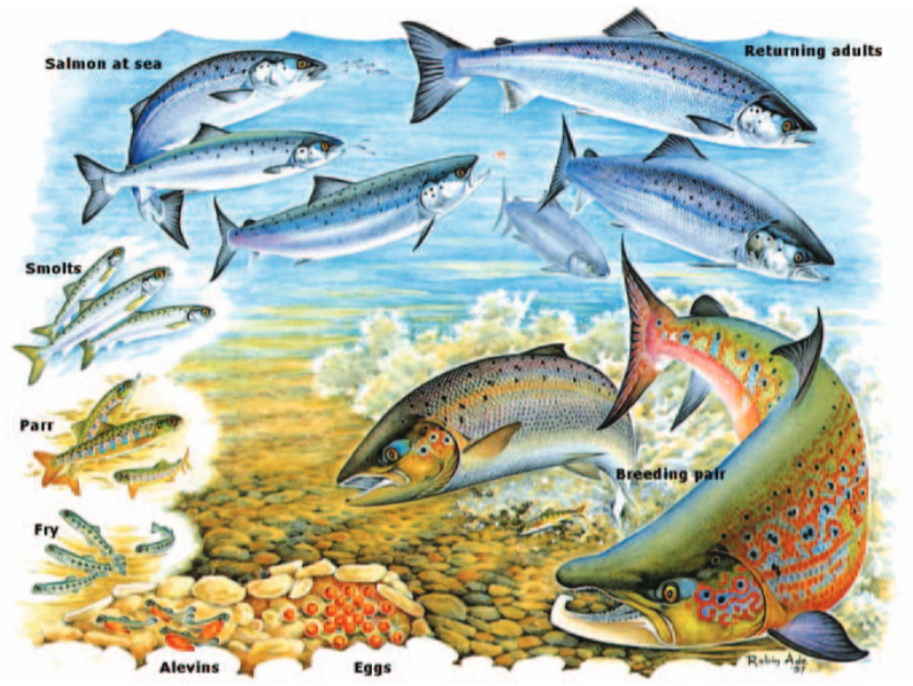

Figure 2.1: Life Cycle of Salmon (Sahlmann, 2013)

2.1.1 Digestive System

The digestive tract of a salmon can be divided into mouth, esophagus (ES), stomach (ST), proximal intestine (PI) with adjacent pyloric caeca, mid intestine (MI), distal intestine (DI) and rectum (Figure 2.2). The gastrointestinal tract (GIT) of a salmon is about 0.8 times the body length, excluding the pyloric caeca. Regulation of feed intake, digestion, absorption and osmoregulation are principle functions of GIT and it is carried out with the help of endocrine cells of the GIT and chemical signals from the corresponding organs (Takei & Loretz, 2010). Yolk sacs attached to ST are present only in the alevins to provide necessary nutrients for their survival until their digestive tracts become fully functional and are ready to intake feed. Protein and lipids comprise the major part of their diet as they are carnivorous. ST and intestine are important for optimal digestion whereas pyloric caeca is for lipid digestion and nutrient absorption (Denstadli et al., 2004; FAO, 2020; NRC, 2011).

Atlantic salmon has small teeth which are usually 4-6 in number and are well developed. Its mouth is quite large and has a pointed and narrow tongue (Fuller et al., 2019). Function of mouth along with the sharp teeth is to assist in catching prey. Mouth also helps in gulping of air, maintaining balance of the swim bladder and in oxygenation of body tissues by helping it to move water over its gills (Federation, 2017). Salmon suck their prey in along with

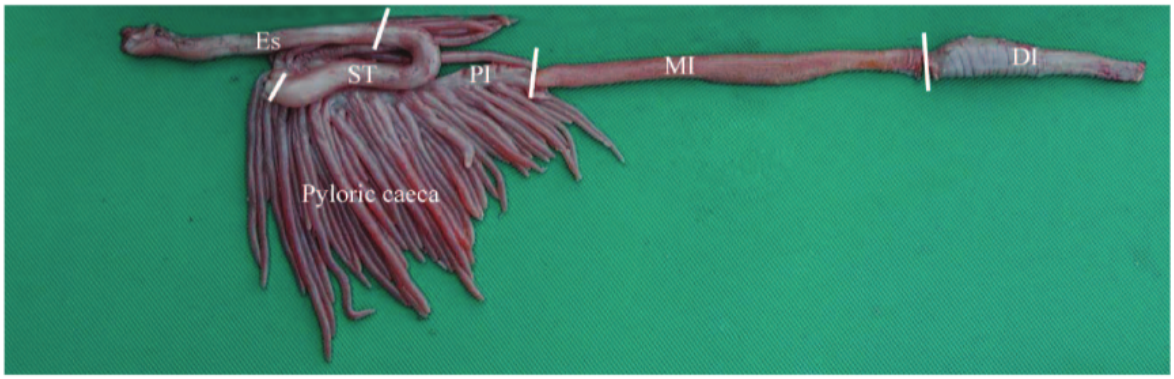

Figure 2.2: Gastrointestinal Tract of Salmon (ES=esophagus, ST=stomach, PI=proximal intestine, MI=mid intestine, DI=distal intestine) (Sahlmann, 2013)

water (NRC, 2011). They select their prey through their vision, however the final prey is decided according to taste (Mearns et al., 1987). Their tongue has the ability to sense taste and is sensitive to some chloride salts, minerals and organic acids but it is insensitive to AA and simple sugars (Sutterlin & Sutterlin, 1970). ES is a straight, thick walled tube with longitudinal folds and its function is to pass food from mouth to ST (Wilson & Castro, 2010). In ST, pepsin in acidic conditions initiates the digestion. When proteins, lipids and carbohydrates reach the PI, trypsin, chymotrypsin, elastase, lipases and α-amylase (from pancreas), and bile from liver are released (Holmgren & Olsson, 2009). pH of ST is 4.5 but it changes to 8 in PI because of the release of bicarbonate and mucus from pancreas and bile (Nordrum et al., 2000). The final digestion of lipids and carbohydrates is carried out by brush border membrane enzymes. PI has finger-like blind-ended projections called pyloric caeca which increases the surface area of PI. Nutrient absorption takes place in the PI with the attached pyloric caeca and also in the DI but in lesser degree (Sahlmann, 2013). DI is the principle site for osmoregulation and secretion of electrolytes (FAO, 2020). It is also able to digest and absorb large molecules such as intact protein (Sire & Vernier, 1992).

2.2 Fish Feed Processing

Fish feed can either be produced by pelleting or extrusion depending upon its physical requirements and target species. Pelleting is simply converting of ground mesh into pellets with the use of moisture, heat and mechanical pressure (Farahat, 2015). Alternatively, extrusion is not only forming the pellets but also cooking them, therefore, requires more

moisture, heat and pressure than pelleting. Pelleted feeds are usually cheaper than extruded feeds, however, extrusion is more frequently used because it can produce any kind of pellets (sinking, floating, buoyant, etc.) depending upon the target species. Pelleting usually results in rapid sinking pellets with lower water stability and less expansion (less the expansion, less the absorption of lipids while coating). Extruded feed results in better water stability, better nutrient retention, and high digestibility, efficiency and conversion rate (Derwent, 2019).

A general fish feed extruder line is shown in Figure 2.3. The main steps involved in fish feed extrusion processing are: receiving, storage, grinding, mixing, conveying, extrusion cooking, drying/cooling, coating, and packaging. Raw materials intake should be 2-3 times more than the production capacity to avoid disruptions in production (Zhenhua, 2020). Grinding of raw materials is very important to improve mixing properties, facilitate conditioning process and better handling of the ingredients. Other benefits of grinding are removal of some moisture, better amalgamation of additives, reduction of clumps, increase of water stability of pellets, feed digestibility, bulk density and better palatability. Proper grinding of ingredients helps in lowering the energy consumption of the feed-mill as well (Hasting & Higgs, 1980; Zhenhua, 2020).

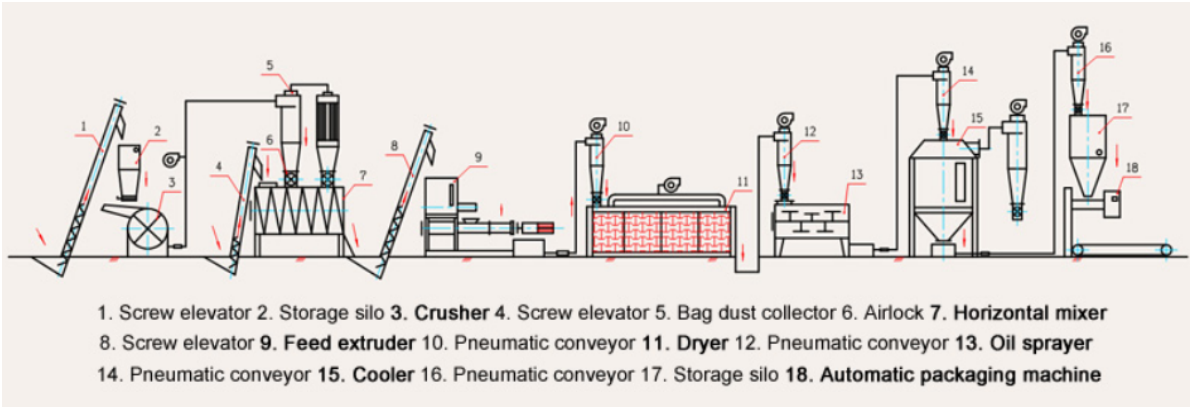

Figure 2.3: Fish Feed Extruder Line (Bridge, 2015)

2.2.1 Extrusion

The first commercial extrusion process of food and feed was performed almost 60 years ago. Extrusion has become the most used cooking process in the commercial aquatic feeds in the recent times (Rokey et al., 2010). Extrusion can simply be defined as a process that cooks the raw material under pressure by passing through a specially designed opening which

allows it to gain a definite shape. Extrusion process is called HTST (high temperature short time) process as the temperature during cooking can reach upto 200°C and the time is usually 5-10s. There are several conditions in the extrusion process that can be controlled to achieve desired results which are mostly interrelated. These conditions include flow rate of the ingredients, particle size of the ingredients, total moisture addition in preconditioner and extruder, retention time of preconditioner and extruder, temperature of barrel in the extruder, configuration of screw elements of extruder, shape and size of die, and time, temperature and air velocity of dryer (Serrano, 1997). A general extruder system is shown in Figure 2.4.

