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Baltic blue mussel (*Mytilus edulis L.*) and black soldier fly (*Hermetia illucens*) combined with pea protein concentrate as protein sources in feed for rainbow trout (*Oncorhynchus mykiss*)

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Keywords: aquaculture, rainbow trout, Baltic blue mussel, black soldier fly larvae, digestibility, protein, fishmeal replacement

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Abstract

A feeding trial was conducted for 9 weeks to test the growth performance, nutrient retention and digestibility in rainbow trout (*Oncorhynchus mykiss*) in order to assess the potential of Baltic blue mussel meal (*Mytilus edulis L*) and black soldier fly larvae (*Hermetia illucens*) meal as protein sources. The dietary treatments consisted of one control diet based on high quality fishmeal, one control diet based on pea protein concentrate for testing dietary palatability and two fishmeal-free experimental diets where approximately 20% of the crude protein from the fishmeal was replaced with the protein from either de-shelled blue mussel meal or larvae meal on a dry matter basis. These four experimental diets were randomly fed to quadruplicate groups of fish. Replacing the dietary fishmeal with mussel meal and larvae meal negatively affected feed intake, final body weight, specific growth rate, feed conversion ratio and nutrient retention of rainbow trout. The apparent digestibility coefficient (ADC) of crude protein, crude lipid, and essential amino acids was not affected by replacing the dietary fishmeal with mussel meal and fish fed mussel meal diet even displayed increased nutrients digestibility, compared with fish fed control diet. Replacing fishmeal with larvae meal did not affect the apparent digestibility coefficient of crude protein.

In conclusion, the ADC values showed that both mussel meal and larvae meal could be regarded as promising protein sources for rainbow trout. However, the growth performance and nutrient retention were negatively affected by low feed intake. The absence of fishmeal and the high inclusion of pea protein concentrate in the diets resulted in the poor palatability and were considered the reasons of low feed intake. Therefore, inclusion of fishmeal or feed stimulants might be needed to increase feed palatability. Furthermore, the inclusion of pea protein should be adjusted to an appropriate level.

Keywords: Aquaculture, rainbow trout, Baltic blue mussel, black soldier fly larvae, digestibility, protein, fishmeal replacement

Sammanfattning

En foderförsök genomfördes under 9 veckor för att testa tillväxtprestanda, näringsinnehåll och smältbarhet i regnbåge (*Oncorhynchus mykiss*) för att bedöma potentialen för Östersjö blå mussla (*Mytilus Edulis*) och amerikansk vapenfluga (*Hermetia Illucens*) som proteinkällor. Kostbehandlingarna bestod av en kontrollfoder baserad på fiskmjöl, en kontrollfoder baserad på ärtprotein koncentrat för testning av smaklighet och två fiskmjölfria försöksfoder där cirka 20% av råproteinet från fiskmjölet ersattes med proteinet från skalad blå musslor eller larvmjöl, på torrsubstans basis. Dessa fyra försöksfoder matades slumpmässigt till fyrdubbla grupper av fisk. Byte av fiskmjöl med musslor och larvmjöl har negativt påverkat foderintag, slutlig kroppsvikt, specifik tillväxthastighet, foderomvandlingsförhållande och näringsinnehåll av regnbåge. Den uppenbara smältbarhetskoefficienten för råprotein, rå lipid och essentiella aminosyror påverkades inte av att ersätta fiskmjöl med blåmusslor och fisk matad med musselmjölfoder, även visat ökad näringsämnes smältbarhet jämfört med fisk matad kontrollfoder. Byte av fiskmjöl med larvmjöl påverkar inte den uppenbara smältbarhetskoefficienten för råprotein.

Sammanfattningsvis visade ADC-värdena att både blåmusslor och larvmjöl kan betraktas som lovande proteinkällor för regnbåge. Växthastigheten och näringsinnehållet påverkades dock negativt av ett lågt foderintag. Frånvaron av fiskmjöl och den höga upptagningen av ärtprotein koncentrat i foder resulterade i den dåliga smakligheten och ansågs orsakerna till lågt foderintag. Därför kan inkludering av fiskmjöl eller foderstimulerande medel behövas för att öka foder smaklighet till fisk. Dessutom bör införandet av ärtprotein justeras till en lämplig nivå.

Nyckelord: Vattenbruk, regnbåge, Östersjö blåmusslor, Amerikans vapen fluga, smältbarhet, protein, fiskmjölsersättning

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1 Introduction

In recent years, aquaculture is the fastest growing food production sector globally (FAO, 2018) and its production provides with 50% of all fish consumed by humans. FAO (2018) shows that production of fish for human consumption grew from 9.0 million tons in 1961 to 151.2 million tons in 2016 globally.

However, the use of fishmeal and fish oil in fish feeds has doubled since 1990s (Naylor *et al.*, 2009). Fishmeal has traditionally been considered an important protein source in aquaculture formulations for both carnivorous and omnivorous species (Glencross *et al.*, 2007) and it is partly dependent on wild-caught fish. Global supplies of fishmeal and fish oil are overstretched and the declines in wild fish stocks together with high demand in the last twenty years have resulted in the price increase of fishmeal (Belforti *et al.*, 2015). As a consequence, the use of fishmeal in aquaculture may be questioned and the substantial effort of identifying a wide range of environmentally friendly and economically viable alternatives to fishmeal and fish oil has been expanded over the past decades.

There are many characteristics to be considered when replacing fishmeal with alternative ingredients, including protein content, possible amino acid deficiency, nutrient digestibility and absorptivity (Gatlin III *et al.*, 2007). Reasonable price, no antinutritional factors and extensive availability are also necessary for the alternative to be a viable replacement for fishmeal.

Plant products including oilseeds, legumes and cereal grains are currently the major alternative protein sources used to substitute for fishmeal in fish aquaculture feeds (Palmegiano *et al.*, 2005). Unfortunately, the presence of antinutritional components and certain nutritional characteristics like inappropriate fatty acids profile and unbalanced essential amino acids limits these plants to be an alternative to fishmeal and fish oil (Gatlin III *et al.*, 2007). Moreover, fish instinctively reject to eat feeds with unpalatable flavor like bitterness and sourness, so factors relating to palatability may impair their feed intake (Nogales-Mérida *et al.*, 2018).

