Utilization of dietary minerals in Atlantic salmon fed blue mussel silage

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Abstract

The salmon farming industry is dependent on fish meal and fish oil derived from wild fisheries to meet the dietary requirements of the fish and ensure nutritional profile of the finished product. Blue mussels are low-trophic organisms with a low use of resources compared to many other farmed species, may serve as a replacement of fish meal as it is comparable in several aspects. Acid silage is a cost-effective means of processing e.g., by-products from the fish processing industry, that is currently used in commercial fish feed production. Consequently, blue mussel silage may also serve as a potential ingredient in fish feed, however, little is known of its mineral availability whether if it differs from that of blue mussel meal.

The aim of this project was to determine a suitable inclusion level of blue mussel silage in the feeds for Atlantic salmon post-smolt based on growth performance, mineral utilization and to compare them between blue mussel silage and blue mussel meal.

To determine a suitable inclusion level of blue mussel silage, a dose-response study with 3, 7 and 11% inclusion levels lasting 10 weeks was performed with Atlantic salmon post-smolt in triplicate groups. One reference diet with no blue mussel and one other diet containing 12% blue mussel meal was used to compare with the groups fed blue mussel silage. Growth, nutrient digestibility, macro- and micro-mineral status of whole fish, liver and plasma were studied.

The results of the experiment showed comparable growth of the blue mussel meal group to the reference. Except for higher Fe status, the micro-mineral status of the blue mussel meal group was unaffected. The blue mussel silage groups showed reduced growth and impaired micromineral status (Fe, Mn, Se and Cu). Selenium status of the silage fed groups was reduced compared to the reference, and an extremely low iron status was observed in all the silage fed groups. Zinc and iodine status were not affected in the silage groups, whereas Cu status was increased. Macro-nutrient (protein and fat) digestibility and macro-mineral (P, Ca, Mg, Na and K) status were not differentially affected in either the blue mussel meal or -silage fed groups. To conclude, no suitable inclusion level of blue mussel silage in Atlantic salmon post-smolt feeds was found due to the negative effects on micromineral utilization. Processing of blue mussels as silage requires further refining and better understanding of the interaction with micro-minerals if blue mussel silage is to be used in feeds for Atlantic salmon.

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APPENDIX I: MINERAL STATUS OVERVIEW ..... **I**

Abbreviations

FM = Fish meal

FO = Fish oil

BM = Blue mussel

BMM = Blue mussel meal

BMS = Blue mussel silage

BMS3 = Blue mussel silage meal, 3% inclusion

BMS7= Blue mussel silage meal, 7% inclusion

BMS11 = Blue mussel silage meal, 11% inclusion

ANOVA = One-way analysis of variance

LR = simple linear regression

Introduction

1.1. Aquaculture and its role in food production

While capture fisheries have remained stable since the 1980s, aquaculture production has grown steadily, and its share of global fish production increased from 26 to 46 percent in the years 2000 - 2018. The share of fish available for human consumption sourced from aquaculture has seen a sharp increase from only 4 per cent in 1950 and 9 per cent in 1980 to 52 per cent as of 2018 (FAO, 2020b). In the future, this share is expected to increase further, and aquaculture is therefore an increasingly important food source for the growing global population.

With a production of 53 million tons in 2018, finfish is the dominating form of farming of aquatic animals, most of which are freshwater species such as carps with China as the biggest producing country (FAO, 2020b). European finfish aquaculture on the other hand is dominated by coastal aquaculture with Atlantic salmon (*Salmo salar*) as the single most valuable species accounting for around 25% of aquaculture output in terms of value (Eurostat, 2019). Other major aquaculture species in Europe include species of trout, gilthead bream (*Sparus aurata*) and European seabass (*Dichentrarchus labrax*).

1.2. Atlantic salmon farming in Norway

Salmon has since 2013 been the largest single fish commodity by value globally (FAO, 2018). Atlantic salmon aquaculture comprised 4.5% of the global finfish aquaculture production in 2018 with Norway and Chile as the two leading producers (FAO, 2020b). Other producing countries include Scotland, the Faroe Islands and Canada. Atlantic salmon farming in Norway started as a small side income for coastal farmers in the 1970's and has grown to become a major export industry, making up over 16% of Norway's exports of mainland goods (SSB, 2017). This development may continue as there is a political ambition to increase the production of Atlantic salmon five-fold by 2050 (Meld. St. 22 (2012–2013)).

1.3. Feeds in salmon aquaculture

The composition of feed used for farmed Atlantic salmon has changed drastically over the last decades with increasing proportions of marine protein and oils being replaced by plant-based alternatives. Fishmeal (FM) and fish oil (FO) produced from pelagic fisheries or by-products from the fish processing industry have historically been main ingredients in feed as they are good sources of protein, lipids, vitamins, and minerals required for growth of fish.

This however is no longer the case as the amount of marine protein in the feed has declined from over 65% in 1990 to around 14% in 2016 and the amount of marine oils have been reduced from 24% to around 10% in the same period (Figure 1)(Ytrestøyl, Aas, & Åsgård, 2015; Aas, Ytrestøyl, & Åsgård, 2019). The shift from a mainly marine based diet to a progressively more plant-based diet for farmed salmon is mainly due to the increased price of fish meal caused by fluctuations in supply and increased demand (Shepherd & Jackson, 2013). The industry's dependency on marine ingredients with its effects on wild fish stocks is also often raised as an argument against the sustainability of salmon farming (Deutsch et al., 2007; Naylor & Burke, 2005; Tacon, 2011).