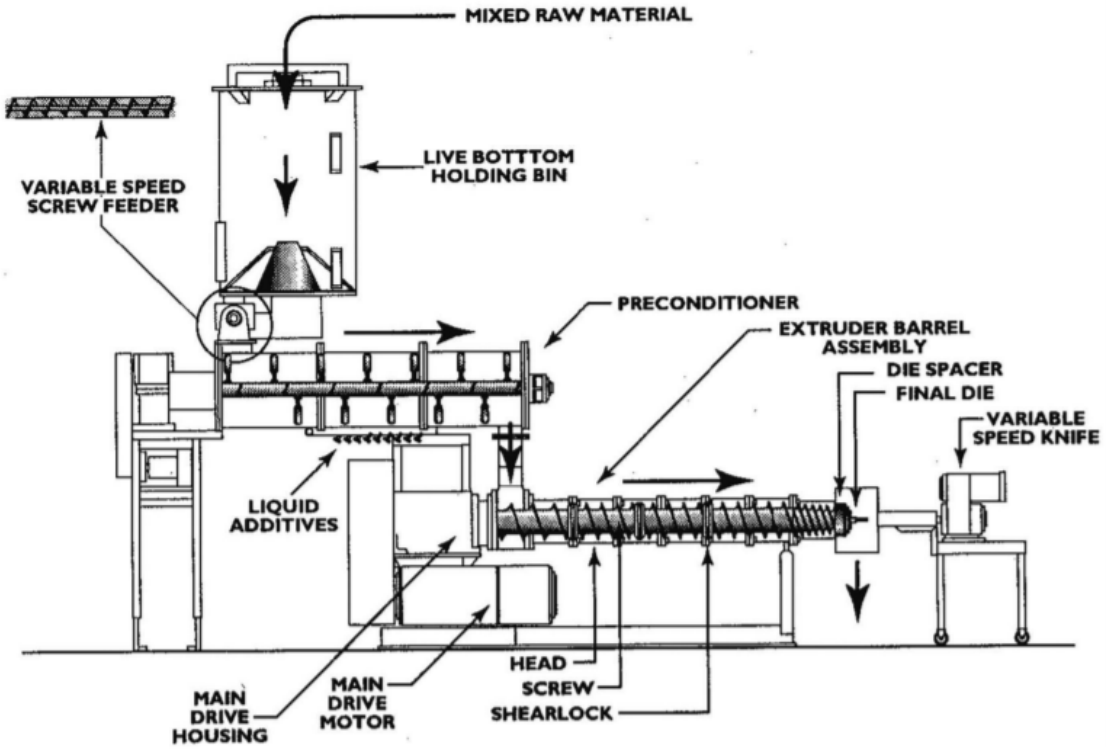

Figure 2.4: A General Extruder System (Fang & Hanna, 2010)

An extruder consists of a feeder, preconditioner, extrusion cooker and die. The function of the feeder is to uniformly pass the ingredients to the preconditioner and then to the extrusion cooker. It controls the product rate of the system. The flow of the feeder should not be interrupted. Before extrusion, ingredients must pass through preconditioner for moisture addition. This helps in achieving better quality of the final product and reduces abrasion of the extruder. Here, moisture is introduced in the form of water and/or steam and moisture content of the product after preconditioner is 10-25%. Water is added from the top of the preconditioner through spray nozzles whereas steam is added from the bottom. Nozzles aid in minimizing mixing load of the preconditioner by atomizing the water stream. Steam

should be continuous and condensate free. Another type of preconditioner is the pressurized preconditioner which provides high discharge temperature but has high potential of nutrients destruction and high operation cost. Using steam alone increases the moisture by 5-7%, therefore, to achieve higher moisture content, water should be used. Steam and water are usually balanced in the preconditioner to obtain a temperature of 70-90°C. Liquid such as fat is added near the end of preconditioner because fat can cover feed particle and decrease gelatinization by reducing moisture absorption and thermal energy transfer (Rokey et al., 2010). Starch gelatinization is an irreversible process of swelling of starch granules and disruption of the crystalline regions due to heat and moisture which allows more water absorption. Starch undergoes structural changes during extrusion such as gelatinization, melting and fragmentation. Factors that influence these changes are shear forces, residence time, shear rate, temperature, screw speed, amylose to amylopectin ratio and moisture content of ingredient used. When there is limited moisture, complete gelatinization does not occur. When temperature increases, starch granules become mobile and the crystalline regions start to melt. Fragmentation of the granules occur due to shear (Lai & Kokini, 1991).

The feed mixture then reaches the extruder barrel where final product is produced. The first section of the barrel acts as a feeding zone. The other section is the processing zone where dough is formed. Screw elements can be chosen according to its function, for instance conveying backward or forward. This along with the steam injection creates mechanical energy and increase temperature. Steam addition aids in the cooking process as well. Extrudate (product from extruder) before the die usually has 125-150°C temperature, 23-28% moisture content and 34-37 atmospheric pressure. Then the dough reaches the die from where it gains a definite shape and expansion because of pressure and shear. The die opening can manipulate shape and expansion of the final product (Rokey et al., 2010). Temperature rise just after the die can reduce viscosity and increase expansion which causes more brittleness in the pellets (Sørensen, 2012). Speed of the knife adjacent to the die determines the length of the pellet (Rokey et al., 2010).

Downscaling of the Extrusion Procedure

Downscaling of extrusion is performed when there is less feed material throughput and when a new formulation needs to be developed before execution. Batch sizes are also easy to adjust

by changing the total process time in downscaling. Essential parts of the extruder needs to be optimized when downsizing. Volume, heat transfer, mass transfer, feeding equipment and sectional design are some of the factors affecting scale-up or scale-down. Residence time in the extrusion is affected when feed materials, especially in cohesive powder forms, changes the feeding rate. This might alter qualities and properties of the final product. Barrel, screws and die of the downscaled extrusion might cause some changes in factors such as surface to volume ratio which then affects friction and heat transfer. Therefore, a uniform feeding rate should be achieved which also helps in achieving uniform moisture content of the extrudates (Muehlenfeld & Thommes, 2011).

2.2.2 Post-Extrusion

To reduce moisture content of the product to a stable moisture content, drying is required after extrusion. The main reason for this is to prolong the shelf-life of the product by reducing the water activity. Cross-flow dryer is considered the most effective for drying wet extrudates (Zhenhua, 2020). The moisture content of dried extrudates should be less than 10% to minimize bacterial and mold growth (Rokey et al., 2010).

In addition, liquid fat, vitamins or flavors can be coated externally after drying if required. Post liquid application by coating will not only avoid the risk of damaging heat sensitive ingredients, but also improve the palatability and reduce powder generation of the finished product. After coating, extruded aquafeed is cooled and packaged as required. Packaging prevents from oxidation and degradation of the feed and increase the shelf-life of the final product. Sealing the bag by sewing and heating plastic film lining has increased the shelf-life by 50-100% (Zhenhua, 2020).

2.3 Pellet Quality

Some of the important functional properties of a fish feed are moisture content, hardness, durability, water stability, water holding capacity, bulk density and sinking velocity. The pellet quality depends upon the type of feed ingredients used, screw configuration of extrusion and

extrusion parameters. Criteria for these qualities can differ according to the fish that is being fed. For instance, shrimps are bottom feeders and slow eaters, therefore their feed must have high water stability. On the other hand, salmon feed on the pellets when they are slowly sinking to the bottom therefore their feed must be slow-sinking.

Moisture content of feed determines its shelf-life and that of fish feed should be less than 10% to avoid moulding (Terpstra, 2015). Hardness of a feed shows its ability to withstand breakage during storage. It depends upon the moisture and temperature of the extrusion process. Higher the moisture and the temperature, harder the product (Delgado & Reyes-Jáquez, 2018). Higher durability or hardness is observed when there is less expansion of the pellet (Sørensen, 2012). Moisture and temperature of extrusion also affect the water stability of the product. Lower moisture and temperature in the extrusion results in lower water stability and softer product (Delgado & Reyes-Jáquez, 2018). High water stability of a feed is important for less disintegration of pellet and minimum leaching of nutrients.

Durability can help measure the amount of fines and breakages of pellets. In an experiment carried out by Haubjerg et al. (2015), higher durability was observed in the pellets which were viscoelastic meaning they were able to return back to their original state after deformation. This high durability was because of higher cohesiveness. Hilton et al. (1981) observed that extruded pellets were more durable than steam pellets. Extruded pellets also resulted in better water stability and water absorption than steam pellets. Durability, hardness, expansion and bulk density can be changed by altering the screw configuration of the extrusion (Sørensen, 2012). Bulk density and expansion of a pellet are related to each other and they determine its floatability or sinking velocity. A sinking pellet has more bulk density and less expansion. Likewise, expansion of a pellet is also related to its capacity of oil coating. More the expansion, more it can hold the oil (Sørensen, 2012).

2.4 Insects in Fish Feed

Insects are considered potential ingredients in animal and fish feed. They can be reared on bio-wastes and are capable of converting the wastes into useful and valuable resources. They have fast growth and reproduction rate and are highly efficient in feed conversion.

Feed conversion of mealworm was found to be more efficient on diets with high protein (Broekhoven et al., 2015) whereas, BSF was found to be more efficient in feed conversion than yellow mealworms and house crickets (Oonincx et al., 2015). They are rich in nutrients; they contain 42-63% crude protein and upto 36% lipid (Makkar et al., 2014). Carbohydrate level is usually low in insects. The level of chitin is different among different insect species (Tran et al., 2015). Insects are mostly deficient in methionine and lysine (except silkworm), therefore their supplement is required for better performance of animals and fish. Most insect meals are also deficient in calcium (except BSFL) and should be supplemented for better growth of fish and animals (Makkar et al., 2014). The chemical compositions of different insects are shown in Table 2.1.

Table 2.1: Chemical composition of different Insects (Makkar et al., 2014), cited by (Tran et al., 2015)

Constituents	**Black soldier fly larvae**	**Housefly maggot meal**	**Mealworm**	**Locust meal**	**House cricket**	**Mormon cricket**	**Silkworm pupae meal**	**Silkworm pupae meal (defatted)**
				DM %				
Crude Protein	42.1 (56.9)*	50.4 (62.1)	52.8 (82.6)	57.3 (62.6)	63.3 (76.5)	59.8 (69.0)	60.7 (81.7)	75.6
Lipids	26.0	18.9	36.1	8.5	17.3	13.3	25.7	4.7
Calcium	7.56	0.47	0.27	0.13	1.01	0.20	0.38	0.40
Phosphorus	0.90	1.60	0.78	0.11	0.79	1.04	0.60	0.87
Ca:P ratio	8.4	0.29	0.35	1.18	1.28	0.19	0.63	0.46

* Values in parentheses are calculated values of the defatted meals.

There are several experiments carried out in fish using insects as a feed ingredient. However, the commission regulation (EU, 2017) limits only the use of insects which are reared on plant origins. This is to avoid any possible risk of cross-contamination with other protein. The seven insect species that can be used in feeds are BSF, common housefly, yellow mealworm, lesser mealworm, house cricket, banded cricket and field cricket. BSFL has been used in feeds for channel catfish *(Ictalurus punctatus)*, blue tilapia *(Oreochromis aureus)*, rainbow trout *(Oncorhynchus mykiss)*, Atlantic salmon *(S. salar)*, turbot *(Psetta maxima)* and crustaceans. House fly maggots and pupae have been used in African catfish *(Clarias gariepinus)*, Nile tilapia *(Oreochromis niloticus)*, crustaceans and shrimps. Likewise, mealworm has been used for African catfish *(C. gariepinus)*, gilthead sea bream *(Sparus aurata)*, rainbow trout *(O. mykiss)* and European sea bass *(Dicentrarchus labrax)*. Locust, grasshoppers and crickets have been used for African catfish *(C. gariepinus)*, walking catfish *(Clarias batrachus)* and Nile tilapia *(O. niloticus)*. Similarly, carps *(Cyprinus carpio)*, silver barb *(Barbonymus gonionotus)*, mahseer *(Tor khudree)*, tilapia *(Oreochromis mossambicus)*, catfish, Japanese

sea bass *(Lateolabrax japonicus)* and crustaceans have been fed with silkworm. These experiments resulted that these insects can partially or fully replace FM depending upon the fish species and have resulted in good palatability (Makkar et al., 2014).

2.5 Black Soldier Fly Larvae

BSF, *H. illucens*, was originally found in warm, tropical and subtropical zones of America but now can be found in many parts of the world. The optimum temperature and relative humidity for rearing BSFL are 29-31°C and 50-70% respectively (Makkar et al., 2014). It has been used in animal feed since decades but are now primarily used due to their ability of converting high quality protein from several food wastes (Belghit et al., 2019a). It also has a potential of proper manure management as Sheppard et al. (1994) reported a conversion of manure into larval mass containing 42% protein and 35% fat. It lowered manure accumulation by more than 50% and eradicated house fly breeding as well. BSFL contains high amount of protein and lipids which is around 40-45% and 30-35% on dry weight basis (Rana et al., 2015). It has a well-balanced AA profile, wide range of minerals and is also rich in saturated fatty acids (SFA), especially lauric acid, C12:0 (Belghit et al., 2019a). It contains 11-15% ash, 4.8-5.1% calcium, and 0.6% phosphorous as well (Rana et al., 2015). Feed of BSFL greatly affects its nutrition value. For instance, if it is fed with fish offal and meat leftovers, level of omega-3 fatty acids (FA) content can be high (St-Hilaire et al., 2007). BSFL can therefore add value to the leftovers/wastes and convert them to useful product namely insect biomass. Similarly, insect frass (excrements) are easily manageable compared to the original waste (Rana et al., 2015). In addition, BSFL rearing is environmentally friendly and sustainable. BSFL rearing is also not labor intensive as larvae migrates from waste pit to a clean collecting vessel before it turns into pupae and adults (Sheppard et al., 1994) which is termed as 'self-harvesting'. Adults do not bite and are not a vector of diseases (Rana et al., 2015). All these advantages of BSFL make it a suitable alternative ingredient in fish feed.