Insects have been highlighted by lots of researchers (de Haro *et al.*, 2014; Lock *et al.*, 2016; Sánchez- Muros *et al.*, 2016) and they are identified as one of the renewable feed sources for fish due to their high and valuable protein contents. Furthermore, blue mussel could also be one of promising candidates to replace fishmeal. It has been evaluated as a replacement for fishmeal in feeds for organic poultry (Jönsson & Elwinger, 2009), Arctic charr (*Salvelinus alpinus*) and Eurasian perch (Langeland *et al.*, 2016).

Black soldier fly (*Hermetia illucens*) larvae (BSFL) meal and Baltic blue mussel (*Mytilus edulis* L.) meal were used in this paper. The aim of this project was to estimate the potential of Baltic blue mussel meal and BSFL meal as protein sources in feed for rainbow trout (*Oncorhynchus mykiss*) by assessing the growth performance, nutrient retention and digestibility. The results of this project aim to expand the knowledge on use of sustainable resources in aquaculture feeds.

2 Background

2.1 Aquaculture and rainbow trout

Aquaculture is a well-established staple food producer despite being the youngest animal production sector (Beveridge, 2008). The Figure 1 shows that production from capture fisheries and aquaculture in 2004 was 85.5 and 18.3 million tons respectively but by 2016 the figures became to 90.9 and 80 million tons respectively, with aquaculture supplying 47% of the total.

Moreover because of growing population, increasing incomes and pursuing healthier diets, the human consumption of fish is also on the rise after a little fluctuation (Figure 1). Firstly, fish provide people with high quality protein with balanced amino acid profile compared with beef, pork and poultry production (Brunner *et al.*, 2009). Then oily fish is one of the sources of omega-3 fatty acids (Kris-Etherton *et al.*, 2002), which are associated with health benefits. Finally, enormous employment opportunities from construction to farm management have been developed by the rapidly growing aquaculture sector (Khan *et al.*, 2011). Therefore, aquaculture has the potential to be an important food producer nutritionally and economically.

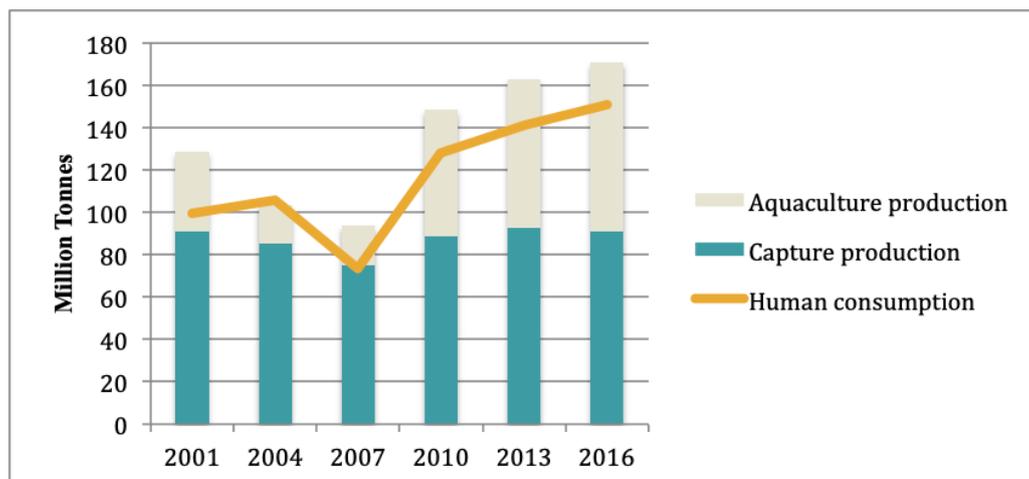


Figure 1 The production from capture fisheries and aquaculture and human consumption of fish

Global aquaculture is dominated by Asia, contributing to over 90% of global aquaculture production; with China is by far the world's top producer and around 50 million tons aquaculture production was produced in 2016 (FAO, 2018). Except Asia, aquaculture has also grown substantially in Europe, from about 2 million tons aquaculture production in 2004 to around 3 million tons in 2016 (FAO, 2018). Aquaculture production in Sweden has kept an increasing trend, which increased from 4919 tons in 2007 (Sweden Statistics, 2008) to 12800 tons in 2017 (Sweden Statistics, 2018).

Rainbow trout dominates the aquaculture production in Sweden, which accounted for 88% of the cultivated fish in 2017 (Sweden Statistics, 2018). It is probably the most widely introduced salmonid fish species in the world, and is has been introduced into at least 99 different countries (Stanković *et al.*, 2015). As cold-water specie, rainbow trout can survive in water between approximately 10 °C and 20 °C, so it has the advantage of maintaining relative high growth rate at low temperature for aquaculture in the Nordic

region like Sweden. Also, rainbow trout has been regarded as one of the sources of biological value protein and this fish species is rich in polyunsaturated fatty acid (PUFA) content (Secci *et al.*, 2019).

2.2 Protein sources

2.2.1 Fishmeal

Fishmeal is considered one of the most adequate and sought protein sources for aquaculture since its high-quality protein content with the excellent composition of essential amino acids and high digestibility (Oliva-Teles *et al.*, 2015). Fishmeal is also an excellent source of lipids, minerals (including calcium and phosphorus) and vitamins (including cobalamin, niacin, choline, pantothenic acid and riboflavin) (Miles and Chapman, 2006). Highly digestible fish lipids are rich sources of PUFAs, with omega-3 fatty acids being predominant (Miles and Chapman, 2006). Therefore, it is not surprising that fishmeal has been used as the major dietary protein source for many important farmed fish, especially for carnivorous fish.