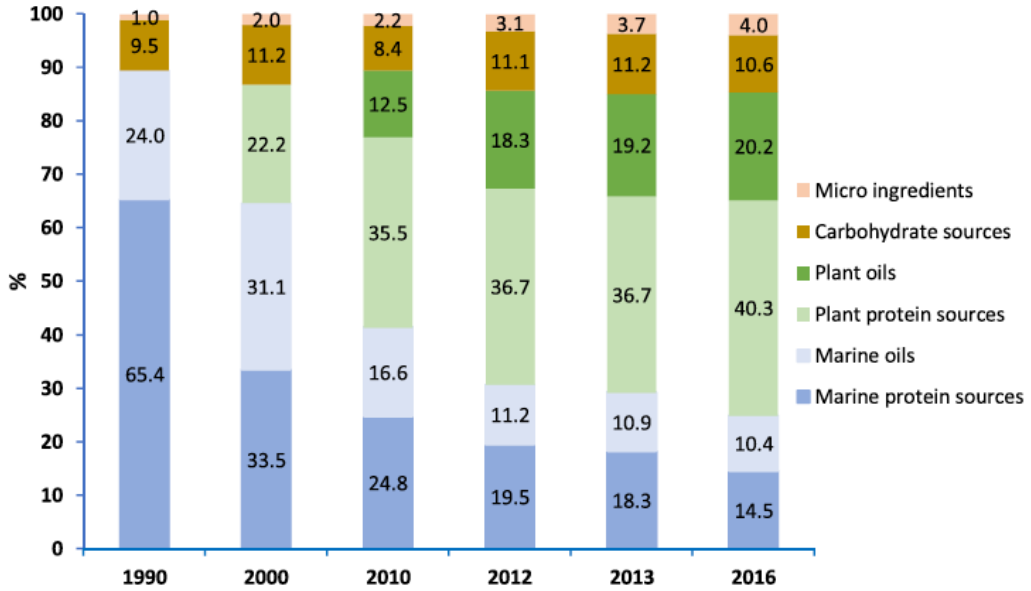

Figure 1. Ingredient sources in feed for Norwegian farmed salmon as percentage of feed from 1990 to 2016. From "Utilization of feed resources in the production of Atlantic salmon (*Salmo salar*) in Norway: An update for 2016" by Aas, T. S., Ytrestøyl, T., & Åsgård, T., 2019, *Aquaculture Reports*, *15*. doi:10.1016/j.aqrep.2019.100216

This shift in feed composition is not problem-free however, as plant protein sources like soy or other plant derived ingredients may contain antinutritional factors that can affect the availability and uptake of certain nutrients like minerals and trace elements (Kaushik, 1990). Plant meals are also problematic in the sense that the production of particularly soy may compete with human food crops or be a driver for deforestation of rainforest leading to increased greenhouse gas emissions and habitat loss (Winther, Hognes, Jafarzadeh, & Ziegler, 2020). Due to the challenges associated with increased plant material in the salmonid diet, efforts have been put towards finding alternative and more sustainable feed resources from lower trophic marine sources with low environmental footprints and high nutritional value. One such alternative is the blue mussel (*Mytilus edulis*).

1.4. Blue mussels: a potential feed source

The blue mussel (*Mytilus edulis*) is a common saltwater mussel found from the tidal zone down to about 10m depth throughout the Atlantic. In Europe, France, Italy, and Spain are leading producers of blue mussel and the closely related Mediterranean mussel (*Mytilus galloprovencialis*) (FAO, 2020a). Production of blue mussel in Norway is relatively small compared to other European countries, but the potential for production is much larger than what is produced today (Agnalt et al., 2018). Blue mussel production is favorable in several aspects, mainly that it doesn't require active feeding, doesn't use fresh water, fertilizers, or pharmaceuticals in contrast to many other cultured species or feed crops. As primary consumers, blue mussels feed on phytoplankton and other seston and can by doing so combat coastal eutrophication by binding phosphorous and nitrogen (Lindahl et al., 2005; Stadmark & Conley, 2011).

Blue mussel has a long history as a human food source and is rich in protein, essential fatty acids like EPA and DHA, the vitamins D, A and B12 as well as trace elements like selenium and iodine (Berge & Austreng, 1989; Kikuchi & Furuta, 2009b). The biochemical composition of blue mussels varies with feed availability, reproduction cycle (de Zwaan & Zandee, 1972; Smaal & Van Stralen, 1990) and is largely dependent on the time of harvest. The nutrient profile of blue mussels is seasonal, and the composition of ash free dry meat varies between 51.8 - 82.4% for protein, 8.6-35.8% for carbohydrates and 2.6-12.7% for lipids while the ash content in dry meat varies between 4.2-14% (Okumuş & Stirling, 1998). The amino acid profile of the blue mussel is considered similar to that of fish meal (Berge & Austreng, 1989), however some

free amino acids like taurine and lysine are present in higher concentrations in blue mussels than in fish meal (Árnason et al., 2015). This may be favorable as these amino acids have attractant properties (Adams, Johnsen, & Zhou, 1988; Gaylord, Teague, & Barrows, 2006). Blue mussels can therefore work as an attractant in diets where the fish meal content is low (Nagel et al., 2014).

Publications on the topic of blue mussel as an alternative to fish meal is relatively limited but work so far indicates that blue mussel meal can partly replace FM in diets for several species of fish using mostly growth performance and feed efficiency as the main response criteria (Table 1). According to Berge & Austreng (1989) feeding rainbow trout with feed containing up to 45% blue mussel meal made from whole, crushed mussels resulted in enlarged livers, lower feed utilization and a tendency towards poorer growth. Meal produced from deshelled blue mussels have however been fed to arctic char (*Salvelinus alpinus*) up to 40% of the diet with no clear effects on either growth or welfare (Vidakovic et al., 2016). The negative health effects associated with high inclusion rates of blue mussels in salmonid feeds as described by Berge & Austreng (1989) may therefore be caused by the high degree of crushed shells in the experimental diet. While BMM has successfully been incorporated in feed for several fish species, not much is known of how diets containing blue mussel affect the mineral status in fish. When producing FM usually the whole fish or by-products are utilized, including bones and vertebrae, which have relatively high mineral content. Blue mussels however are devoid of bony structures but possess a shell consisting mostly of calcium carbonate and other minerals. Under the production of blue mussel meal however, usually only the meat of the mussel is utilized. How this affects the mineral profile of the feed and subsequent mineral status of fish fed diets containing whole or deshelled mussels is an open question.

Table 1. Literature on the use of blue mussels in fish diets: The table shows fish species, source of mussel protein (crushed or de-shelled mussels), inclusion percentages in diets and the response variables used. Diet BM data is the type of blue mussel added to the diet (meal or extract) with the inclusion (%) used in the respective studies.