2.5.1 Fractions of Black Soldier Fly Larvae

BSFL can be fractionated into valuable biomolecules namely protein, lipid and chitin. Several extraction methods such as chemical or enzymatic extraction can be carried out to obtain these fractions.

Lipid Fraction

BSFL oil is a high quality lipid source and is often compared to soy oil because of its good lipid synthesizing properties from carbohydrate of the larvae (Mai et al., 2019). BSFL does not contain mycotoxins or pesticides and has higher saturated fat content compared to other insects (Purschke et al., 2017). It has a good balance of saturated and unsaturated FA and its FA composition depends on the FA composition of the substrates that are used to rear BSFL (Makkar et al., 2014). The unsaturated FA are oleic acid (18:1, n-9) and linoleic acid (18:2, n-6). BSFL oil is also rich in medium chain FA such as lauric acid which has antimicrobial function and can be used as nutritional supplements. However, the crude oil obtained contains non-triacylglycerol components including FA, odorous residue, pigments and gummy materials which have negative effects on taste, appearance and nutritional value. Therefore, they must be eliminated before its application in food/feed industry or any other industries through a refining process (Mai et al., 2019).

In a study carried out by Matthäus et al. (2019), BSFL fat was extracted using an oil seed screw press and the lipid fraction contained lauric acid (48%), myristic acid (11%) and palmitic acid (16%), which are similar to that of palm kernel and coconut fat. The content of tocopherols and tocotrienols were low (64.7 mg/kg) whereas the sterol content was comparable to other common plant oils (3557 mg/kg). The dominant saturated triacylglycerols were LaLaLa (27.6%), LaLaM (16.0%) and LaMM (15.1%), where La is lauric acid and M is myristic acid. Cholesterol content of BSFL fat is usually between 12.0 mg/100 g and 28.2 mg/100 g, which is low compared to other animal fats (Liland et al., 2017; Ramos-Bueno et al., 2016).

The effect of diets with BSFL oil was studied in juvenile Jian carp (*Cyprinus carpio* var. Jian) by Senlin et al. (2016) which resulted in similar growth, nutrition utilization and serum biochemical parameters to fish fed diets with soy. In another study by Dumas et al. (2018),

diets containing mechanically extracted oil from BSFL oil fed to rainbow trout (*Oncorhynchus mykiss*) did not affect relative body protein content or gut histology of trout. Hydroxyproline content was increased in the fish fed with BSFL oil. Kumar et al. (2020) reported some immunological benefits of BSFL oil in diets for rainbow trout such as improved immunity and reduced inflammation. Another study by Mohamad-Zulkifli et al. (2019), showed that digestibility of lipids in hybrid grouper (*Epinephelus fuscoguttatus x E. lanceolatus*) was higher in diets containing oven-dried BSFL than spray-dried BSFL, suggesting that drying method could affect nutritional value of the BSFL.

Protein Meal

BSFL contain most of the EAA and they are present in higher level than in soybean meal (SBM). BSFL are also rich in natural antibiotics (Hodar et al., 2020). However, nutritional value of BSFL protein can depend upon its drying process. In a study carried out by Huang et al. (2019), the effect of two drying methods, conventional drying method (60°C) and microwave drying method were studied. EAA to total AA ratio of both methods were found to be higher than 40%. Lysine and valine were found to be the first limiting AA for conventionally dried BSFL protein and microwave dried BSFL protein respectively. *In vitro* DIAAS (Digestible Indispensable Amino Acid Score) values of proteins from both methods were found to be higher than 75%. The *in vitro* digestibility of BSFL protein was found to be better for conventionally dried protein whereas microwave drying resulted in compact and larger particle size of the protein. In another study by Mohamad-Zulkifli et al. (2019) with hybrid grouper (*Epinephelus fuscoguttatus x E. lanceolatus*), ADC of crude protein was higher in the fish fed with reference diet (FM and SBM) than oven-dried and spray-dried BSFL diets.

BSFL meal in diets of rainbow trout resulted in reduced intestinal enteritis which was otherwise seen when SBM was used in their diets. Better immunity and lowered inflammation was observed when BSFL meal was included along with SBM (Kumar et al., 2020). In a study carried out by Xiao et al. (2018), yellow catfish (*Pelteobagrus fulvidraco*) obtained better growth performances and immune indexes with 25% replacement of FM with BSFL meal. In another study with juvenile barramundi (*Lates calcarifer*) reared in freshwater by Katya et al. (2017), a 100% replacement of FM with BSFL meal resulted in higher level of EAA in

the whole body of fish, however, they concluded that the optimum level of replacement with BSFL meal in the fish without any negative effects on whole body and AA composition should be between 28.4 to 50%. Similarly, Maina (2020) stated that BSFL meal inclusion in the diets of African catfish had no adverse effects on nutrients composition and EAA concentration increased with increasing amount of BSFL meal in the diets.

Chitin

Chitin is an aminopolysaccharide polymer found abundantly in crustaceans, fungi and insects (Elieh-Ali-Komi & Hamblin, 2016). It is insoluble in usual solvents like water, mild acidic or basic solutions, organic solvents, etc. (Roy et al., 2017). Therefore, various modifications such as enzymatic or chemical deacetylation are carried out to convert it into soluble derivatives such as chitosan which is the most common derivative (Liu et al., 2012). Chitin has various functional assets namely potential prebiotic, antimicrobial, antiviral and antifungal agent. However, it is considered as an ANF and is classified as a non-digestible fiber (Hahn et al., 2018). Its low digestibility is the reason why its usage in feed stuff is limited (Rust, 2003). On the other hand, some studies (Gutowska et al., 2004; Henry et al., 2015) have used chitin in feeds of animals (such as fish) by degrading it with the help of chitinolytic enzymes. However, there is no assurance of a complete degradation of chitin by chitinase activity in fish (Ringø et al., 2012).

Chitin is a polysaccharide of glucosamine and N-acetylglucosamine, both of which contain nitrogen (N) atoms. Chitin content varies according to life stage, hard cuticles have lower chitin (15-30%), higher protein content (70-85%, dry basis) and higher water (40-75%) whereas soft cuticles have higher chitin (50%), lower protein content (50%) and lower water (12%) (Nogales-Mérida et al., 2019; Vincent & Wegst, 2004). The digestibility of protein depends on the amount of AA bound to chitin (Finke, 2007). But they can be available to fish because of the chitinolytic enzymes such as chitinase and chitobiase in their stomach and intestine respectively (Lindsay, 1983). When replacing fish meal with krill meal containing chitin completely in salmon feed, growth and lipid utilization decreased (Olsen et al., 2006). High krill meal also reduced growth in rainbow trout (*Oncorhynchus mykiss*) (Wojno & Dabrowska, 1984), however, Lellis and Barrows (2000) reported that 6% of chitin supplementation increased growth in rainbow trout juveniles. This might indicate that chitin

may be digested and may enhance fish performance under certain conditions. 10% chitin inclusion resulted in increased growth in red sea bream (*Pagrus major*) (Kono et al., 1987). Danulat (1987) states that Alantic cod (*Gadus morhua*) digests chitin effectively.

2.5.2 Black Soldier Fly Larvae in Salmon Nutrition

Several studies have been carried out to study the effect of dietary inclusion of BSFL replacing traditional protein sources partially or completely in fish, including salmon. Lock et al. (2014) observed that dietary replacement of 25%, 50% and 100% by BSFL meal increased the feed conversion ratio of Atlantic salmon. Further, they observed that BSFL did not affect histology and sensory quality of the fillets of Atlantic Salmon. Belghit et al. (2019a) observed that replacing FM with insect meal did not compromise growth performance, feed utilization, nutrient digestibility, liver traits and sensory qualities of the fillet in salmon. They also remarked BSFL meal as a nutritionally suitable protein source for sea water Atlantic salmon. Similarly, Li et al. (2020) observed no adverse effect on the gut health of Atlantic salmon when fish meal was totally replaced with BSFL meal. Fish fed with BSFL meal diet resulted in reduced enterocyte steatosis in the proximal intestine and increased relative weight of DI. In addition, Fisher et al. (2020) reported that the digestibility coefficients of BSFL meal was higher than 75%. 20% inclusion of BSFL meal resulted in similar growth performance of salmon as the fish fed with control diet. Further, Bruni et al. (2020) reported no reduction in the physicochemical quality of fillets of Atlantic salmon when they were fed with diets containing total BSFL meal as a substitute of FM.

In our previous study Weththasinghe et al. (2021), the effect of increasing dietary inclusion of two differently processed BSFL (i.e. BSFL meal and paste) on extrusion parameters, pellet quality, growth performance, nutrient digestibility and utilization in Atlantic salmon was studied. In this study, BSFL meal replaced 6.25%, 12.5% and 25% of protein content whereas BSFL paste replaced 3.7% and 6.7%. Results showed that increasing BSFL inclusion in the diet decreased the efficiency of processing parameters in terms of specific mechanical energy, temperature, torque, pressure of die and residence time which then led to decreased cooking and expansion of the pellet. This was because of increased lipid content in the extruder with increased BSFL inclusion. Less cooking resulted in poor quality of the pellets and therefore

lesser hardness, durability and water stability. Feed intake was not affected by the dietary inclusion of both BSFL meal and paste. Protein and lipid digestibilities were reduced with increasing level of BSFL meal and paste which was most probably because of chitin as chitin lowers the nutrient availability in the intestinal tract of salmon. However, apparent protein retention was not affected by dietary inclusion of BSFL meal and paste, but apparent lipid and energy retention were decreased with increasing dietary inclusion of BSFL meal. Overall, inclusion of upto 12.5% of insect meal and 6.7% of insect paste resulted in better growth performances compared to the control fed salmon.

Chapter 3

Materials and Methods

The feed production was carried out at Centre for Feed Technology (Fôrtek) - NMBU. The fish experiment was carried out at Center for Fish Research, NMBU on a Recirculating Aquaculture System (RAS).

3.1 Raw Materials

The major ingredients used in experimental diets were corn gluten, FM, SPC, wheat flour, wheat bran and fish oil. In addition, BSFL products used were full-fat BSFL meal, defatted BSFL meal, dechitinized BSFL meal, BSFL oil and BSFL exoskeleton (chitin). Diets are shown in Table 3.1. Micro ingredients used were yttrium oxide (Y_2O_3), MCP, vitamins/minerals mix, choline chloride and L-methionine.

3.2 Production of BSFL fractions

The BSFL fractions were produced at HiProMine S.A., Robakowo, Poland. The feed for BSFL contained wheat middlings (17%), fresh apples (15%), fresh carrots (50%), fresh potatoes (15%) and fresh cabbages (20%) to balance its dry matter content which was 22%. Pre-consumed waste of fresh vegetable and fruit mix were ground using miller (2000 rpm, 55 kW; HPM milling system, Robakowo Poland) in order to pass it through 2 mm screen. It was fed ad libitum to BSFL. Contamination from animal products were completely avoided, in accordance with an EC regulation (no. 1069/09). On the seventh day of rearing, larvae were harvested, sieved using a 3 mm screen and washed with water on a drum separator at 90°C for

10 min using HPM cleaning system, Robakowo, Poland.