However, the availability of fishmeal is limited in recent years because the average levels of capture fisheries have decreased globally. Tacon and Metian (2009) reported that over 36.2% of the total world fisheries catch was destined for fishmeal production in 2006 and more than 75% of the world fish stocks were fully exploited or overexploited. From environmental and ethical standpoints, it is unsustainable and incorrect to overfish for aquaculture feed when this source could be consumed directly by humans. Thus, in order to reduce the dependency of aqua-feeds on fishmeal, looking for alternative ingredients with high-quality protein like insect and blue mussel should be a high research priority.

2.2.2 Baltic blue mussel

The blue mussel meal is one of the promising candidates to replace fishmeal because of its high protein content (64.5% of dry matter), with an appropriate amino acid profile similar to fishmeal (Berge & Austreng, 1989). Berge & Austreng (1989) also reported that the meat of blue mussel has low fat content but the requirement for essential fatty acids in rainbow trout can be satisfied with the favorable lipid composition. Vidakovic *et al* (2015) showed that the growth performance was unaffected when 40% of fishmeal was replaced by de-shelled blue mussel in the diet for Arctic charr.

Blue mussel farming has experimentally been started along coastlines of the Baltic Sea as a tool to achieve nutrients reduction. The Baltic Sea is considered one of the world's most polluted seas with environmental threats such as persistent pollution and eutrophication caused by the intensification of nitrogen and phosphorous (Cheng *et al.*, 2017). Filter feeders live and grow by filtering off phytoplankton in the water (Newell, 2004) and it has been shown to improve the water quality and transparency (Haamer & Rodhe, 2000). *Mytilus edulis* is a native mussel species from the Baltic Sea (Stadmark & Conley, 2011) and they are relatively easy to cultivate (Jönsson & Elwinger, 2009), the substrate for them are typically ropes and nets. However, blue mussels from the Baltic Sea are too small and fragile to consume by human because of the low water salinity, so

these less valuable blue mussels could be reintroduced back into the food chain in the form of meal (Cheng *et al.*, 2017). In the long run, blue mussel not only could be a high-quality protein source but also have ability to reduce the nutrient contents in the Baltic Sea.

2.2.3 Black soldier fly larvae

As one of the potential protein sources, BSFL has been studied and identified for aquaculture (Van Huis, 2013). They consist of approximately 42% protein and 29% fat even when they are fed plant-based waste like rice straw (Wang & Shelomi, 2017). Insect meals present different profile of amino acids depending on insect species and some insects have limiting lysine, tryptophan and histidine (Sánchez-Muros *et al.*, 2014). However, BSFL as an exception has more interesting amino acid composition, which could meet the requirements of most fish (Magalhães *et al.*, 2017). Therefore, the larval stage may be the most interesting as an alternative to fishmeal and fish oil. In addition, BSFL can be fed on different kinds of inedible organic material and can be utilized in small-scale waste management. It is reported that BSFL has been beneficial in solving environmental problems in term of waste management, for example reduced nitrogen and phosphorus waste (St-Hilaire *et al.*, 2007).

However St-Hilaire *et al.* (2007) found that fish fed feed containing BSFL meal had low omega-3 fatty acids in the rainbow trout fillets, which could be an obstacle towards effective substitution of fishmeal and fish oil. High biological value protein and omega-3 fatty acids content in rainbow trout play an important role in human nutrition (Secci *et al.*, 2019). Omega-3 fatty acids have been linked to healthy benefits throughout life because it benefits fetal development (Swanson *et al.*, 2012), reduces cardiovascular disease, inflammatory disease (Ruxton *et al.*, 2004) and prevents Alzheimer's disease (Swanson *et al.*, 2012).

On the other hand, St-Hilaire and Cranfill (2007) also found that the amount of omega-3 fatty acids in BSFL increased to about 3% after feeding them approximately 10% fish offal. This means that omega-3 fatty acids in substrate could be accumulated by BSFL, which could turn the BSFL meal into a reliable alternative to fish meal and fish oil for rainbow trout. Blue mussel as a marine source also rich in omega-3 fatty acids besides fish and fish oil (Vaidya & Cheema, 2014). Therefore, the Baltic blue mussel was used in present trial as a substrate including omega-3 fatty acids for BSFL to accumulate. This may increase value of the BSFL nutritionally and provide a possible stream of high value fatty acids into the aquaculture production cycle.

3 Material and methods

3.1 Facilities and fish

The experiment was carried out at the aquatic facility, Swedish University of Agricultural Sciences, Uppsala, Sweden, using rainbow trout from commercial producer, Vilstena fiskodling AB (Fjärdhundra, Sweden). Before transferring into experimental tanks, fish were kept in a holding tank connected to a recirculated aquaculture system (RAS). Two days prior to the start of the experimental, 192 rainbow trout with a start body weight (SBW) between 65.9 ± 21.9 g (mean \pm SD) were netted from the holding tank and lightly anesthetized with 40 mg/L tricaine methanesulphonate (MS-222 Western Chemical Inc., Ferdale, WA, USA) before being randomly distributed to 16-200 L experimental tanks (12 fish per tank). Each experimental tank was equipped with feed waste and faeces collectors (Hølland teknologi, Sandnes, Norway) and an automatic belt-feeder (Hølland teknologi, Sandnes, Norway). All the fish were individually tagged with passive integrated transponders (Biomark, Inc., Bose, Idaho, USA). Each tank was supplied with a partial RAS and an addition of fresh municipal water with one liter per minute. During the experiment, water temperature and dissolved oxygen levels were checked daily in 4 random tanks and maintained at 11 ± 1 °C and 10.5 ± 1 mg/L, respectively (HQ40D Portable Multi Meter, Hach, Loveland, CO, USA). Lighting was automatically controlled and maintained on a 12:12 diurnal cycle. The experiment was conducted according to the laws and regulations overseen by the Swedish Board of Agriculture and approved by the Ethical Committee for Animal Experiments in Uppsala, Sweden (dnr 5.8.18-16347/2017).