Fish species	BM source	Diet BM (%)	Response variables	References
Rainbow trout (*Oncorhynchus mykiss*)	Whole	BMM (45)	ADCs, CF, FCR, HSI, PC	(Berge & Austreng, 1989)
Japanese flounder (*Paralichthys olivaceus*)	de-shelled	BMM (50)	WG, PER, FE, FBW	(Kikuchi & Sakaguchi, 1997)
		BME (20)	HC, WG, FE, FC	(Kikuchi, Ueda, S, & Takeda, 2002)
Tiger puffer (*Takifugu rubripes*)	de-shelled	BME (20)	GP, HC, FE	(Kikuchi & Furuta, 2009a)
		BMM (20)	GP, HC, FE	(Kikuchi & Furuta, 2009b)
Turbot (*Scophthalmus maximus*)	de-shelled	BMM (25)	HSI, SGR, CF, FCR	(Weiß & Buck, 2017)
		BMM (8)	ADCs, PC, HC, Histology	(Nagel et al., 2014)
Eurasian perch (*Perca fluviatilis*)	de-shelled	BMM (30)	ADCs	(Langeland et al., 2016)
Arctic charr (*Salvelinus alpinus*)	de-shelled	BMM (40)	GP, ADC, IBF	(Vidakovic et al., 2016)

Notes:
ADCs = apparent digestibility coefficients, BMM = blue mussel meal, BME = blue mussel extract, CF = condition factor, FBW = final body weight, FCR = feed conversion ratio, FE = feed efficiency, GP = growth performance, HC = hematological characteristics, IBF = Intestinal barrier function, PC = proximate composition, PER = protein efficiency ratio, SGR = specific growth rate, WG = weight gain.

1.5. Blue mussel silage processing

One method of producing feed containing blue mussel is by first processing mussels into silage. Making silage involves mincing the raw material before adding an acidic solution (like formic acid or other organic acids), and occasionally stirring until the raw material is liquefied. Adding acid lowers the pH which inhibits bacterial growth and prevents purification of the raw material. Endogenous proteolytic enzymes then break down the organic material in the silage resulting a liquefied solution of low molecular weight peptides and free amino acids (Toppe, Olsen, Peñarubia, & James, 2018). This may affect digestibility of the feed as short chain peptides or free amino acids are absorbed more readily compared to intact protein (Gilbert, Wong, & Webb, 2008). Several studies however have shown successful use of low or moderate amounts of fish silage or fish protein concentrates in fish feed (Olsen & Toppe, 2017). In one experiment with pigs, blue mussel silage yielded comparable results to that of fish silage (Nørgaard, Petersen, Tørring, Jørgensen, & Lærke, 2015). Silage processing may be relevant to blue mussel production as blue mussels are highly perishable and must be processed quickly after harvest to avoid rapid deterioration. It is estimated that around 27% of harvested blue mussels are not fit for human consumption with much of it simply treated as compost or waste (Naik, Mora, & Hayes, 2020). As silage is nutritionally stable over time it can serve as an intermediary stage in production of feed and other products where the access to raw material is seasonal or where the volumes of raw material do not justify the cost of having an associated feed mill. Silage may therefore facilitate greater resource utilization in the blue mussel industry and production of fish feed.

High contents of fish bones is known to affect the silage process by increasing the buffering capacity of the silage (Arason, 1994). Similarly, calcium carbonate present in blue mussel shells may also affect the buffering capacity when producing silage of blue mussels. If the silage is produced from the meat only, however, the processes that take place may be different to that of producing fish silage. Whether this affects availability of certain nutrients such as minerals remains to be studied.

1.6. Mineral requirements of Atlantic salmon

Minerals are essential for biological function and growth in all animals as they are part of skeletal formation, enzymes, and many other biological systems. Meeting the mineral requirements of fish is important as reduced availability and retention of minerals can lead to production related diseases such as bone deformities and reduced growth and welfare in general (Baeverfjord et al., 2019). While fishmeal is regarded as a good source of minerals for fish, changes in the composition of formulated feeds as described earlier has altered the mineral profile of feeds increasing the need for revisiting mineral requirements in fish (Antony Jesu Prabhu, Lock, et al., 2019; Lorentzen & Maage, 1999).

Macro-minerals

Minerals are divided into macro- and micro-minerals according to their function, concentration and dietary requirements. The macro-minerals occur in the fish body in concentrations of the order of grams per kg wet weight and include the electrolytes sodium (Na), potassium (K) and chlorine (Cl), and the structural elements calcium (Ca), magnesium (Mg) and phosphorous (P). The electrolytes are essential for the osmotic balance and acid-base equilibrium in fish. These ions are abundant in seawater as well as in many feed ingredients and so supplementation is usually not necessary (Lall, 2003). The structural elements calcium and phosphorous are important for development and maintenance of the skeletal system, whereas magnesium is part a range of biological processes such as energy metabolism and protein synthesis (Lall, 2003).

Micro-minerals

Micro-minerals are present at the level of milligrams or micrograms in fish and include essential elements such as zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), iodine (I) and selenium (Se). These elements are essential as they form part of metalloenzymes, work as co-enzymes or otherwise are crucial for normal development, growth, and reproduction (Lall, 2003). For most micro-minerals, the diet is the main route of uptake while the renal- and entero-hepatic systems are important for regulation and homeostasis (Bury, Walker, & Glover, 2003; Hambidge, 2003; Wood, 2011). Some micro-minerals such as iron, copper and selenium may be taken up through the gills, but at levels too low to cover the needs of the animal in the long run. Consequently, if the feed is too low in certain minerals, or the uptake and utilization is hindered, symptoms of deficiency may develop. While many symptoms of mineral deficiency are non-specific, such as reduced growth or loss of appetite, others are more distinct, such as microcytic anemia or

cataracts in fish deficient in iron/copper or zinc, respectively (Andersen, Maage, & Julshamn, 1996; Kamunde, Grosell, Higgs, & Wood, 2002). Mineral status of whole body or specific tissues are considered better indicators than clinical symptoms as changes in mineral concentration in the fish precede more pronounced clinical effects (Shearer, 1984). Reported concentrations of macro- and micro-minerals in Atlantic salmon is shown in Table 2 below.