A batch of BSFL was divided into two parts for further processing; first part was used to produce full-fat meal and part of it was defatted to produce defatted meal and oil, and the second part was used to produce dechitinized meal and chitin fractions. To produce full-fat meal, BSFL biomass were first dried for 1 h at 130°C and then for 23 h at 80°C until it reached a constant weight using an air flow dryer chamber (HiProMine S.A., Robakowo, Poland) and a part of it was defatted with oil press (Reinartz, model AP14/22, Neuss, Germany) to produce low-fat meal and oil. To produce dechitinized meal and chitin fractions, BSFL biomass was mechanically dechitinized using food press twin-screw processor with 0.3 mm screen diameter (Angel Juicer, model 7500, Busan, Korea). All the products were stored at 4°C before their use in feed production.

3.3 Feed Formulation

The diets were formulated based on the nutrients and energy requirements of the salmon. There were six experimental diets: control diet (Diet 1), full-fat BSFL diet (Diet 2), defatted BSFL diet (Diet 3), dechitinized BSFL diet (Diet 4), BSFL oil diet (Diet 5) and exoskeleton BSFL diet (Diet 6). The ingredient compositions of the experimental diets are presented in the Table 3.1. 15% of the total protein of the control diet was replaced by BSFL protein in diets. The diets were formulated to be isolipidic and isoenergetic.

3.4 Feed Processing

The ingredients (FM, wheat bran, MCP) were ground in a small horizontal hammer mill (Alpine, UPZ-mølle, Denmark) with a perfocon 0.5 mm sieve. The ground ingredients were then weighed manually and mixed in an ISDECA mixer (60-liter paddle-mixer, Fôrtek, Forberg, Norway) (Figure 3.1) for 3 min. For diet 2 and 4, the ingredient mixes were again ground in the hammer mill to avoid any problems in the extruder (coarse particles of the diet mix can result in softer pellets because of less integration between the ingredients). However, the sieve was clogged inside the hammer mill for diet 2 mix which might be due to high

Table 3.1: Composition of Different Diets

Diet	**1**	**2**	**3**	**4**	**5**	**6**
Ingredient (g/100g)	**Control**	**Full-fat**	**Defat**	**Dechitin**	**BSFL oil**	**Exoskeleton**
Corn gluten	5.50	4.54	4.54	4.54	5.50	5.32
FM	22.50	18.57	18.57	18.57	22.50	21.78
SPC	34.50	28.48	28.48	28.48	34.50	33.39
Wheat flour	14.65	14.65	14.65	14.65	14.65	14.65
Full-fat BSFL meal	0.00	20.36	0.00	0.00	0.00	0.00
Defatted BSFL meal	0.00	0.00	14.89	0.00	0.00	0.00
Dechitinized BSFL meal	0.00	0.00	0.00	24.53	0.00	0.00
BSFL oil	0.00	0.00	0.00	0.00	7.85	0.00
BSFL exoskeleton	0.00	0.00	0.00	0.00	0.00	7.20
Wheat bran	5.04	1.13	2.31	1.60	4.74	0.49
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15
Fish oil	16.00	10.47	14.75	5.82	8.45	15.36
Yttrium	0.01	0.01	0.01	0.01	0.01	0.01
Vit/min	0.65	0.65	0.65	0.65	0.65	0.65
MCP	0.80	0.80	0.80	0.80	0.80	0.80
SUM	100	100	100	100	100	100

1: Control Diet. 2: Full-fat BSFL Diet. 3: Defatted BSFL Diet. 4: Dechitinized BSFL Diet. 5: BSFL Oil Diet. 6: Exoskeleton BSFL Diet.
FM: Fishmeal
SPC: Soy Protein Concentrate
MCP: Monocalcium Phosphate
Corn gluten; Baolingbao Biology, Shangdong Yucheng, China
LT fishmeal; Norsildmel AS, Bergen, Norway
SPC (Tradkon SPC HC-200, Sojaprotein); Becej, Serbia
Wheat flour 78%, batch number: 5093060546; Norgesmøllene, Bergen, Norway
Wheat bran; Norgesmøllene, Bergen, Norway
Fish oil; Norsildmel AS, Bergen, Norway
Yttrium oxide (Y_2O_3); Metal Rare Earth Limited, Shenzhen, China
MCP; Yara, Animal Nutrition, Helsingborg, Sweden
Vitamins/minerals mix (Vit/min premix, Farmix; Per kg feed; retinol 2,500.0 IU, cholecalciferol 32,400.0 IU, α-tocopherol SD 0.2 IU, menadione 40.000 mg, thiamine 15.0 mg, riboflavin 25.0 mg, d-Ca-pantothenate 40.002 mg, niacin 150.003 mg, biotin 3,000.0 mg, cyanocobalamin 20.0 mg, folic acid 5.0 mg, pyridoxine 15.0 mg, ascorbate polyphosphate 0.098 g, Cu: Cu sulphate $5H_2O$ 11.998 mg, Zn: Zn sulphate 89.992 mg, Mn: Mn(II) sulphate 34.993 mg, I: K-iodine 1.999 mg, Se: Na-selenite 0.200 mg, Cd Max. 0.0003 mg, Pd max. 0.028 mg, Ca 0.915 g, K 1.380 g, Na 0.001 g, Cl 1.252 g); Trouw Nutrition, LA Putten, The Netherlands
Choline chloride (70%, C_5H_14ClNO, 139.6g/mol); Vilomix, Hønefoss, Norway
L-methionine; Bestamino^{TM} Cj Cheiljedang, Seoul, Korea
BSFL ingredients; HiProMine S.A., Poznań, Poland.

amount of exoskeleton in the mix. The sieve was then manually cleaned. There was no such problem for milling of diet 4 mix.

Figure 3.1: ISDECA Mixer

Figure 3.2: Twin Screw Extruder

The mixes were then extruded with a twin-screw extruder (Bühler BCTG 62, 5 sections,

72 kW DC, Bühler, Switzerland) (Figure 3.2). The extrusion process was conducted in the reduced capacity without a pre-conditioner. Therefore, a feeder (K-tron) fed manually in the first chamber of the extrusion with a speed of 7Hz for diet 1, 6Hz for diets 3, 5 and 6 and 8Hz for diets 2 and 4. The feeding rate was 35, 40, 30, 40, 30 and 30kg/h respectively. The screw configuration and the temperature profile of the extruder are shown in Figure 3.3 and Figure 3.4 respectively.

Screw Configuration

							90°	90°	R					R					Lenght
40	40	60	60	60	80	80	20	20	120	100	80	60	40	60	100	80	80	80	1260
R	R	R	R	R	R	R	L	L	POLY	R	R	R	R	POLY	R	R	R	R	
																UC	UC		

L - Left (backward) turn of the element
UC - **U**nder **C**ut (means more free volume in the extruder)
Numbers are representing the lenght of the Archimedean Spiral (screw alike) for each screw element

Screw configuration - remarks:
Red arrow = spacer

Figure 3.3: Screw Configuration of the Extruder

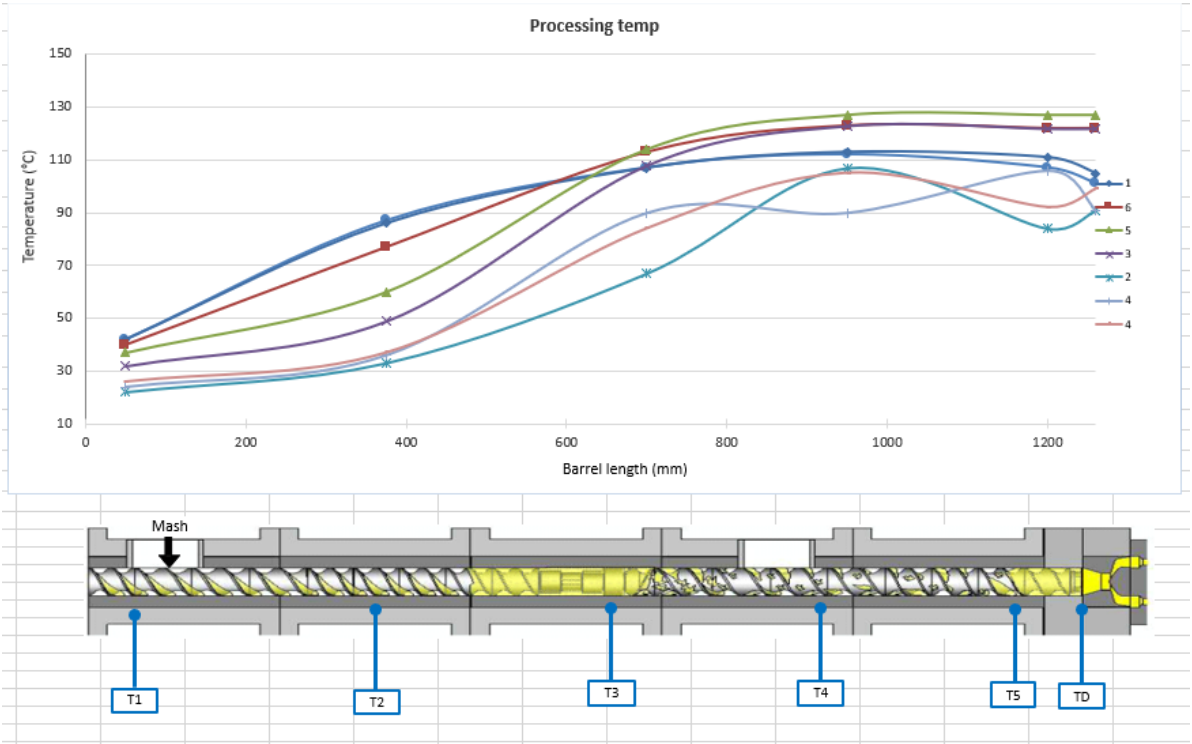

Figure 3.4: Temperature Profile of the Extruder

After extrusion, the pellets were dried in batch driers. Experimental dryer (Ziehl Abegg

Ventilator and Fan) (Figure 3.5) was used until the moisture content of the diets reached 8-10%. The moisture content was analyzed using moisture analyzer (MB25, Ohaus, Nänikon, Switzerland). Then for vacuum coating, the fish oil and insect oil were manually heated. The fish oil was heated to 40°C whereas the mixture of fish oil and insect oil was heated to 50°C. The heated oil was then poured into a pressurized tank (30 L) with a nozzle (nozzle type: 6508) for vacuum coating (Gentle Vacuum Coater, GVC – 80 prototype, Fôrtek, Amandus-Kahl) (Figure 3.6). At first, vacuum was created to approx 0.15 bar and turned off, and then a small opening in the valve was made to release the pressure. This took around 2-3 min and meanwhile, oil and the pellets inside the tank was rotating at around 18 rpm.

Figure 3.5: Experimental Dryer

Figure 3.6: Vacuum Coater

3.5 Physical Quality Analysis of Pellets

Pellet quality measurements such as moisture content, bulk density, hardness, durability, water stability, expansion, and sinking speed were performed.

3.5.1 Moisture content and Bulk Density

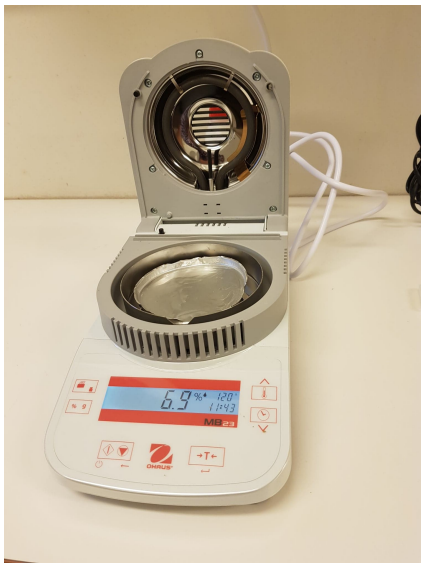

Figure 3.7: Moisture Analyzer

Figure 3.8: Bulk Density Measurement

Moisture content of the pellets was measured using a moisture analyzer (MB23, Ohaus, Switzerland) (Figure 3.7). Bulk density of the pellets was measured using a scale (Figure 3.8) in grams per liter (g/L).