3.2 Diets and faeces collection

In this experiment the dietary treatments consisted of two reference diets (control diet and PPD) and two test diets. The control diet was formulated to resemble a commercial diet for rainbow trout and the protein was mainly supplied from fishmeal. The PPD was formulated in a similar manner but with no fishmeal, as the main protein source was pea protein concentrate in order to test for effects on dietary palatability. The two test diets were formulated based on the control diet, but replacing approximately 20% of the crude protein (CP) from the fishmeal with a protein from the test ingredient on a dry matter (DM) basis. The test diets contained either de-shelled blue mussel meal (MD) or BSFL meal (LD). Mussel meal was made using Baltic blue mussel (Musselfeed AB company,

Ellös, Sweden) and the BSFL was produced at Swedish University of Agricultural Sciences, Uppsala, Sweden (Department of Energy and Technology; Environmental Engineering Unit). All diets contained 5 g/kg titanium dioxide (TiO₂) as an indigestible marker to determine the nutrient digestibility. The formulation of experimental diets is given in Table 1.

Table 1 Feed formulation (g/kg as is) of the experimental diets

Ingredients	Control diet	MD	LD	PPD
Fish meal	280	-	-	-
Fish oil	50	55	20	50
Pea protein concentrate	280	395	425	550
Wheat meal	160	140	135	155
Larvae meal	-	-	200	-
Mussel meal	-	165	-	-
Cellulose meal	30	30	25	30
Titanium dioxide	5	5	5	5
Rapeseed oil	115	130	110	130
Mineral/ vitamin premix	20	20	20	20
Gelatin	60	60	60	60

MD = diet with blue mussel (*Mytilus edulis* L) meal.

LD = diet with black soldier fly (*Hermetia illucens*) larvae meal.

PPD= diet with pea protein concentrate

All the diets were processed at the laboratory of the Swedish University of Agricultural Sciences, Uppsala, Sweden. Firstly, all the dry dietary ingredients, except gelatin, were weighed and mixed in a horizontal paddle mixer (Elektroheliolux, Electrolux, Stockholm, Sweden) for 20 minutes. Then the mixed ingredients were divided into 10 equal parts and each part was moved into a bench top mixer (Hugin, Coop Sweden AB, Stockholm, Sweden). The gelatin was dissolved in warm water firstly and then transferred to a microwave oven for additional heating until it was thoroughly dissolved. The fish oil and dissolved gelatin were finally added to the bench top mixer and mixed thoroughly. The mash was divided into several firm doughs manually, which was then pressed through a single-screw meat grinder with a 3 mm die (Nima Maskinteknik AB, Örebro, Sweden) and air-dried for 12 hours at 50 °C. After that, small pellets were cut in a blender (Livesmedelsteknik AB, Stockholm, Sweden). The resulting pellets were sieved and stored in 4 plastic containers.

In order to avoid effects of handling stress on feed intake, the fish had a 2-day acclimation period. After two days, the four experimental diets were randomly fed to quadruplicate groups of fish at 10:00 am every day by automatic belt- feeders until the satiation levels were established. Daily feeding rate was 1.5% of their body weight, which was in excess. After feeding, uneaten feed pellets collected by faeces collectors were gathered and stored at 12:00 every day and faeces collection was performed at 8:00am on the following days. Both feed waste and faeces were separately stored at -25 °C until analysis.

Table 2 Chemical composition (g/kg DM) and energy content (MJ/kg DM) of fishmeal, blue mussel (*Mytilus edulis*) meal and black soldier fly (*Hermetia illucens*) larvae meal

	Feed ingredients		
	Fish meal	Mussel meal	Larvae meal
Crude protein	763	666	433
Sum of amino acids	713	526	336
Crude lipid	100	77	262
Ash	148	117	198
Gross energy	22.1	19.1	24.6
Indispensable amino acids			
Arginine	44.0	36.4	15.8
Histidine	15.3	10.8	10.3
Isoleucine	30.9	23.4	16.2
Leucine	59.6	36.7	24.0
Lysine	59.9	41.4	22.9
Methionine	22.6	12.0	7.2
Phenylalanine	30.9	23.1	15.6
Threonine	34.0	27.9	14.5
Valine	37.8	27.1	22.6
Sum	335	238.8	149.1
Dispensable amino acids			
Alanine	47.5	30.8	27.1
Aspartic acid	72.9	63.0	34.3
Cysteine+Cystine	6.7	6.4	3.3
Glutamic acid	109.7	71.7	38.7
Glycine	47.3	35.0	19.9
Ornithine	<0.01	9.2	1.1
Proline	32.6	23.9	19.9
Serine	33.1	26.1	14.5
Tyrosine	27.8	21.4	28.2
Sum	377.6	287.5	187.0

3.3 Sampling of fish and relative body indices

All fish were anaesthetized with 40 mg/L MS-222 and weighted individually at the beginning of the experiment after each period and in the end of the experiment.

At the termination of the experiment, five fish for reference whole-body analysis and five fish for fillet fatty acid analysis were netted randomly from the holding tank, euthanized with an overdose of 300mg/L MS-222 solution. Additionally, 10 fish from each experimental tank were randomly selected and euthanized as previously described, with 5 fish for whole-body analysis and 5 fish for collecting data on hepatosomatic index (HSI) and viscerosomatic index (VSI). The whole gastrointestinal tract and liver from these 5 fish were removed and weighed. During sampling, all of the samples were temporarily held on ice and then transferred to -25°C until biochemical analysis. The remaining 2 fish from each experimental tank were placed back into the holding tank after weighing.

3.4 Sample preparation and chemical analysis

Feed were milled and stored before the analyses. Faeces were freeze-dried, weighed, milled and stored until required for analysis.