While dietary requirements of fish have been established for several minerals (Table 3), factors such as water quality, life stage and interaction between nutrients in the feed may influence the actual requirements of the animal and how much of the minerals present in the feed that the fish can utilize (Lall, 2003; Lall & Milley, 2008). When evaluating new ingredients or means of processing, such as blue mussel silage, it is therefore of importance to study how it affects the mineral properties of the feed and ultimately the mineral status of the fish.

Table 2: Macro- and mineral concentrations in whole body, liver, and plasma of Atlantic salmon

	Whole body	Liver (Mean ± SD)	Plasma (mean ± SD)
Macro-minerals			
Ca	3 - 5.5	-	-
Na	1.5 - 2	-	-
K	2.3 - 4	-	-
Mg	0.3 - 0.5	-	-
P	4 - 5	-	-
Micro-minerals			
Mn	1.5 - 3	0.85 ± 0.2	1.3 ± 0.1
Fe	10 - 20	96 ± 45 (56 -102)[2]	10.9 ± 0.5
Cu	1 - 3	67 ± 44 (70 - 190)[3]	25.6 ± 3.3
Zn	25 - 60	27.6 ± 7	319 ± 86
Se	0.2 - 0.4	2 ± 0.6	3.3 ± 0.7
I[1]	0.10 - 0.12	-	-

Notes: Whole body and liver macro-minerals are presented as g/kg ww. Microminerals are mg/kg wet weight whereas plasma values are μmol/L. Values are from Antony Jesu Prabhu, Schrama, and Kaushik (2016). [1]Normal ranges for iodine are from Antony Jesu Prabhu, Lock, et al. (2019).[2]Hepatic Fe concentration from Andersen et al. (1996). [3]Hepatic Cu concentration from Lorentzen, Maage, and Julshamn (1998).

Table 3:. Mineral requirements of Atlantic salmon (*Salmo salar*).

	Requirement (mg/kg DM)	Estimate based on	References	Other species (mg/kg) DM[1]
Macro-minerals				
Ca	-	-	-	1.9 - 10
Mg	0.33	WB-Mg, Pl-Mg, Ver-Mg	(El-Mowafi & Maage, 1998)	0.24 - 1.35
K	-	-	-	2 - 10
P	5.6 6 9 9	Ver-Ash Ver-Ash WG WB-P	(Vielma & Lall, 1998) (Ketola, 1975) (Bishop & Lall, 1977) (Aasgaard & Shearer, 2003)	2.0 - 15
Microminerals				
Zn	37-67	WB-Zn, Sr-Zn	(Maage & Julshamn, 1993)	8.6 - 240
I	0.7 - 1.6	WB-I	(Antony Jesu Prabhu, Lock, et al., 2019)	1 - 1.1
Fe	60 - 100	H, Hep-Fe	(Andersen et al., 1996)	30 - 330
Mn	7.5 - 10.5 15 4.9 - 5.7	WB-Mn WB-Mn, Ver-Mn WB-Mn, Ver-Mn, Plasma-Mn, Bile-Mn	(Maage, Lygren, & El-Mowafi, 2000) (Lorentzen, Maage, & Julshamn, 1996) (Antony Jesu Prabhu, Silva, et al., 2019)	2.4 - 25
Se	<1.2 0.65 ± 0.18	WG, H-GPx Liver-Se, Kidney-Se, WB-Se Plasma-Se, GRH	(Lorentzen, 1994) (Antony Jesu Prabhu et al., 2020)	0.1 - 12
Cu	8.5 - 13.7	Liver-Cu	(Lorentzen et al., 1998)	2.0 - 18

Notes: GRH, glutathione redox homeostasis; hematological parameters, H; hepatic iron concentration Hep-Fe; hepatic glutathione peroxidase activity, H-GPx; Pl-Mg, plasma magnesium concentration; serum zinc concentration, Sr-Zn; Ver-Mg, vertebral Mg content; Ver-Ash, vertebral ash content; Ver-Mn, vertebral manganese content; whole body iodine concentration, WB-I; whole body Mn concentration, WB-Mn; whole body selenium concentration, WB-Se; whole body zinc concentration, WB-Zn; weight gain, WG.[1]The "Other species" column contain ranges of reported mineral dietary requirements for other species than Atlantic salmon, see Antony Jesu Prabhu et al. (2016) for further details. For P-requirement of other species than A.salmon see Antony Jesu Prabhu, Schrama, and Kaushik (2013).

2. Thesis aim and objectives

The aim of the project was to determine the suitable inclusion level of blue mussel silage in the feed for Atlantic salmon post-smolt and compare the mineral status of fish fed blue mussel silage with blue mussel meal and fish fed a commercially relevant diet.

The objectives of the project were to:

- determine the optimal inclusion level of blue mussel silage using a dose-response study
- compare macro-nutrient digestibility and performance of fish fed BMM and BMS
- compare status of the macro- and micro-minerals in fish fed BMM and BMS.

The experiment was based on the following hypotheses:

$H0_1$: Replacement of fish meal by blue mussel silage in the feed for Atlantic salmon post-smolt does not affect growth performance and feed utilization.

$H1_1$: Replacement of fish meal by blue mussel silage in the feed for Atlantic salmon post-smolt does affect growth performance and feed utilization.

$H0_2$: The processing method of blue mussel used in feed for Atlantic salmon post-smolt does not affect growth performance and feed utilization.

$H1_2$: The processing method of blue mussel used in feed for Atlantic salmon post-smolt does affect growth performance and feed utilization.

3. Material and Methods

3.1. Experimental diets

Five diets were prepared for the experiment by Cargill Norway. One commercially relevant diet as reference with 25% FM, one diet containing 12% blue mussel meal (TripleNine, Denmark), and three diets containing 3, 7, and 11% blue mussel silage (Lerøy/Ocean Forest AS). The blue mussel silage was made from undersized mussels from a commercial blue mussel farming operation by Blå Biomasse A/S in Limfjorden, Denmark. The blue mussels were crushed and mechanically separated into three phases: shell, byssus threads and meat. The meat was made into silage by mixing with formic acid. The blue mussel silage was carefully evaporated and mixed with soy protein concentrate (SPC) on low heat to increase the dry matter content before including it in the feed. To determine apparent availability of minerals, yttrium oxide was added as an inert marker to all diets. The formulation, proximate composition and mineral composition of the experimental diets are shown below in Table 4 and Table 5, respectively.