3.5.2 Hardness

Hardness was measured using Tinius Olsen Texture Analyzer (H5KT, Salfords, England) (Figure 3.10). First, length and diameter of 30 pellets per diet was measured using a digital caliper (Figure 3.9). Then, 15 pellets per diet with average length and diameter ($\pm$0.2mm) were selected and placed across the diameter of the compression platen. The peak force applied to break down each pellet was recorded. Hardness was expressed as the maximum force per pellet length (N/mm).

Figure 3.9: Digital Caliper

Figure 3.10: Texture Analyzer

3.5.3 Durability

Durability of pellets was measured in triplicates on a Doris Pellet Tester (AKVAsmart, Bryne, Norway) (Figure 3.11). First, 100 g of feed sample was taken for each replicate. Next, the samples were sieved using Retsch Vibratory Sieve Shaker (AS 200 Control, Germany) (Figure 3.12). Two different sizes of sieves were used; 2 mm screen (whole pellets) and 1 mm screen (small broken pellets) along with the bottom dust pan. Sieving was carried out for 60 s at 1.2 amplitude.

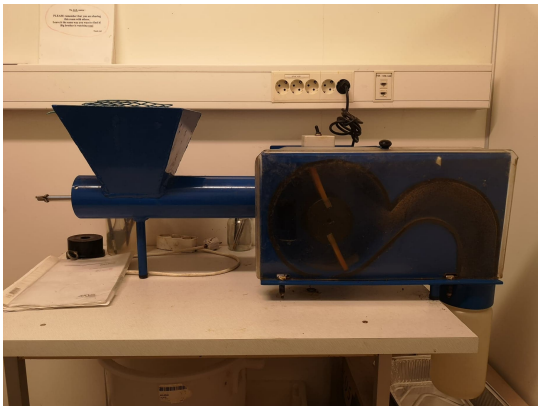

Figure 3.11: Doris Pellet Tester

Figure 3.12: Vibratory Sieve Shaker

3.5.4 Water Stability

The water stability of pellets was measured using Julabo SW22 Shaking Water Bath (Figure 3.13). It was measured according to Baeverfjord et al. (2006) with some modifications. The sample size was 20 g. Reverse Osmosis (RO)-water (300 ml) was used in the beakers and placed inside the water bath in 3 parallels. The test was done for 2 incubation times, i.e. 30 min and 60 min at a temperature of 25°C. Shaking was set to 120 rpm. After incubation, the triplicates were dried in an oven for 20 h at 100-104°C. Weight of the remains in the baskets and dry matter content of the pellet were then used to calculate water stability.

Figure 3.13: Shaking Water Bath

3.5.5 Expansion

The width of 30 random pellets per diet were measured using a digital caliper. The expansion was then calculated as

$$Expansion(\%) = \frac{Pellet\ width - die\ diameter}{die\ diameter} \times 100$$

3.5.6 Sinking Speed

A plastic tube of 1.2 m length (Figure 3.14) was filled with drinkable tap water. The tube had a 1 m marking. One pellet at a time was dropped into the tube and the time required for the pellet to travel 1 m was noted. For this measurement, 10 pellets per diet were used.

Figure 3.14: Tube used to measure Sinking Speed

3.6 Fish Experiment

In the fish experiment, 900 Atlantic salmon were distributed in 18 circular tanks (50 fish per tank) of approximately 230 L whereas 15 fish were sampled for whole body composition analysis. FINQUEL® vet. aquatic anaesthetic was used as a sedater during weighing and selection of the fish. The initial biomass for each tank was approximately 1.4 kg and the average initial weight of the fish was approximately 28 g. The average water temperature of tanks during the experiment was 15°C. The experimental period lasted for eight weeks. For feeding, automatic and electrically driven belt feeders were used. Feeding started at 08:25 and ended at 14:25 each day, and the belts paused at intervals of every 90 s for 180 s. Fish were fed *ad libitum* (i.e. 10% excess). Faeces and uneaten pellets accumulated on the wire screen and uneaten pellets were separated, collected and weighed. Oxygen saturation (%), oxygen

amount (mg/L), temperature, and water flow were checked daily after feeding. Saturation level was maintained at above 80% by increasing the water flow. Mortality of fish was checked daily after feeding. The experiment was carried out for 62 days.

3.6.1 Recovery and Water Absorption of the Diets

For the recovery test, about 10 g of feed were placed in the water tanks. The feed that accumulated on the screen was weighed and then dried in an oven for 11 h. Recovery per tank was then calculated using the weight of the dry feed and its dry matter. Recovery test shows the amount of particles that leached out of the feed pellets in the tanks. To calculate water absorption of the diets, about 5 g of the uneaten feeds were taken in containers. The diets were then kept in an oven at 100-104°C for 12 h and then weighed.

3.6.2 Sampling

At the end of the trial, 5 random fish per tank were sampled for analysis of whole body composition. Rest of the fish were weighed and stripped for faeces three times i.e. 0, 7 and 14 days after sampling for whole body composition. For sedation, 0.8 - 1.0 g/10L of FINQUEL® vet. was used.

3.7 Chemical Analysis of Feed Ingredients, Feed and Faeces

Chemical analysis of dry matter, ash, crude protein, crude lipid, starch, energy, yttrium oxide and minerals, total phosphorous, acid detergent fiber (ADF), AA and FA was carried out at LabTek-analyselab at the department of animal and aquacultural sciences, NMBU. The measurement values of different chemical analysis and AA analysis are mentioned in the Table 3.2 and Table 3.3 respectively.

Table 3.2: Chemical Analysis of Diets

Diet	**1**	**2**	**3**	**4**	**5**	**6**
	Control	**Full-fat**	**Defat**	**Dechitin**	**BSFL oil**	**Exoskeleton**
Dry matter (g/kg)	916	919	930	929	933	917
Ash (g/kg)	67.0	66.0	67.7	72.3	67.1	66.1
Crude protein (g/kg)	466.1	444.1	459.6	466.0	465.5	472.7
Starch (%)	13.1	12.2	12.4	12.4	12.6	11.7
Lipid (g/kg)	131.0	179.0	162.0	144.5	158.0	154.0
Yt (mg/g)	0.080	0.068	0.079	0.082	0.081	0.079
Total-P (mg/g)	11.3	11.0	11.0	12.4	11.2	11.0
Ca (%)	1.11	1.19	1.29	1.28	1.28	1.19
Mg (%)	0.180	0.194	0.202	0.234	0.173	0.170
ADF (g/kg)	56.0	38.3	39.3	26.7	32.0	45.7
Chitin (%)	NP	1.44	1.44	0.53	NP	1.43

1: Control Diet. 2: Full-fat BSFL Diet. 3: Defatted BSFL Diet. 4: Dechitinized BSFL Diet. 5: BSFL Oil Diet. 6: Exoskeleton BSFL Diet.

NP: not present

ADF: Acid Detergent Fiber

Chitin content in diets was calculated using the chitin content in insect ingredients and their inclusion level in each diet.

Dry matter content was measured by oven drying method at 104°C until the sample reached a constant weight. Ash content was measured in a furnace (Nabertherm) at 550°C for around 20 h. Nitrogen content was measured by Kjeldahl method. Crude protein was then estimated by the formula Nitrogen*6.25. Solvent extraction method (ASE® 350 Accelerated Solvent Extractor, Nerliens Mezanski) was used to measure the crude lipid amount (Schäfer, 1998). Starch content was determined according to McCleary et al. (1994) with some alterations. α–amylase and amyloglucosidase was added to breakdown the starch into glucose. Color spectrometer (RX4041 Randox Daytona+, England) was then used to measure the glucose content. Energy content was measured by bomb calorimetry (PARR 6400 Bomb calorimeter) in which samples were burned under a closed environment and the amount of released/absorbed heat was measured. Yttrium oxide is used as an indigestible marker to calculate the nutrient digestibility. Yttrium oxide and minerals

namely calcium (Ca) and magnesium (Mg) were measured using spectrophotometric method (Microwave plasma atomic emission spectrometer; MP-AES 4200, Agilent Technologies, USA) after acid decomposition in a microwave digestion system (Start D, Milestone Srl, Italy). Total phosphorus (P) was measured by combustion and acid digestion and then using a spectrophotometric kit (PH8328, Randox laboratories, County Antrim, UK). Chitin content of the insect ingredients was estimated according to Finke (2007).

Tryptophan content was measured using a Dionex Ultimate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) and with a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan). All other AA were determined using a Biochrom 30 AA Analyzer (Biochrom Ltd., Cambridge, UK). FA content was measured according to O'Fallon et al. (2007) using Trace Gas Chromatography Ultra (Thermo Fisher Scientific, US) which converted FA into FAME (Fatty Acid Methyl Esters).

Table 3.3: Amino Acids Analysis of Diets

Diet		**1**	**2**	**3**	**4**	**5**	**6**
		Control	**Full-fat**	**Defat**	**Dechitin**	**BSFL oil**	**Exo-skeleton**
Cysteine	g/kg	4.51	4.06	4.11	4.27	4.44	4.31
Methionine		9.80	8.95	8.97	9.39	9.03	9.37
Aspartic acid		41.25	36.81	38.20	39.88	41.24	40.48
Threonine		15.89	14.78	15.10	15.62	15.84	15.95
Serine		18.36	16.60	17.11	17.06	18.02	18.49
Glutamic acid		80.50	70.77	73.15	75.51	79.96	78.95
Proline		21.23	20.47	21.66	20.68	21.60	23.19
Glycine		17.01	16.37	16.66	16.29	16.90	17.81
Alanine		19.61	19.92	20.24	19.14	19.62	21.11
Valine		15.48	15.18	15.37	15.15	15.58	16.39
Isoleucine		17.84	16.55	17.02	17.53	17.97	17.93
Leucine		33.99	30.51	31.20	32.28	33.95	33.63
Tyrosine		12.12	14.12	14.30	12.45	12.51	14.84
Phenylalanine		19.91	17.63	18.13	19.00	19.75	18.81
Histidine		10.71	10.36	10.48	10.55	10.71	10.92
Lysine		26.19	24.74	24.92	25.91	25.97	25.77
Arginine		27.23	24.17	24.64	25.69	27.15	26.34
SUM AA		391.64	361.99	371.26	376.39	390.25	394.28

1: Control Diet. 2: Full-fat BSFL Diet. 3: Defatted BSFL Diet. 4: Dechitinized BSFL Diet. 5: BSFL Oil Diet. 6: Exoskeleton BSFL Diet.