DM was determined after drying the samples at 103 °C in a drying oven for 16h and then cooled in a desiccator and weighted. Ash content was determined after combustion in a muffle furnace at 550 °C for 3h until the contents had a white color, then cooled in a desiccator and weighted. The CP content was analyzed by Kjeldahl method (N×6.25; Nordic committee on food analysis, 1976) following acid digestion, using a 2020 digester and a 2400 Kjeltex Analyser unit (FOSS Analytical A/S, Hillerød, Denmark). Gross energy (GE) was determined using an isoperibol bomb calorimeter (Parr 6300; Parr Instrument Company, Moline, IL, USA) and expressed as MJ/kg. Crude lipid (CL) content was determined by acid hydrolysis and extraction (Official Journal of the European Communities, 1998) using a Soxtec2 system (Hydrotec 8000 and a Soxtec 8000 Extraction Unit; FOSS Analytical A/S, Hillerød, Denmark). Amino acid content in feed and faeces were analyzed by SS-EN ISO-13903 (2005) method (Eurofins Food & Feed Testing Sweden, Linköping). TiO₂ was qualified spectrophotometrically after a method described by Short *et al* (1996) with boiling ashed samples in 7.4 M sulphuric acid for 3h.

3.5 Calculations

Growth performance and nutrient utilization of the experimental fish were based on the following parameters:

$$\text{Weight gain (WG, \%)} = ((\text{FBW} - \text{SBW}) / \text{SBW}) \times 100$$

$$\text{Specific growth rate (SGR, \% / d)} = 100 \times ((\ln \text{FBW} - \ln \text{SBW}) / \text{T})$$

$$\text{Feed conversion ratio (FCR)} = \text{FI} / \text{WG}$$

$$\text{Nutrient retention (NR, \%)} = (\text{Nutrient retained in the body} \times 100) / \text{Nutrient ingested}$$

Where FBW is the final body weight (g), SBW is the initial body weight (g), T is the rearing period (days) and FI is the total feed intake (g).

The somatic indexes were obtained as follows:

$$\text{Hepatosomatic index (HSI, \%)} = (\text{LW} / \text{FW}) \times 100$$

$$\text{Viscerosomatic index (VSI, \%)} = (\text{VW} / \text{FW}) \times 100$$

Where LW is the liver weight (g), VW is the viscera weight (g) and FW is the fish weight (g).

The ADCs of each nutrient and energy content in the test diets were calculated using the standard formula (Maynard & Loosli, 1969):

$$\text{ADC (\%)} = 100 \times (1 - (\text{F} / \text{D} \times \text{Di} / \text{Fi}))$$

Where F= % nutrient (or kJ/g gross energy) in faeces; D= % nutrient (or kJ/g gross energy) in diet; Fi= % titanium dioxide of faeces; Di= % titanium dioxide of diet.

3.6 Statistical analysis

The statistical analysis was performed using Graphpad prism 7.04, for windows (Graphpad Software, La Jolla, CA, USA). The effect of experimental diets on growth performance, apparent digestibility coefficients, nutrient retention and fatty acid deposition was evaluated using one-way ANOVA followed by Tukey's multiple comparison tests. The model included a fixed factor of experimental diet and tank was used as experimental unit. A significance of $P < 0.05$ was chosen for all analyses performed.

4 Results

4.1 Chemical composition of feed ingredients and experimental diets

The chemical compositions of feed ingredients and experimental diets are shown in Table 2 and Table 3, respectively. The CP content of the feed ingredients was the highest in fishmeal and the lowest in larvae meal, which contained around 130 g/kg DM less CP than fishmeal. The total amount of amino acids was also the highest in fishmeal, followed by mussel meal and larvae meal. The profile of indispensable amino acid (IAA) varied, with the total amount of IAA in fishmeal was 335 g/kg DM and only 149 g/kg DM in larvae meal. Compared with fishmeal, the methionine level in both mussel meal and larvae meal was low. In addition, larvae meal contained much higher CL than fishmeal and mussel meal and CL in larvae meal was even three times more than that in mussel meal. The gross energy content varied between 19.1 MJ/kg DM (mussel meal) and 24.6 MJ/kg DM (larvae meal). The highest and lowest ash content was found in larvae meal (198 g/kg DM) and mussel meal (117 g/kg DM). The experimental diets were formulated to contain equal amounts of nutritional ingredients. Therefore, each of the diets had a comparable level of CP, CL, GE and IAA with the exception of ash, which was relatively low in MD (38 g/kg DM). The least abundant IAA was methionine in these four experimental diets and the most abundant IAA was leucine. The CP content of experimental diets varied from 505 g/kg DM (LD) to 519 g/kg DM (control diet), the sum of amino acids content varied from 428 g/kg DM (MD) to 468 g/kg DM (control diet) and the CL content varied from 215 g/kg DM (control diet) to 225 g/kg DM (LD). The experimental diets had similar GE levels, which were 24.5 MJ kg⁻¹ DM in control diet, 24.6 MJ kg⁻¹ DM in MD and 24.8 MJ kg⁻¹ DM in LD.

Table 3 Chemical compositions of experimental diets expressed as g/kg DM; amino acids expressed as g/kg DM; energy content expressed as MJ/kg DM

	Control diet	MD	LD	PPD
Dry matter (%)	93.9	94.2	94.2	95.1
Crude protein	519.0	508.0	505.0	523.0
Sum of amino acids	467.8	427.8	443.4	495.5
Crude lipid	215.0	223.0	225.0	240.0
Ash	55.0	38.0	58.0	30.0
Gross energy (MJ kg ⁻¹)	24.5	24.6	24.8	25.5
Indispensible amino acids				

Arginine	31.0	29.3	27.8	33.8
Histidine	9.6	8.6	9.5	10.2
Isoleucine	19.7	18.5	19.4	21.9
Leucine	37.9	33.7	36.4	41.5
Lysine	34.1	30.1	29.9	34.6
Methionine	9.2	6.9	6.3	7.3
Phenylalanine	22.4	21.9	23.2	27.0
Threonine	20.8	18.8	20.1	21.2
Valine	24.1	22.0	24.1	25.8
Sum	208.8	189.8	196.7	223.3
Dispensable amino acids				
Alanine	27.8	22.9	25.7	25.0
Aspartic acid	47.9	49.3	50.6	57.7
Cystein + Cystine	4.6	4.7	5.5	5.8
Glutamic acid	71.7	61.7	60.7	71.5
Glycine	37.2	31.6	32.8	34.1
Proline	28.7	25.5	26.5	29.4
Serine	22.0	22.1	22.7	26.3
Tyrosine	19.1	19.0	21.8	22.4
Ornithine	< 0.1	1.2	0.4	< 0.1
Sum	259.0	238.0	246.7	272.2

MD = diet with blue mussel (*Mytilus edulis L*) meal.