Table 4: Formulation and proximate composition of experimental diets

	Reference	BMM12	BMS3	BMS7	BMS11
Fish oil	10.2	10.1	10.3	10.4	10.4
Rapeseed oil	13.9	13.2	13.3	12.4	11.6
Fishmeal LT (?)	25.0	13.0	20.3	15.4	10.5
Soy protein concentrate (SPC)	20	12.3	15.5	8.2	-
Raw wheat	11.0	11.0	11.0	10.4	10.5
Other plant proteins[1]	16.8	24.3	17.8	21.2	24.9
Micro-ingredients	3.17	4.11	3.30	3.45	3.62
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
Blue mussel meal		12			
Blue mussel silage			1	1	1
Dried blue mussel silage + SPC			7.5	17.5	27.5
Protein (g/100g ww)	48	46	48	46	45
Lipid (g/100g ww)	27	27	30	26	24
Carbohydrate (g/100g ww)[2]	12.6	15.4	8.9	13.9	18.3
Ash (g/100g ww)	7.4	6.6	7.1	7.1	6.7
Energy (J/100g ww)	23 700	24 100	23 700	23 000	23 200
Dry matter (%)	95	95	94	93	94

Notes: Ingredients are listed as percentages of whole feed. [1]Wheat gluten meal, pea protein concentrate- and guar meal. [2]By calculation: carbohydrate (%) = dry matter (%) - protein (%) - lipid (%) - ash (%).

Table 5: Mineral composition of experimental diets

	Reference	BMM12	BMS3	BMS7	BMS11
Ca	14.4	11.9	12.2	11.7	10.9
Na	4.0	4.5	4.8	5.8	6.3
K	11.4	8.6	10.7	10.3	8.8
Mg	2.3	2.0	2.2	2.3	2.2
P	13.8	13.6	12.3	12.2	11.5
Mn	54.1	98.4	57.0	53.5	60.4
Fe	198.6	304.3	222.5	262.7	313.9
Cu	10.5	10.9	10.1	10.6	11.2
Zn	168.0	170.8	161.4	165.4	167.5
Se	0.9	1.0	0.9	0.9	0.9
I	3.9	4.6	5.5	7.0	11.0

Notes: Table shows analyzed levels of macro- and micro minerals in experimental diets. Ca, Na, K, Mg and P are listed as g/kilogram as is, whereas Mn, Fe, Cu, Zn, Se and I data are listed as mg/kg as is.

3.2. Experimental design

The trial was designed as a dose-response study with 4 dietary groups in triplicate for the mussel silage, and in addition one level of blue mussel meal (12%) close to the highest blue mussel silage inclusion level (11%). The trial was conducted at the Institute of Marine Research, Matre research station and lasted for 70 days from December 2020 to February 2021. Sixty-five Atlantic salmon (*Salmo salar*) post smolts weighing 206 ± 11g were kept in each of 15 quadrangular 1.5m^3 glass fiber tanks. The tanks were supplied with a flow through system with seawater at 8-9°C and a salinity of 34ppt. The fish were kept under a 24:0 light regime to promote growth and were fed in excess two meals per day (morning and afternoon). Uneaten pellets were collected after each meal for estimation of feed intake measurements as described by (Helland, Grisdale-Helland, & Nerland, 1996).

3.3. Sampling procedure

The fish were starved for 24 hours prior to sampling and were euthanized by an overdose (6ml/L) of trichina methane sulphonate (Finquel, MSD Animal Health). Individual blood samples were drawn with heparinized syringes from the caudal vein of 5 fish per tank. Plasma samples were separated from blood by centrifugation (13200 RPM, 2min, 4°C) and were kept on dry ice before transfer to -80°C until further analysis. Viscera, heart, and liver of 5 fish per tank were weighed and registered to calculate somatic indices. Liver samples from 5 fish per tank were harvested and immediately frozen on dry ice and stored at -80°C until further analysis. For nutrient digestibility analysis, feces were stripped from all the fish at the end of the trial. Additionally, 5 fish per tank (n = 15 per treatment) were collected for analysis of whole-body composition. Whole fish samples were homogenized and subsequently freeze-dried for 48 hours (Bulk dryer, Labconco) before determination of mineral content. Liver samples were pooled and analysed for mineral content as is.

3.4. Mineral analysis

The concentration of macro-minerals (Ca, Na, K, Mg, P) and micro-minerals (Mn, Fe, Cu, Zn, Se and I) were determined in feed and feces samples as well as targeted tissues (whole fish, liver, and plasma) with inductively coupled plasma mass spectrophotometry (ICP-MS) after microwave digestion. Further, yttrium was also analyzed in the feed and feces samples to determine the apparent digestibility of macro-nutrients and apparent availability of minerals from the feed. An account of the samples analyzed in given in the table below, following which a description of the methods is also provided.

Table 6: Overview of samples and analyses used in the experiment.

	No. of samples	Nature of the sample	Analytical replicates	Alkali	Metal	Iodine	Yttrium
Feed	5		2	x	x	x	x
Whole fish	15	pooled	2	x	x		
Liver	15	pooled	2		x	x	
Plasma	75	individual	1		x	x	
Feces	15	pooled	2		x		x