3.8 Calculations

$$Expansion(\%) = \frac{Pellet\ width - die\ diameter}{die\ diameter} \times 100$$

$$SME \quad (Wh/kg) = (2 \times \pi \times 60^{-1}) \times (S_{\text{rpm}} \times Tk_{\text{nm}} \times T_{\text{t/h}}{}^{-1})$$

$$where,\ SME = Specific\ Mechanical\ Energy$$

$$S_{\text{rpm}}\ is\ screw\ speed,\ Tk_{\text{nm}}\ is\ Torque\ and\ T_{\text{t/h}}\ is\ throughput$$

$$SGR \quad (\%body\ weight/day) = \frac{ln\,(FBW) - ln\,(IBW)}{Total\ Experimental\ Days} \times 100$$

$$where,\ SGR = Specific\ Growth\ Rate$$

$$FBW = Final\ Body\ Weight$$

$$IBW = Initial\ Body\ Weight$$

$$Feed\ intake,\ FI\ (gm\ DM/fish) = \frac{Total\ feed\ intake}{Number\ of\ fish\ per\ tank}$$

$$FCR \quad (gm/gm) = \frac{Feed\ intake}{Final\ body\ weight - Initial\ body\ weight}$$

$$where,\ FCR = Feed\ Conversion\ Ratio$$

$$Geometric\ mean = \sqrt{Final\ Body\ Weight \times Initial\ Body\ Weight}$$

$$Hepatosomatic\ index = \frac{Liver\ weight}{Body\ weight} \times 100$$

$$Condition\ factor = \frac{Body\ weight}{Length^3}$$

$$Recovery\ (\%) = \frac{Dried\ Feed}{Amount\ of\ feed\ taken \times Dry\ Matter} \times 100$$

$$ADC\ of\ nutrients\ (\%) = \left[1 - \left(\frac{D_{\mathrm{i}}}{F_{\mathrm{i}}} \times \frac{F_{\mathrm{n}}}{D_{\mathrm{n}}}\right)\right] \times 100$$

$$where,\ ADC = Apparent\ Digestibility\ Coefficient$$

$$D_{\mathrm{i}}\ and\ F_{\mathrm{i}} = concentration\ of\ marker\ in\ diet\ and\ faeces\ respectively$$

$$and\ D_{\mathrm{n}}\ and\ F_{\mathrm{n}} = concentration\ of\ nutrients\ in\ diet\ and\ faeces\ respectively$$

$$Faecal\ excretion\ of\ minerals\ or\ N\ (\%) = 100 - ADC\ of\ minerals\ or\ N$$

$$where,\ ADC = Apparent\ Digestibility\ Coefficient$$

$$Protein\ or\ Lipid\ Efficiency\ Ratio\ (g/g) = \frac{FBW - IBW}{FI \times \frac{Protein\ or\ Lipid\ content\ in\ Feed}{100}}$$

$$where,\ FI = Feed\ Intake$$

$$FBW = Final\ Body\ Weight$$

$$IBW = Initial\ Body\ Weight$$

$$Apparent\ Nutrient\ Retention\ (\%\ intake) = \frac{(FBW \times FN)\ -\ (IBW \times IN)}{FI \times \frac{Nutrient\ content\ in\ Feed}{100}} \times 100$$

$$where,\ FN = Final\ nutrient\ content\ in\ fish$$

$$IN = Initial\ nutrient\ content\ in\ fish$$

3.9 Statistical Analysis

Growth performance and nutrient digestibility data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The p-values below 0.05 was considered as significant. IBM SPSS Statistics 27 software was used for statistical analyses.

Chapter 4

Result

4.1 Feed Production Parameters and Pellet Quality

The extrusion parameters used in the production of diets are shown in Table 4.1. BSFL oil diet had numerically the highest energy inputs such as SME, torque, die temperature, bar pressure and drive power whereas dechitinized BSFL diet had the lowest. Lipid percentage in the mash of dechitinized diet was the highest (13%), therefore, screw speed was increased in order to achieve desirable physical pellet quality. The values of different physical quality measurements are shown in Table 4.2. Durability, hardness, expansion and water stability of dechitinized and full-fat diets were numerically lower than other diets. Particle size distribution of pellets after Doris test and water stability are shown in Figure 4.2 and Figure 4.1 respectively.

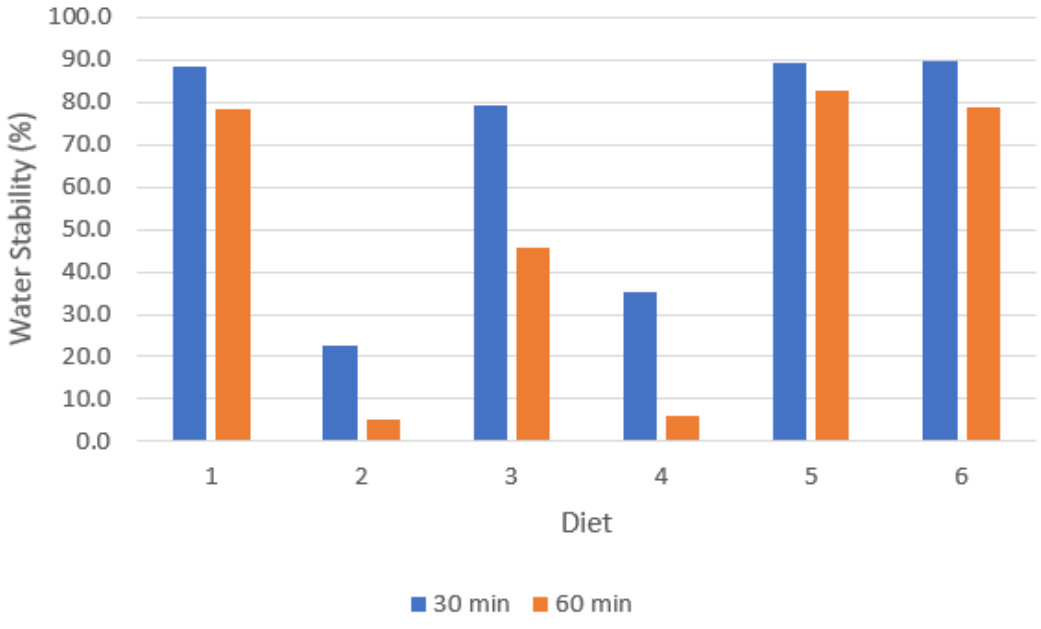

Figure 4.1: Water Stability of Pellets

Table 4.1: Extrusion Parameters of Different Diets

Diet	**1**	**2**	**3**	**4**	**5**	**6**
	Control	**Full-fat**	**Defat**	**Dechitin**	**BSFL oil**	**Exoskeleton**
Die size	2.5	2.5	2.5	2.5	2.5	2.5
Number of dies	4	4	4	4	4	4
Feeder (kg/h)	35	40	30	42	30	30
Feeder (Hz)	7	8	6	8.5	6	6
Barrel 1 (°C)	42	22	32	24	37	40
Barrel 2 (°C)	86	33	49	36	60	77
Barrel 3 (°C)	107	67	108	90	114	113
Barrel 4 (°C)	113	107	123	90	127	123
Barrel 5 (°C)	111	84	122	106	127	122
Die temp. (°C)	105	91	122	91	127	122
Die pressure (bar)	23	4	12.3	3.9	25.2	22.4
Barrel Heating	Yes	No	Yes	No	Yes	Yes
Pressure, barrel 4 (bar)	0.5	0.31	1.14	0.3	1.5	1.2
SME (Wh/kg)	449	510	616	480	771	630
Drive power (kW)	6.5	7.6	8.8	7.7	10.7	9
Torque (%)	53	43	48	37	60	49
Screw speed (rpm)	270	400	400	450	385	400
Extr. water (kg/h)	14	15.5	14	16.3	14	14
Knife speed (rpm)	1450	1850	1450	1850	1450	1450
Number of knifes	6	6	6	6	6	6

1: Control Diet. 2: Full-fat BSFL Diet. 3: Defatted BSFL Diet. 4: Dechitinized BSFL Diet. 5: BSFL Oil Diet. 6: Exoskeleton BSFL Diet.

SME: Specific Mechanical Energy

Table 4.2: Physical Pellet Quality Measurements

Diet	**1**	**2**	**3**	**4**	**5**	**6**	**SEM (±)**
Parameter	**Control**	**Full-fat**	**Defat**	**Dechitin**	**BSFL oil**	**Exoskeleton**	
Moisture Content (%)	4.8	5.3	5.0	5.0	4.8	6.9	0.33
Bulk Density (gm/l)	566.7	586.3	540.3	571.3	528.7	560.7	4.80
Hardness (N)	18.2	12.3	13.4	11.1	23.6	14.0	0.82
Durability (%)	97.7	96.4	98.0	93.7	98.6	98.3	0.42
Expansion (%)	18.4	1.6	17.6	-0.8	32.4	22.0	0.89
Sinking Speed (s/m)	10.0	12.2	11.1	12.8	12.7	11.9	0.25

Moisture content, hardness, durability, expansion and sinking speed were measured in coated pellets whereas bulk density was measured in uncoated pellets.

1: Control Diet. 2: Full-fat BSFL Diet. 3: Defatted BSFL Diet. 4: Dechitinized BSFL Diet. 5: BSFL Oil Diet. 6: Exoskeleton BSFL Diet.

SEM: Standard Error Mean

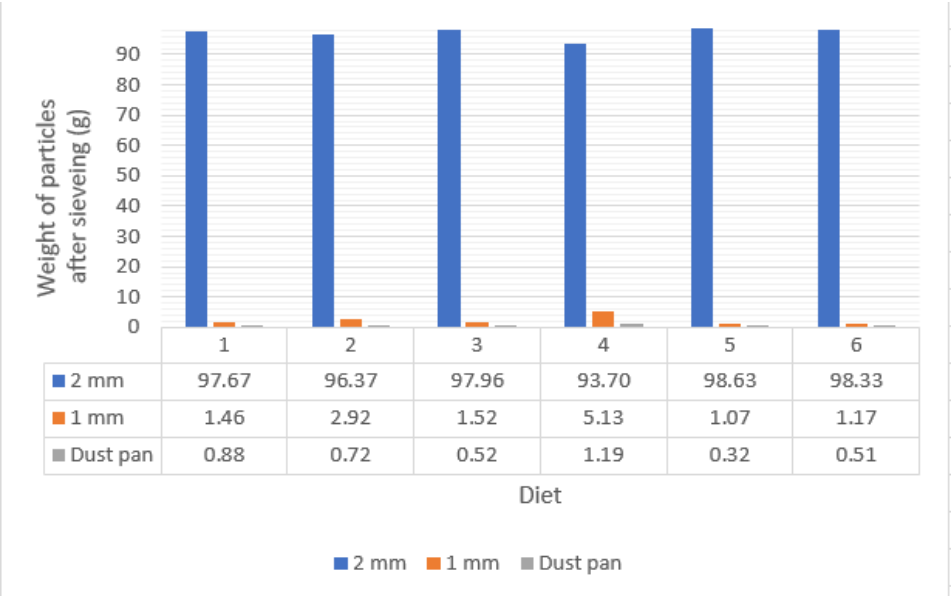

Figure 4.2: Particle Size Distribution of Pellets after Doris Test

4.2 Fish Performance

The number of fish that died within the experiment period was four. The values for performance indicators of fish are shown in Table 4.3. There were no differences in FBW, BWG and SGR between fish fed with defatted, BSFL oil and exoskeleton diets, and control diet. FBW, BWG and SGR were higher in fish fed full-fat and dechitinized BSFL diet compared to fish fed control diet. FI was similar among different diets, except higher for fish fed full-fat BSFL diet. Likewise, there were no significant differences in FCR between fish fed with BSFL diets and control diet. FCR was higher in fish fed full-fat diet compared to dechitinized and exoskeleton diet.

4.3 Nutrient Digestibility

The values of apparent digestibility coefficients are shown in Table 4.4. The dry matter, ash, starch and lipid digestibilities in BSFL diets were not different from control diet. However, protein digestibility of fish fed with defatted, dechitinized and exoskeleton diets were reduced compared to control diet.Full-fat and BSFL oil diets had similar protein digestibility as control diet.