LD = diet with black soldier fly (*Hermetia illucens*) larvae meal.

PPD= diet with pea protein concentrate

4.2 Growth performance and feed utilization

Results of the statistical analysis of growth parameters and nutrient retention are present in Table 4. According to the results, the growth responses in terms of FBW, SGR and FCR significantly differ, especially between fish fed control diet and PPD. Fish fed LD and PPD had significantly difference FBW, compared with fish fed control diet and MD, though FBW for fish fed MD was significantly low compared with fish fed control diet. Fish fed PPD had significantly high FCR, followed by fish fed LD and MD. There were no significantly differences in both HIS ($p=0.4693$) and VSI ($p=0.0353$).

Except for the control diet, the three experimental diets especially PPD were not well accepted by the fish therefore low feed intake was observed. The only mortality observed was in fish fed PPD, with two recorded deaths.

Data on nutrient retention are summarized in Table 4, which was obviously affected by dietary treatment and decreased significantly from control diet to PPD. The CP retention ranged from 36.95% in fish fed control diet to 2.05% in that fed PPD. The GE retention ranged from 35.65% in fish fed control diet to -5.57% in fish fed PPD. Fish fed LD had significantly lower CL retention, which was -8.90%.

Table 4 Growth performance, relative organ weight and nutrient retention

	Control diet	MD	LD	PPD	P-value
SBW (g)	65.30 ^a	72.30 ^a	66.30 ^a	59.70 ^a	0.1457
FBW (g)	146.60 ^a	106.60 ^b	84.40 ^c	69.50 ^{cd}	<0.0001
SGR (%/day)	1.33 ^a	0.64 ^b	0.40 ^c	0.26 ^d	<0.0001
FCR (g/g)	0.93 ^c	1.40 ^b	1.90 ^{bc}	2.79 ^a	<0.0001
HIS (%)	1.23 ^a	1.17 ^a	1.10 ^a	1.22 ^a	0.4693
VSI (%)	12.36 ^{ab}	11.72 ^{bc}	11.70 ^{ac}	10.92 ^c	0.0353
Nutrient and energy retention (%)					
CP	36.96 ^a	22.20 ^b	12.19 ^c	-	<0.0001
GE	35.65 ^a	22.66 ^b	5.63 ^c	-	<0.0001
CL	52.29 ^a	30.80 ^b	-8.92 ^c	-	<0.0001
Mortality (%)	0	0	0	4.2%	

SBW, start body weight; FBW, final body weight; SGR, special growth rate; FCR, feed conversion ratio.

HIS, hepatosomatic index; VSI, viscerosomatic index.

a, b, c in the same row indicate significant differences ($P < 0.05$).

MD = diet with blue mussel (*Mytilus edulis L*) meal.

LD = diet with black soldier fly (*Hermetia illucens*) larvae meal.

PPD= diet with pea protein concentrate

4.3 Apparent digestibility coefficients of diets

The ADC values of DM, CP, GE, CL and IAA are reported in Table 5. Significant differences between the ADC for DM in experimental diets were observed ($P < 0.0001$) and MD and LD had the highest and the lowest ADC for DM, respectively. There were no significant differences in the digestibility of GE, CL ($P = 0.0001$) between fish fed control diet and MD, but the ADC for CP in MD was significantly higher than that in control diet and LD.

According to the results, the ADC for sum of IAA did not significantly differ between diets and the highest ADC for sum of IAA (96.7%) was observed for MD and the lowest ADC (94.8%) for sum of IAA was found for larvae meal diet. The MD displayed higher ADC values for all amino acids and the LD displayed lower ADC values for all amino acids compared with the control diet.

Table 5 Apparent digestibility coefficients (%) for MD, CP, GE, CL and indispensable amino acids of experimental diets

	Control diet	MD	LD	P-value
ADC for DM	78.0 ^b	80.1 ^a	75.2 ^c	<0.0001
ADC for CP	94.4 ^b	96.3 ^a	93.5 ^b	0.0001
ADC for GE	81.7 ^a	82.9 ^a	77.9 ^b	0.0001
ADC for CL	86.8 ^a	85.4 ^a	77.0 ^b	0.0009
ADC for IAA				
Arginine	97.0 ^b	97.9 ^a	96.6 ^b	0.0002
Histidine	95.8 ^a	96.6 ^a	94.8 ^b	0.0013
Isoleucine	95.2 ^a	96.1 ^a	93.9 ^b	0.0014

Leucine	96.2 ^a	96.7 ^a	94.8 ^b	0.0013
Lysine	97.2 ^a	97.5 ^a	96.0 ^b	0.0019
Methionine	95.0 ^{ab}	96.1 ^a	94.0 ^b	0.0102
Phenylalanine	95.6 ^{ab}	96.5 ^a	94.6 ^b	0.0059
Threonine	94.9 ^a	95.1 ^a	92.9 ^b	0.0011
Valine	95.2 ^a	95.9 ^a	93.7 ^b	0.0007
Sum of IAA	96.0 ^a	96.7 ^a	94.8 ^b	0.0014

a, b, c in the same row indicate significant differences ($P < 0.05$).

MD = diet with blue mussel (*Mytilus edulis L*) meal.

LD = diet with black soldier fly (*Hermetia illucens*) larvae meal.

5 Discussion

5.1 Growth performance and nutrient retention

The main aim of this project was to assess the potential of Baltic blue mussel meal and BSFL meal as protein sources in feed for rainbow trout. Chemical analyses revealed that both blue mussel meal and BSFL meal could be potential ingredients based on the protein content. The sum of amino acids in both blue mussel meal and BSFL meal were lower than that found in fishmeal, but they had relevant essential amino acid profiles to satisfy the requirement of rainbow trout. However, methionine and lysine, two limiting amino acids in rainbow trout (Gaylord & Barrow, 2009) are present in lower amounts in both blue mussel meal and BSFL meal when compared to fishmeal.