3.4.1. Multi-element determination with inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion

Chemicals and reagents

The chemicals and reagents used for the mineral analysis were the certified reference materials (CRM) oyster tissue (OT, CRM 1566, NIST) and TORT3 (National Research Council, Canada), concentrated nitric acid (HNO3, Suprapur), hydrogen peroxide (H_2O_2, 30%p.a.ISO), Milli-Q® water. For determination of the elements Fe, Cu, Zn, Mn and Se (ICP - Metal) the following standards were used: multi element standard (Spectrascan, 1000 mg/L Al, Fe, Mg, Zn, 50mg/L As, Ba, Cu, Mn, Se, Sr and 10 mg/L Ag, Cd, Co, Cr, Mo, Ni, Pb, U, V), rhodium (Spectrascan, 1012 ± 4 mg/L in 5% HCl), germanium (Spectrascan, 1000 ± 3 µg/L in H_2O) and Thulium (Certipur, 1000 ± 5 mg/L in 3% HNO_3). For determination of the elements Ca, Na K, Mg and P (ICP - Alkali) Spectrascan multi standard: Na (500 ± 3 mg/l), Mg (250 ± 1 mg/l), K (500 ± 3 mg/l), Ca (250 ± 1 mg/l) og P (500 ± 3 mg/l) was used and Scandium (Sc) Spectrascan 1006 ± 3 mg/l in 5% HNO_3 was used as internal standard for correction of the procedure. As there is no certified reference for determination of yttrium (ICP-yttrium) an in-house standard made of pulverized fish muscle with yttrium added prior to homogenization was used. Rhodium (Rh) Spectrascan 1012±4 mg/l in 5 % HCl was used as internal standard for ICP-Yttrium. The procedure for sample preparation and element determination for the methods ICP-Alkali, ICP-metal and ICP-Yttrium is the same and is described below.

Sample preparation and microwave digestion

Approximately 0.2g of freeze-dried sample or approximately 0.4 - 0.5 mL of thawed plasma was weighed in a 15mL quartz tube into which 2mL nitric acid 69% Suprapur (HNO_3) was added. The tubes were placed in a positioning rack then digested by microwave digestion in a Milestone UltraWave. The samples were diluted to 25ml in a volumetric flask with deionized and filtrated water (Milli-Q® water) and transferred to a 50mL falcon tube. The digested samples were stored at room temperature until the element determination was performed.

Element determination by inductively coupled plasma-mass spectrometry

For element determination an ICP-MS iCap Q (Thermo Fisher) with collision cell and FAST SC-4 DX autosampler was used. The tuning of the ICP-MS was performed using a tuning solution (1 ppb tuning solution B, Thermo Fisher, in 2% HNO3 and 0.5% HCl) prior to analysis. Data were collected and processed using the Qtegra ICP-MS software (Thermo Scientific, version 2.10.3324.83).

3.4.2. Iodine determination with ICP-MS after basic extraction

Chemicals and reagents

The chemicals and reagents used for analysis were TMAH - tetramethylammonium hydroxide ultra-pure Wako (TMAH 25%) CAS-nr:200-882-9, tellurium standard solution from NIST H_6TeO_6 1000mg/l in HNO_3 0.5 mol/l. Fish muscle ERM-BB422 from the National Institute of Standards and Technology (NIST) was used as the standard reference material and iodine standard (I) Spectrascan 997 ± 3µg/ml Tekno lab (Art.nr SS-11I) was used to ensure reliability of the method and results obtained.

Sample preparation and basic extraction

Approximately 0.2g of homogenized sample material was weighed in a 50ml falcon tube. Five milliliters of deionized and filtrated water (Milli-Q® water) and 1ml of TMAH 1% were then added. The sample tubes were shaken using a mini shaker to ensure homogeneity and then put into a hot water bath (Grant OLS 200) with continuous shaking at 100 rpm at a temperature of 90 ± 3°C for 3 hours. After cooling in a fume cupboard, the samples were diluted to 25ml in a volumetric flask and transferred back into the centrifugal tube. The samples were then centrifuged at 8000rpm for 10 minutes in a Thermo Fisher centrifuge Heraeur x IR and rotor Fiberlite F13-14 x 50 cy. Part of the sample solution was then filtered through a 0.45µm syringe filter to a 15ml falcon tube for further analysis.

Iodine determination by inductively coupled plasma-mass spectrometry

Iodine determination is performed by ICP-MS with stock tuning solution (100 ml) part #5188-6564 10 mg/ l Ce, Co, Li, T l and Y, and tellurium solution from NIST $H_6TeO_6$1000 mg/l HNO_3 0.5 mol/ l (Art. No. 1195140100) as internal standard to correct the baseline for operation. Data were collected and processed using the software MassHunter 4.5 Workstations (Agilent

Technologies Inc., version C.01.05). To assess accuracy of the analysis the sample material fish muscle (ERM- BB422; National Institute of Standards and Technology) was used.

3.5. Calculations

Growth performance, feed utilization, and fish body indices were calculated using following equations.

The specific growth rate was calculated as percentage growth per day:

$$SGR = (\ln BW_{Final} - \ln BW_{Initial} \times d^{-1}) \times 100.$$

where BW_{final} and $BW_{Initial}$ are final and initial weights in grams, and d is sum of experimental days.

The condition factor (K) was calculated as

$$K = 100 \times BM_{Final} \times Length^{-3},$$

where BM_{Final} is the final body weight in grams and Length is the fork length in centimeters.

The total feed intake (TFI) was calculated as an estimate of dry matter content of the waste feed (obtained in the recovery test) as described by Helland et al. (1996):

$$\text{Total feed intake (g)} = ((A \times A_{DM}/100) - (W \times W_{DM}/R))/ A_{DM}/100$$

Where A is weight of air-dry feed(g), A_{DM} is dry matter content of air-dry feed (%), W is weight of waste feed collected (g), W_{DM} is dry matter content of waste feed (%), and R is recovery of dry matter of waste feed (%).

$$\text{Recovery (\%)} = 100 \times (W \times W_{DM})/(A \times A_{DM})$$

Average daily feed intake per kg biomass (DFI) was calculated from recorded daily feed intake and estimated daily biomass from SGR using the equation

$$\ln W_{dayX} = (SGR/100) \times (1 + \ln W_{day(X-1)}$$

Where W_{DayX} is the biomass on a given day.

The feed conversion ratio (FCR) was calculated as:

$$FCR = W_{Diet} \times (BW_{Final} - BW_{Initial})^{-1},$$

where W_{Diet} is the weight of administered feed throughout the trial (feed eaten) in grams, BW_{Final} and $BW_{Initial}$ are the final and initial biomasses of the fish in grams, respectively.