Table 4.3: Performance of Fish fed on Experimental Diets

Diet	**1**	**2**	**3**	**4**	**5**	**6**	**SEM (±)**	**p-value**
Performance Indicator	**Control**	**Full-fat**	**Defat**	**Dechitin**	**BSFL oil**	**Exo-skeleton**		
Initial Body Weight (g)	28.32	28.29	28.31	28.33	28.27	28.31	0.01	0.142
Final Body Weight (g)	98.4^{c}	114.8^{a}	104.2^{bc}	107.1^{b}	99.8^{bc}	104.7^{bc}	1.41	<0.001
Body Weight Gain (g)	70.1^{c}	86.6^{a}	75.9^{bc}	78.7^{b}	71.5^{bc}	76.4^{bc}	1.41	<0.001
Specific Growth Rate (%)	2.01^{c}	2.26^{a}	2.10^{bc}	2.14^{b}	2.03^{bc}	2.11^{bc}	0.02	<0.001
Feed Intake (g/fish)	53.1^{b}	68.8^{a}	55.9^{b}	59.4^{b}	55.7^{b}	57.1^{b}	1.31	<0.001
Feed Conversion Ratio (%)	0.76^{ab}	0.80^{a}	0.74^{b}	0.76^{ab}	0.78^{ab}	0.75^{b}	0.01	0.013

1: Control Diet. 2: Full-fat BSFL Diet. 3: Defatted BSFL Diet. 4: Dechitinized BSFL Diet. 5: BSFL Oil Diet. 6: Exoskeleton BSFL Diet.
SEM: Standard Error Mean
p-value: p-value for one-way ANOVA. The letters a-c denote significant differences among diets. Values in the same row that share same superscripts are not statistically different ($p<0.05$) according to Tukey's multiple comparison test.

Table 4.4: Nutrient Digestibility of Fish fed with Experimental Diets

Diet	**1**	**2**	**3**	**4**	**5**	**6**	**SEM (±)**	**p-value**
Apparent Digestibility Coefficients	**Control**	**Full-fat**	**Defat**	**Dechitin**	**BSFL oil**	**Exo-skeleton**		
Dry Matter	73.5	75.6	74.8	74.3	73.5	73.9	0.27	0.175
Ash	26.1^{abc}	29.8^{a}	29.4^{a}	28.6^{ab}	22.0^{bc}	21.1^{c}	0.97	0.003
Starch	84.2	82.0	82.8	83.2	84.9	81.6	0.37	0.054
Lipid	96.4	97.3	96.8	96.2	96.5	96.8	0.21	0.771
Protein	89.4^{a}	88.0^{abc}	87.4^{bc}	86.9^{c}	89.0^{ab}	86.7^{c}	0.28	0.001

1: Control Diet. 2: Full-fat BSFL Diet. 3: Defatted BSFL Diet. 4: Dechitinized BSFL Diet. 5: BSFL Oil Diet. 6: Exoskeleton BSFL Diet.
SEM: Standard Error Mean
p-value: p-value for one-way ANOVA. The letters a-c denote significant differences among diets. Values in the same row that share same superscripts are not statistically different ($p<0.05$) according to Tukey's multiple comparison test.

4.4 Nutrient Utilization

Table 4.5: Nutrient Retention Parameters of Fish fed with Experimental Diets

Diet	**1**	**2**	**3**	**4**	**5**	**6**	**SEM (±)**	**p-value**
Nutrient Retention Parameter	**Control**	**Full-fat**	**Defat**	**Dechitin**	**BSFL oil**	**Exo-skeleton**		
Protein Efficiency Ratio (g/g)	2.60^{ab}	2.61^{ab}	2.75^{a}	2.64^{ab}	2.57^{b}	2.59^{ab}	0.02	0.028
Lipid Efficiency Ratio (g/g)	9.3^{a}	6.5^{d}	7.8^{c}	8.5^{b}	7.6^{c}	8.0^{c}	0.21	<0.001
Apparent Protein Retention (%)	48.4	47.4	50.5	48.6	48.6	48.3	0.32	0.087
Apparent Lipid Retention (%)	132.5^{a}	88.3^{c}	107.7^{b}	114.8^{ab}	112.0^{b}	103.8^{bc}	3.46	<0.001
Apparent Phosphorus Retention (%)	43.4^{b}	47.2^{b}	55.9^{a}	47.3^{b}	44.0^{b}	45.9^{b}	1.16	0.003

1: Control Diet. 2: Full-fat BSFL Diet. 3: Defatted BSFL Diet. 4: Dechitinized BSFL Diet. 5: BSFL Oil Diet. 6: Exoskeleton BSFL Diet. SEM: Standard Error Mean

p-value: p-value for one-way ANOVA. The letters a-d denote significant differences among diets. Values in the same row that share same superscripts are not statistically different ($p<0.05$) according to Tukey's multiple comparison test.

The values for nutrient retention parameters are shown in the Table 4.5. There were no differences in protein efficiency ratio (PER) between fish fed with BSFL diets and control diet, but fish fed defatted diet had higher PER than control diet. On the other hand, all BSFL diets had lower lipid efficiency ratio (LER) than control diet, where full-fat diet had the lowest. There were no significant differences in apparent lipid retention between fish fed with dechitinized and control diet. However, the other BSFL diets showed lower lipid retention compared to control diet. There were no differences in apparent phosphorus retention between fish fed with full-fat, dechitinized, BSFL oil and exoskeleton diets, and control diet, however, fish fed defatted diet had higher P retention than control diet.

Chapter 5

Discussion

5.1 Feed Production Parameters and Pellet Quality

High lipid in mash prior to extrusion can affect production parameters and the following pellet quality. In the present study, full-fat and dechitinized BSFL diets had higher BSFL inclusion level (20.4% and 25% respectively). Energy inputs of these diets such as SME, torque, die temperature, bar pressure and drive power were lower compared to the other dietary treatments. This further shows that higher BSFL inclusion in diet resulted in lower energy inputs during extrusion. Lipid levels were high in the mash of full-fat and dechitinized BSFL diets (8% and 13% respectively) due to high lipid level in full-fat and dechitinized BSFL meals respectively. This resulted in increased lubrication during extrusion. Therefore, screw speed was increased for these diets, however, decrease in die pressure and torque resulted in a decreased SME. Similar changes were observed by Weththasinghe et al. (2021). Higher lipid content (more than 7%) acts as a lubricant as it becomes difficult to convert mechanical energy into required heat for cooking (Riaz, 2000) and therefore reduces friction in the extruder. This leads to decrease in SME, dough temperature, torque, bar pressure and drive power. Extruder water was also higher for full-fat and dechitinized diets than others, and similar to lipid, it can also reduce friction. Moisture is required for starch gelatinization and protein denaturation. Same as lipids, high moisture content decreases the required mechanical energy (Riaz, 2000). This can also lead to lower dough temperature and leads to reduced starch gelatinization, expansion and pellet quality (Morken et al., 2012). As a result of increasing screw speed of the extruder was increased to minimize this effect of high lipid and moisture during extrusion. High screw speed can also lead to higher torque, thus increase in viscosity causes more friction (Huang et al., 1995) and further to increased dough temperature and

then finally to more expansion. The temperature of the extruder barrels was manipulated to possibly obtain required bulk density and pellet quality. Increased screw speed to compensate for high lipid diets does not seem to be enough which is indicated by lower last barrel and die temperature, SME and torque. Modification of screw configuration might have resulted in better results as it is crucial for better extrusion parameters and pellet quality (Gogoi et al., 1996). Hardness and durability were also lower in the same diets (full-fat and dechitinized diets), indicating that high lipid content affects the physical quality of pellets. This was in line with Sørensen et al. (2009), as reduced physical quality of pellets was observed in FM diet containing higher lipid content. On the contrary, durability and hardness of pellets did not reduce with inclusion of BSFL meal (Weththasinghe et al., 2021).

In the present study, the high lipid content in the mash resulted in lower expansion of the pellets. High lipid and water content also resulted in lower pellet hardness and durability. This is in line with Hansen et al. (2010) and Hansen et al. (2011). Increasing the amount of lipid inclusion in the mash seemed to result in reduced cooking of the dough in the extruder and therefore lowered durability, hardness, expansion and water stability of the pellets. Similar results were obtained by Weththasinghe et al. (2021). Decreased filling rate in the extruder can be caused by increased screw speed. This decrease in the degree of filling in the extruder barrel can further reduce temperature of the dough because of insufficient heat transfer from the barrel (Huang et al., 1995). In accordance to the present study, Ilo et al. (2000) and Hansen et al. (2011) also observed reduction in expansion of pellets with increasing dietary lipid level. High lipid content increases lubrication in the extruder barrel, which further reduces dough temperature, starch gelatinization and expansion of the diets (Lin et al., 1997; Schweizer et al., 1986). As a result of reduced expansion of diets with higher lipid content (full-fat and dechitinized diets), there was reduction in pellet water stability as well. However, extrusion processing parameters (Baeverfjord et al., 2006) and composition of ingredients (Lim & Cuzon, 1994) can also influence water stability of diets.

5.2 Fish Performance

As indicated by FI, palatability of the diets was only affected by dietary inclusion of full-fat BSFL in feed and not by the inclusion of other BSFL fractions. Previous results showed

no effect of full-fat BSFL meal diets (Weththasinghe et al., 2021) and partially defatted meal diet (Belghit et al., 2019a) on FI of Atlantic salmon. Although, Belghit et al. (2018) observed no effect on FI of Atlantic salmon by dietary inclusion of BSFL oil which is in line with this present study. On the other hand, FI of juvenile Japanese seabass increased with increasing dietary inclusion level of defatted BSFL meal (Wang et al., 2019). The present results show that FCR had low variation among the dietary treatments. However, full-fat BSFL meal resulted in higher FCR than defatted and exoskeleton diets. In accordance with the present result, full-fat BSFL diet (Weththasinghe et al., 2021) and partially defatted BSFL diet (Belghit et al., 2019a) had no significant effect on FCR in Atlantic salmon. No effects of dietary inclusion of BSFL oil on FCR in Atlantic salmon was also observed by Belghit et al. (2018) which is in line with the present study. In another study carried out by Karlsen et al. (2017), SGR and FCR were not affected in Atlantic salmon with inclusion of different levels of chitin in their diet. Similarly, growth performance in terms of FBW, FI, SGR and FCR was also not affected in Jian carp by the inclusion of BSFL meal in the diet (Zhou et al., 2018). In the present study, performance of fish in terms of BWG and SGR was significantly higher for fish fed full-fat and dechitinized meal diets compared to the FM control. Similar result of improved BWG and SGR were seen in Siberian sturgeon (*Acipenser baerii*) when fed with full-fat BSFL meal diet (Rawski et al., 2020).

Chitin is found abundantly in insects (Elieh-Ali-Komi & Hamblin, 2016) and BSF consists of 8-26% chitin depending upon its different life stages (Soetemans et al., 2020). Chitin is indigestible to some fish species because of lack of chitinolytic activity in the digestive tract (Rust, 2003). Growth decreased in salmon (Olsen et al., 2006) and rainbow trout (Wojno & Dabrowska, 1984) when high amount of krill meal containing chitin was included in their diet. Decrease in growth of Atlantic salmon was also observed by (Karlsen et al., 2017) when chitin level in feed was 2% and 5%. However, in the present study, there was no differences in fish performance between dechitinized and exoskeleton diets indicating that the amount of chitin (0.5% and 1.4% respectively) had no effect on Atlantic salmon. When Atlantic salmon was fed with high level of dechitinized BSFL meal, growth of the fish was not affected (Belghit et al., 2018). Atlantic salmon have low ability to digest chitin as 12.9-40% chitin digestibility has been reported (Olsen et al., 2006). Karlsen et al. (2017), on the other hand, discussed that poor utilization of chitin could lead a reduced energy intake and therefore, growth can be affected at high inclusion levels of chitin. This implies that the chitin content in diets of the

present study was not high enough to reduce growth of Atlantic salmon.