After 9 weeks of feeding, results show poor growth based on FBW, SGR and FCR when approximately 20% of the total protein was provided by mussel meal and larvae meal. The primary reason for such performance might be the low feed intake. In present study, average 75.39g control diet was consumed per fish during the whole period, but only 48.46g MD, 34.23g LD and 26.84g PPD per fish were consumed, respectively. From figure 2, it is clear that the SGR decreased gradually while the feed intake decreased. Various studies have demonstrated similar effect of fishmeal-free diet on feed intake. For example, Teskeredzic *et al* (1995) and Médale *et al* (1998) mentioned that rainbow trout fed plant-based diets without fishmeal showed a significant decrease in feed intake and fish growth was also negatively affected. Similarly, a reduced feed intake was observed in Atlantic Cod when fishmeal level was low in the diet and was probably due to palatability problems (Abigail and David, 2011). In our study, 100% of fishmeal was replaced by mussel meal, larvae meal and pea protein concentrates. However, few studies have attempted complete substitution of fishmeal (Kissil *et al.*, 2000) because fishmeal is considered an important ingredient that can make the fish feed more agreeable to the taste and is considered a good attractant in aquaculture (Thiessen *et al.*, 2003). Therefore, the absence of fishmeal in MD, LD and PPD could be one of the reasons of decreased feed intake.

The low feed intake could also be attributed to the high inclusion levels of pea protein in MD, LD and PPD. In present study, feed intake was obviously decreased when pea protein levels increased gradually in the experimental diets. Similarly, Tibaldi *et al* (2005) mentioned that markedly feed intake influence was observed when sea bass (*Dicentrarchus labrax*) fed feed with high level of pea protein concentrate. Further more, when the pea protein concentrate was included to replace 90% of fishmeal protein, the

FCR, protein retention and gross energy retention were negatively affected (Tibaldi *et al.*, 2005). This has also been confirmed in the present study by adding the fish group fed PPD. Feed acceptance of pea protein concentrate may be affected by antinutritional factors (sinapine, tannins and glucosinolates), and the bitter, astringent taste attributing to soyasaponin I (Thiessen *et al.*, 2003). Some studies have reported that pea protein concentrate could be a suitable ingredient for use in rainbow trout and Atlantic salmon diets without adverse effect on growth performance, but the recommended inclusion level was only 20% (Thiessen *et al.*, 2003; Øverland *et al.*, 2009). Except pea protein, other plant proteins like rapeseed products have also been found to reduce feed acceptance and growth performance of trout (Nagel *et al.*, 2014).

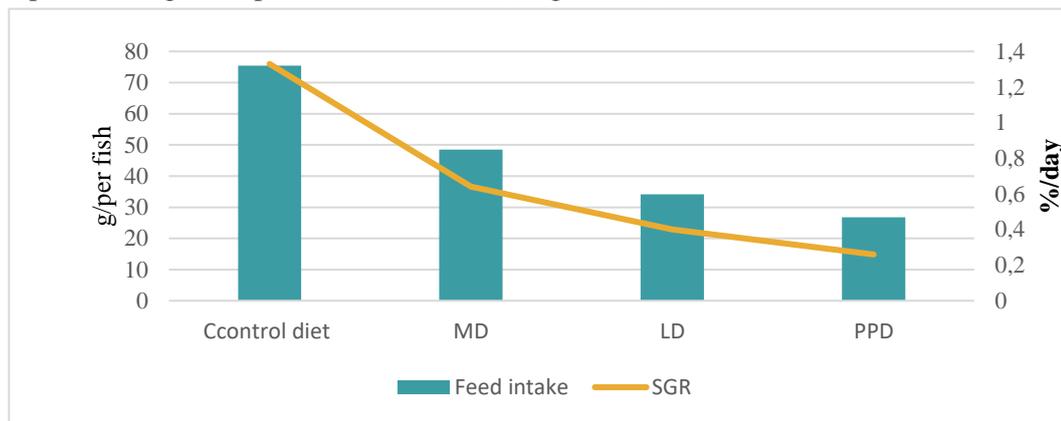


Figure 2 The effects of feed intake on specific growth rate (SGR)

In order to enhance the acceptability of aqua diets based on new protein sources, feed stimulants or attractants could be recommended. Tiril *et al.* (2008) showed that feed intake was significantly higher in rainbow trout fed the diet with betaine supplementation than in rainbow trout fed the fishmeal based diet. Therefore, betaine could be an effective feeding attractant to improve palatability and intake of economically fishmeal-free diets for rainbow trout.

Additional reason for negative growth performance could be the limiting levels of methionine in the diets. The experiments conducted on European sea bass, juvenile Cobia (*Rachycentron canadum*) and fingerling rohu (*Labeo rohita*) found that the methionine deficiency were responsible for growth problems and negative feed efficiency (Wu *et al.*, 2017). The methionine requirement was estimated for rainbow trout juveniles, being 0.7% in the diet on a dry-matter basis (NRC, 2011). However, dietary methionine levels in both MD and LD in present study did not meet the requirements for rainbow trout. It is well known that methionine as an essential amino acid affects growth performance because methionine can increase digestive and absorptive function. Enzymatic antioxidant capacity can also be improved by methionine, which in turn protects intestine from lipid peroxidation and protein oxidation (Wu *et al.*, 2017). Additionally, Rolland *et al.* (2015) conducted a study on rainbow trout and reported that feed intake and growth performance (weight gain and growth rate) were significantly affected by limited dietary methionine supplement. Therefore, when feeding non-fish meal based diets, it is important to balance the essential amino acids. For fishmeal-free feeds for rainbow trout, multiple amino acid supplementations could be recommended. Several studies have been undertaken to evaluate the growth performance of trout as affected by amino acid supplementations. Davies and Morris (1997) reported that the rainbow trout fed soya-

based diets with multiple amino acid supplementations had positive growth performance (improved percentage WG and SGR). Also for fishmeal-based diet Yamamoto *et al* (2005) mentioned that protein utilization in rainbow trout could also be positively improved by essential amino acid supplementation.