The hepatosomatic indexes (HSI), cardio somatic indexes (CSI) and visceral somatic indexes (VSI) were calculated as percentages of the final weight:

$$HSI = (W_{Liver} / BW_{Final}) \times 100$$

$$CSI = (W_{Heart} / BW_{Final}) \times 100$$

$$VSI = (W_{Viscera} / W_{Final}) \times 100$$

where $W_{Liver,}$ W_{Heart} and $W_{Viscera}$ are the weights of the liver, heart, and viscera at the end of the trial respectively, and BW_{Final} is the final body weight.

Apparent digestibility coefficients (ADC) of dry matter, fat and protein were calculated as the ratio between the inert marker (yttrium) and the nutrient in question (dry matter, protein, or fat)

$$ADC = (1- [(\text{feed marker content} \times \text{fecal marker content}) \times (\text{feed nutrient content} \times \text{fecal nutrient content})^{-1}]) \times 100$$

Apparent availability coefficients (AAC) were calculated as the ratio between the inert marker (yttrium) and the minerals within diet and feces

$$AAC = (1- [(\text{feed marker content} \times \text{fecal marker content}) \times (\text{feed mineral content} \times \text{fecal mineral content})^{-1}]) \times 100$$

3.6. Statistical analysis

For all data sets Levene's test was used to assess the homogeneity of variance and Shapiro Wilk's test was used to check the normality of the data. Growth performance, somatic indices whole body and tissue mineral data of all the groups were analyzed using One-Way ANOVA (ANOVA) and the significant results were followed up by Tukey's honestly significant differences post-hoc test. To evaluate dose-dependent responses to the blue mussel silage feed groups, simple linear regression (LR) was performed with the reference and silage groups with the silage inclusion percentage on the x-axis (0, 3, 7 and 11). All statistical analyses were performed, and regression graphs were designed using Graph Pad Prism 8 software (Version 9.2.0 (283). San Diego, California USA). Significance was set at $p < 0.05$ for all statistical tests.

4. Results

4.1. Growth Performance Indicators

The mean initial body weight (IBW) of the reference group was 210 ± 6g. The IBW of the reference was not significantly different from any of the other experimental groups ($p > 0.05$, ANOVA).

The mean final body weight (FBW) of the reference group was 485 ± 14g. The final weight was lower in all the other experimental groups with the BMM12 group at 426 ± 98g, and 351 ± 39g the BMS11 group. The final body weights (FBW) were not significantly different between the groups ($p > 0.05$, ANOVA), but there was a dose-dependent decline in the fish fed blue mussel silage ($p = 0.0001$, $R^2 = 0.78$, LR).

The weight gain (WG) of the reference group was 275 ± 8g, whereas the BMM12 was a bit lower at 220 ± 94g, but not statistically significant due to high variation from random tank effects. The weight gain in the silage groups were even lower at 221 ± 23g, 184 ± 26g and 148 ± 22g in the BMS3, BMS7 and BMS11 groups, respectively. Although the groups weren't significantly different ($p > 0.05$, ANOVA), a significant decline was seen in the silage groups upon regression analysis ($p < 0.0001$, $R^2 = 0.96$, LR)

During the experiment, the specific growth rate (SGR) of the reference group was 1.3 ± 0.1, whereas the SGR of the BMM12 group was somewhat lower at 1.1 ± 0.5. The lowest SGR was seen in the BMS11 group at 0.8 ± 0.1. When comparing all the groups, SGR was not significantly different between the groups ($p > 0.05$, ANOVA), but a dose dependent decline was seen in the silage groups, with regression ($p < 0.0001$, $R^2 = 0.9$, LR).

The condition factor (K) of the reference group was 2.6 ± 0.1 and was not significantly different from that of any of the other groups ($p > 0.05$, ANOVA), but a dose-dependent decrease was observed in the silage groups ($p = 0.03$, $R^2 = 0.4$, LR).

Neither the total feed intake (TFI) or the daily feed intake adjusted for biomass (DFI) were significantly different between the treatments ($p > 005$, ANOVA), however, the TFI showed a dose-dependent decrease in the silage groups ($R^2 = 0.4$, $p < 0.05$)

The feed conversion ratio (FCR) of the reference group was 0.7 ± 0.01, whereas that of the BMM12 group was 0.8 ± 0.2. The highest FCR was seen in the BMS11 group at 1.1 ± 0.3. The FCR of the experimental groups were not significantly different from each other ($p > 0.05$, ANOVA), but a dose-dependent increase was seen in the silage groups ($p < 0.01$, $R^2 = 0.6$). A summary of the growth performance indicators is displayed in Table 7 below.

Table 7: Growth Performance Indicators of Atlantic salmon post smolt fed blue mussel meal and graded inclusion of blue mussel silage.

	Reference	BMM12	BMS3	BMS7	BMS11	ANOVA	Regression
IBW (g)	210 ± 6	206 ± 13	211 ± 8	201 ± 12	204 ± 17	n.s.	n.s.
FBW (g)	485 ± 14	426 ± 98	432 ± 24	385 ± 37	351 ± 39	n.s.	$R^2 = 0.78$, $p = 0.0001$
WG (g)	275 ± 8	220 ± 94	221 ± 23	184 ± 26	148 ± 22	n.s.	$R^2 = 0.96$, $p < 0.0001$
SGR	1.3 ± 0.1	1.1 ± 0.5	1.2 ± 0.03	1.0 ± 0.1	0.8 ± 0.1	n.s.	$R^2 = 0.9$, $p < 0.0001$
K	1.3 ± 0.01	1.2 ± 0.1	1.3 ± 0.03	1.2 ± 0.01	0.9 ± 0.3	n.s.	$R^2 = 0.4$, $p = 0.03$
TFI (kg)	11.9 ± 0.3	11.9 ± 1.4	10.2 ± 0.4	10.2 ± 0.4	10.1 ± 1.4	n.s.	$R^2 = 0.4$, $p < 0.05$
DFI (%)	0.6 ± 0.3	0.4 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	n.s.	n.s.
FCR	0.7 ± 0.01	0.8 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	1.1 ± 0.3	n.s.	$R^2 = 0.6$, $p < 0.01$

Notes: IBW = initial body weight (g), FBW = final body weight (g), WG = weight gain (g), SGR = specific growth rate, K = condition factor, TFI = total feed intake (kg), DFI(%) = daily feed intake as percentage of biomass, SGR = specific growth rate. Data is presented as mean ± SD. Means with different superscripts are significantly different ($p < 0.05$) from the Tukey HSD test. The column labeled "Regression" gives R^2 and p-value for linear regression performed for the reference and silage groups with silage inclusion percentage as x-variable (0, 3, 7 and 11).