Dietary inclusion of full-fat BSFL meal (20%) resulted in better growth performances in Atlantic salmon compared to other dietary treatments. In contrast to the present study, dietary inclusion of 25% of partially defatted BSFL meal resulted in impaired growth in Atlantic salmon post smolts (Lock et al., 2016). In the present study, BSFL diets had higher lipid level in the diets than control diet. Among the BSFL diets, full-fat BSFL diet had the highest level of lipid (17.4%) and resulted in better growth performances in fish compared to the other diets. Similar result was observed in a study by Hamre et al. (2004) where high lipid level in diet had positive effect on growth and feed conversion of Atlantic salmon. In a study by Einen and Roem (1997) in Atlantic salmon, higher growth was observed in large sized fish with diet containing lower dietary protein and higher dietary lipid whereas growth in small sized fish was improved with diet containing higher dietary protein and lower dietary lipid. Utilization of dietary energy can depend on the growth rate of fish as slow-growing fish are most likely to utilize a high dietary energy for maintenance than fast-growing fish (Einen & Roem, 1997). High lipid diets are supplied so that fish utilize energy from fat, making more protein available for the growth (Mock et al., 2018). The present study also shows similar outcome of higher growth rate in fish fed full-fat diet despite its low protein content.

In the present study, dietary inclusion of BSFL oil resulted in similar growth performances to Atlantic salmon fed control diet. Addition of BSFL oil in the diet alters the fatty acid profile; increasing the amount of saturated fatty acids in the diet, mostly lauric acid which is an essential unit of medium chain TAG (Lock et al., 2016). According to Nordrum et al. (2000) and Nordrum et al. (2003), feed intake and growth of Atlantic salmon impaired with the intake of medium chain TAG. On the other hand, according to Belghit et al. (2018), dietary inclusion of BSFL oil produced from BSFL grown on different media had significant effect on growth of Atlantic salmon, as BSFL grown on organic waste had lower SGR and growth index compared to that grown on organic waste and marine macroalgae. Likewise, dietary inclusion of 25% of partially defatted BSFL meal resulted in impaired growth in Atlantic salmon post smolts (Lock et al., 2016) which was also grown on organic waste. On the contrary, in the present study, full-fat BSFL meal diet, showed better fish performance in Atlantic Salmon.

5.3 Nutrient Digestibility

ADC indicates a measure of availability of nutrients in feed (Fagbenro, 2004). DM is an indicator of available nutrients for an animal and ADC of DM indicates a better estimation of quantity of indigestible matters in feed (Fagbenro, 2004; Nennich & Chase, 2019). In the present study, dietary inclusion of different BSFL meal fractions had no effect on ADC of DM in Atlantic salmon. However, Renna et al. (2017) observed higher ADC of DM in rainbow trout when dietary inclusion of partially defatted BSFL meal was 20% whereas Mohamad-Zulkifli et al. (2019) observed lower ADC of dry matter in hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) when dietary inclusion of BSFL meal was 30%. Further, high level of ash in the diet can affect digestibility of DM (Rahman et al., 2016). Low DM digestibility in rainbow trout was observed with high ash content (Bureau et al., 1999). However, in the present study, although ash content of dechitinized diet was higher than other diets, DM digestibility was not affected in Atlantic salmon. Ash is the measure of total minerals in the diet. In the present study, ADC of ash in fish fed BSFL diets were similar to fish fed control diet. However, exoskeleton diets resulted in the lowest ADC of ash whereas full-fat meal and defatted meal inclusion in diets resulted in higher ADC of ash in Atlantic salmon. Ash digestibility in fish is complicated as both minerals from diet and minerals from water need to be considered (Watanabe et al., 1997). Besides, extrusion processing can also have an effect on ADC of nutrients as ADC of phosphorus was reduced in extruded diets for rainbow trout (Cheng & Hardy, 2003). Further, in the present study, dietary inclusion of different BSFL meal fractions had no effect on ADC of starch in Atlantic salmon. On the contrary, previous result observed positive effects on ADC of starch with dietary inclusion of BSFL meal as it was the highest at 25% inclusion level (Weththasinghe et al., 2021). Previously, starch digestibility in salmon varied on source of starch, dietary inclusion level, and processing of the starch in feed (Hua & Bureau, 2009). Gelatinization of starch improved starch digestibility in fish at low inclusion level, and water temperature and faecal collection method were also found to vary starch digestibility. Similarly, in another study by Grisdale-Helland and Helland (1997), high dietary lipid level reduced starch digestibility in Atlantic salmon. However, all the factors did not seem to have any effect on ADC of starch in the present study.

Dietary inclusion of different BSFL fractions had no effect on ADC of lipid in Atlantic salmon. In the previous study, ADC of lipid was reduced in Atlantic salmon with increasing inclusion of BSFL meal (Weththasinghe et al., 2021). Likewise, Karlsen et al. (2017) observed decrease in ADC of lipid in Atlantic salmon when chitin was included in their diet. Besides, Belghit et al. (2019b) reported reduced ADC of lipid in Atlantic salmon with inclusion of BSFL meal and oil in diets for Atlantic salmon which was in contrary to the present study. Furthermore, high dietary inclusion of BSFL meal reduced ADC of lipid in Atlantic salmon (Belghit et al., 2018) which was also in contrast to the present study. Digestibility of lipid can be affected by dietary lipid level, length of FA chain, degree of unsaturation and other constituents of FA (Yuan et al., 2010). According to Olsen (1997), there are two opposite effects of increasing lipid content in fish; it might burden the digestive capacity of fish or provide more time for lipid utilization by slowing down gastric evacuation. Likewise, high saturated FA content of lipid could result in reduced digestibility of lipid (Cho & Kaushik, 1990). However, in the present study, these factors had no effect on lipid digestibility which indicate that inclusion level of BSFL and lipid was not high enough to reduce lipid digestibility.

The ADC of protein in Atlantic salmon fed full-fat meal diet and BSFL oil diet in the present study was similar to control diet, whereas, defatted, dechitinized and exoskeleton diets resulted in reduced ADC of protein compared to control diet fed fish. Renna et al. (2017) observed higher ADC of protein in rainbow trout when dietary inclusion of partially defatted BSFL meal was 20%, whereas 40% inclusion level reduced ADC of protein in rainbow trout. Karlsen et al. (2017) also observed decrease in ADC of protein in Atlantic Salmon when chitin was included in their diet. Similarly, Belghit et al. (2018) also observed reduction in ADC of protein in Atlantic salmon when dietary inclusion of BSFL meal was high. The reduction in ADC of protein might be due to low digestibility of chitin. Chitin contain N and will be included in the analysis of protein in the faeces contributing to an overestimation of faecal protein content (Karlsen et al., 2017). In the insect exoskeleton, chitin is incorporated within a matrix containing protein, lipid and other compounds (Kramer et al., 1995). This might prevent the degrading enzymes to reach their substrates and might arrest intestinal absorption of protein and lipid (Tanaka et al., 1997). This impairs chitin digestibility and also lipid and protein digestibility, thus leading to lower nutrient utilization and growth performances in fish (Henry et al., 2015). This is in line with Hansen et al. (2010) and Karlsen et al. (2017) as lower

ADC of nutrients in Atlantic salmon was observed when fed with chitin containing diets. However, in the present study, the use of dechitinized meal did not improve ADC of protein as dechitinized diet and exoskeleton diet resulted in similar ADC of protein. However, BSFL oil diet and control diet resulted in similar ADC of protein which might be due to absence of chitin in the BSFL oil diet.

5.4 Nutrient Utilization

The PER in Atlantic salmon fed BSFL diets and control diet were similar whereas, dietary inclusion of BSFL fractions had no significant effect on protein retention. Despite lowered ADC of protein in defatted, dechitinized and exoskeleton diets, utilization of protein in fish fed BSFL diets were similar to control diet. This might be due to protein sparing effect by dietary lipid (Cho & Kaushik, 1990) or N-sparing effect by nucleic acid of BSFL, as previous result showed higher N retention in salmon fed bacterial meal even though digestibility of nutrients were lower (Øverland et al., 2010). In the previous study (Weththasinghe et al., 2021), dietary inclusion of BSFL meal had no effect on protein retention in Atlantic salmon which is in line with the present study. Similarly, replacing FM with BSFL meal in the diet of Atlantic salmon had no effect on protein retention of fish (Belghit et al., 2019a; Lock et al., 2016) which is also in line with the present study. Likewise, Belghit et al. (2018) reported no effects of dietary inclusion of BSFL meal on PER of Atlantic salmon pre-smolts whereas, Renna et al. (2017) reported no effect on PER of rainbow trout. Fisher et al. (2020), on the other hand, observed increased PER in Atlantic salmon pre-smolts at 30% BSFL meal inclusion.

Both LER and lipid retention was reduced in Atlantic salmon fed BSFL diets despite of no effect of BSFL diets on ADC of fat. The fish fed full-fat BSFL diet resulted in the lowest LER and lipid retention values. Reduction in LER with inclusion of BSFL fractions is in line with Weththasinghe et al. (2021) and Kroeckel et al. (2012) in Atlantic salmon and juvenile turbot respectively. In the present study, low lipid utilization in fish fed full-fat diet might be due to high lipid content in the diet compared to other diets. This is in line with Cho and Watanabe (1985) and Du et al. (2005) who observed lower lipid retention in rainbow trout and juvenile grass carp (*Ctenopharyngodon idella*) respectively fed higher lipid diets. Lower lipid utilization suggests a higher lipid proportion being used for energy. As discussed above on

fish performance, lauric acid is the most abundant saturated fatty acid. Lauric acid is oxidized more as a energy source than used for lipid deposition and therefore results in lower LER and lipid retention (Belghit et al., 2019b; Renna et al., 2017). Low lipid utilization might be due to presence of chitin in the diets as well. Karlsen et al. (2017) observed negative effects of nutrient utilization in Atlantic salmon when chitin level (chitin from prawn shells) in their diet was more than 1% which is in line with the present study.

Apparent P retention, on the other hand, was higher in Atlantic salmon fed the defatted diet whereas P retention of other diets were similar. Dietary P content, however, was higher in dechitinized diet compared to other diets. In the previous study by (Weththasinghe et al., 2021), total P content were similar in all diets, and dietary inclusion of BSFL meal had no effect on P retention and faecal P excretion of Atlantic salmon. In the present study, despite higher dietary P, dechitinized diet did not improve P retention in Atlantic salmon which suggests that P in the diet was less available to the fish. This is in line with Nordrum et al. (1997) who observed lower absorption of P when excess unavailable dietary P was supplied to Atlantic salmon. Previously, P retention in rainbow trout was significantly higher when ingredients having highly bioavailable P was used in the diet even though the total P content in the diet was lower (Sarker et al., 2011). This might be the reason for higher P retention in defatted diet in the present study despite lower dietary P content than dechitinized diet. Likewise, in rainbow trout, diet containing low FM and low phosphorus ingredients such as defatted SBM and corn gluten meal resulted in higher phosphorus retention (Satoh et al., 2003). Phosphorus from alternate protein sources and monocalcium phosphate were highly digestible in rainbow trout. Meanwhile, phosphorus from plant source is released as waste in fish because they cannot digest phytin (Luzier et al., 1995). Another reason for lower phosphorus absorption could be formation of complexes with other minerals which makes it difficult to digest (Lall, 1991).

Chapter 6

Conclusion

The present study showed a decrease in pellet quality when full-fat and dechitinized meals were used in the diet. Full-fat and dechitinized BSFL diets resulted in better growth performances in Atlantic salmon. Dry matter, starch and fat digestibilities were not affected by inclusion of different fractions of BSFL in the diet, however, defatted, dechitinized and exoskeleton diets resulted in lower protein digestibility. The inclusion of different fractions in the diet did not affect protein retention, however, they reduced lipid efficiency ratio and lipid retention. Further, the defatted BSFL diet had higher phosphorus retention in fish compared to the control diet.

6.1 Future Remarks

To improve pellet quality of diets containing BSFL fractions, especially full-fat and dechitinized diets, further study on modifications of screw configuration and screw elements is required. Further, as expected from the previous study (Weththasinghe et al., 2021), the present study did not result in reduced growth in Atlantic salmon implying that chitin content in the diets were not high enough. Therefore, designing experiments using higher dietary chitin levels should be addressed.

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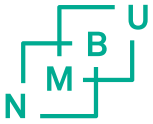

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