Therefore, the detrimental effects of dietary imbalances, antinutritional factors in plant meals and palatability problems on carnivorous species growth should be considered when trying to replace fishmeal with alternative animal-, insects- or plant derived ingredients.

Furthermore, fish fed MD and LD had much higher FCR compared with fish fed control diet in present study. Berge and Austreng (1989) reported that rainbow trout had negatively growth and poor feed efficiency with increasing blue mussel inclusion level, which probably because of the present of high shell content. However, the blue mussel used in present study has been deshelled, so mussel shell was not a reason for high FCR. Deshelled blue mussel was also used by Vidakovic *et al* (2015) as a substitute of fishmeal for Arctic Charr, which showed no significant differences in FCR compared with reference diet. For BSFL, Kroeckel *et al.* (2012) reported that the FCR was affected by larvae meal inclusion level in feed for juvenile turbot. They showed that FCR was obviously higher when the inclusion level reached more than 33%. However, Belghit *et al.* (2019) reported that FCR was not affected by the inclusion of larvae meal (147.5g/kg) in the Atlantic salmon diet. The high FCR of fish fed LD in present study might be explained by the low weight gain due to low feed intake. Therefore, both low WG and low feed intake of MD, LD and PPD in rainbow trout likely induced higher FCR.

As for nutrient and energy retention, both MD and LD were significantly lower than control diet in present study. Previous study (Vidakovic *et al.*, 2015) showed that the retention of crude protein was not affected by the presence of blue mussel, which disagrees with results from the present study. A possible explanation for these values is the metabolic imbalance in fish fed MD and LD due to insufficient feed intake. Due to low intake, requirements for protein and energy needed for growth were not meet, therefore the resulting feed intake was likely insufficient to result in satisfying growth performance but were largely used for sustaining the basic metabolic function of fish. Rychly (1980) reported that nitrogen retention increased with increasing nitrogen consumption, which is in agreement with the results of CP retention in present study. In addition, dietary methionine deficiency could also negatively affect feed conversion and N protein retention (Green & Hardy, 2008). This might also explain the high FCR and low CP retention of fish fed both MD and LD.

5.2 Digestibility

The ADC values of MD in present study were comparable with the ADC values of control diet but there were partly higher than the ADC values reported by Vidakovic *et al.* (2015) for Arctic Charr based on faeces collection from dissection. In fact, all ADC values from current trial are higher than those reported by Vidakovic *et al.* (2015). Different faeces collection methods could explain these differences. Vandenberg & De La Noüe (2001) reported that the ADC values were generally the highest for the column method, which could be due to nutrient leaching when faeces was exposed to water. In contrast, reduced ADC values caused by dissection method were probably because of the intact faeces (Vandenberg & De La Noüe, 2001). Furthermore, Berge & Austreng

(1989) have demonstrated that reduced digestibility of DM was observed with increasing mussel meal content in the diet for rainbow trout. However, the mussel meal used in Berge & Austreng experiment was the whole, milled blue mussel. In the current study, the high DM digestibility (compared with control diet) in MD was probably attributed to the absence of mussel shell. The higher ADCs of methionine and lysine in MD were interesting, as it indicates that mussel meal has a high bioavailability for these two IAA. Overall, the high ADC of protein, lipids, energy and DM of MD indicated that mussel meal is well digested by rainbow trout.

In comparison with MD and control diet, the ADC of LD were relatively low, especially ADC of energy and lipid. Kroeckel *et al.* (2012) observed low digestibility of nutrients when fishmeal was replaced by larvae meal in juvenile turbot, which is in line with the findings from the present study. The chitin content found in their exoskeleton was responsible for the unsatisfactory nutrients digestibility (Renna *et al.*, 2017). Only few published studies have evaluated the chitin content of BSFL meal. Approximately 87g/kg DM of the polysaccharide chitin was observed by Diener *et al.* (2009) in the exoskeleton of the BSFL. However, Kroeckel *et al.* (2012) reported the defatted BSFL used in their experiment contained 96g/kg DM of chitin. Chitinase activity has been reported in some fish species, but the action of breaking down the chitin fraction seems to be limited in rainbow trout (Lindsay *et al.* 1984). Chitin present in larvae meal was considered to negatively affect the absorption of nutrients from the intestinal tract, which therefore decreases CL absorption (Schiaivone *et al.*, 2017). The decreased CL digestibility and low CL retention for LD in the present study could also be explained by the decreased CL digestion due to the present of chitin in larvae meal. Furthermore, low CL digestibility was contributed to the low ADC of DM in LD.

There was no difference for ADC of CP and amino acids between control diet and LD. Other authors, such as Belghit *et al.* (2019) showed that the ADC of CP was not affected by replacing fishmeal with BSFL meal in diets for Atlantic salmon. However, Kroeckel *et al.* (2012) reported that the ADC of CP in juvenile turbot diet containing 30% of larvae meal was lower than diet containing only fishmeal, which could be because of the low ADC of CP in larvae meal itself. Overall, the comparable ADC of CP between control diet and LD in present study indicates the high value of CP from larvae meal in terms of digestibility.

6 Conclusions

In conclusion, and based on overall ADC results, both blue mussel meal and BSFL meal could be used at current inclusion levels in the diets for rainbow trout. Special attention should be directed towards decreased ADC of CL in LD and possible chitin removal should be considered for further trials. Due to lower content of methionine and lysine in mussel meal and larvae meal compared to fishmeal, their supplementation is needed. In addition, use of pea protein concentrate in high percentage for rainbow trout can negatively affect feed intake. Thus, pea protein should be limited to lower inclusion levels to prevent possible feed palatability issues. Furthermore, use of lower levels of fishmeal or possible addition of taste enhancers such as betaine should be considered when composing diets with plant protein sources that may have bitter taste, such as pea protein concentrate.

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