4.2. Somatic Indices

As for the hepatosomatic- (HSI), cardio somatic- (CSI) and visceral somatic indices (VSI) there were no statistically significant differences between the groups ($p > 0.05$, ANOVA), but the CSI showed a dose-dependent increase in the silage groups ($p = 0.02$, $R^2 = 0.08$, LR). Somatic indices of the different groups are displayed in Table 8 below.

Table 8: Somatic indices of Atlantic salmon post smolt fed blue mussel meal and graded inclusion of blue mussel silage.

	Reference	BMM12	BMS3	BMS7	BMS11	ANOVA	Regression
HSI	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	n.s.	n.s.
CSI	0.15 ± 0.027	0.15 ± 0.016	0.16 ± 0.040	0.17 ± 0.030	0.18 ± 0.028	n.s.	R^2 = 0.08, p =0.02
VSI	7.2 ± 1.0	6.7 ± 0.9	6.9 ± 0.4	7.2 ± 0.5	7.3 ± 0.8	n.s.	n.s.

Notes: HSI = hepatosomatic index, CSI = cardio somatic index, VSI = visceral somatic index. Data is listed as mean ± S.D. Means with different superscripts are significantly different ($p < 0.05$). The column labeled "Regression" gives R^2 and p-value for linear regression performed for the reference and silage groups with silage inclusion percentage as x-variable (0, 3, 7 and 11).

4.3. Apparent digestibility

In the reference group the apparent digestibility coefficients were 95.2 ± 0.1, 86.9 ± 1.1 and 94.8 ± 0.5 for dry matter, protein, and fat, respectively. None of the apparent digestibility coefficients were significantly different between groups ($p > 0.05$, ANOVA). No dose-dependent effects were observed in the silage groups for the ADC of fat or protein ($p > 0.05$, LR), but a dose-dependent increase was observed in the silage groups for ADC of dry matter ($p = 0.02$, $R^2 = 0.44$, LR). An overview of ADCs is displayed in table 9 below.

Table 9: Dry matter, fat, and protein apparent digestibility coefficients (ADC) of Atlantic salmon post smolt fed blue mussel meal and graded inclusion of blue mussel silage.

	Reference	BMM12	BMS3	BMS7	BMS11	ANOVA	Regression
Dry matter	95.2 ± 0.1	95.7 ± 0.9	96.1 ± 0.2	96.3± 0.4	96.1 ± 0.1	n.s	R^2 = 0.44, p = 0.02
Total fat	94.8 ± 0.5	95.7 ± 1.9	97.0 ± 0.7	96.8± 1.0	95.7 ± 0.7	n.s.	n.s.
Protein	86.9 ± 1.1	88.4 ± 2.2	88.9 ± 0.5	88.1± 1.0	87.5 ± 0.7	n.s.	n.s.

Notes: Data is listed as mean ± S.D. Means with different superscripts are significantly different ($p < 0.05$). The column labeled "Regression" gives R^2 and p-value for linear regression performed for the reference and silage groups with silage inclusion percentage as x-variable (0, 3, 7 and 11).

4.4. Mineral status and apparent availability

4.4.1. Macro-mineral status

The whole-body Ca, Na, K, Mg and P status in the different treatment groups are listed below (Table 10). No significant differences were observed in whole-body macro-mineral status of any of the dietary groups ($p > 0.05$, ANOVA). No dose-dependent effects on the whole-body macro-mineral status were observed in any of the treatment groups ($p > 0.05$, LR).

Table 10: Whole body mineral composition of Atlantic salmon post smolt fed blue mussel meal and graded inclusion of blue mussel silage.

	Reference	BMM12	BMS3	BMS7	BMS11	ANOVA	Regression
Ca	2.1 ± 0.6	2.1 ± 0.4	1.7 ± 0.4	2.0 ± 0.6	1.7 ± 0.1	n.s.	n.s.
Na	0.9 ± 0.03	0.9 ± 0.01	0.9 ± 0.03	0.9 ± 0.04	1.0 ± 0.1	n.s.	n.s.
K	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.4	n.s.	n.s.
Mg	0.4 ± 0.02	0.3 ± 0.1	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.04	n.s.	n.s.
P	3.4 ± 0.3	3.5 ± 0.2	3.2 ± 0.2	3.3 ± 0.3	3.2 ± 0.2	n.s.	n.s.

Notes: Data are means ± SD of pooled samples of 5 fish with 3 tanks per diet group. All values are listed as gram per kilogram wet weight (g/kg ww). Means with different superscripts are significantly different ($p < 0.05$). The column labeled "Regression" gives R^2 and p-value for linear regression performed for the reference and silage groups with silage inclusion percentage as x-variable (0, 3, 7 and 11).

4.4.2. Micro mineral status

Manganese (Mn)

Manganese concentrations in the reference group were 1.0 ± 0.3 mg/kg ww, 1.2 ± 0.1 mg/kg ww and 0.8 ± 0.2 μmol/L in whole body, liver, and plasma samples, respectively. The Mn AAC of the reference group was -29 ± 32%. In the BMM12 group the whole-body Mn concentration was 1.3 ± 0.2 mg/kg ww whereas the liver and plasma Mn concentrations were 1.1 ± 0.01 mg/kg ww and 0.9 ± 0.3 μmol/L, respectively. None of the analyzed tissues from the BMM12 group were significantly different from those of the reference group ($p > 0.05$, ANOVA).

The Mn AAC for the BMM12 group was not significantly different from the reference at 21 ± 17 ($p > 0.05$, ANOVA). Manganese concentration in the BMS3, BMS7 and BMS11 were as follows 0.8 ± 0.1 mg Mn/kg ww, 0.8 ± 0.2 mg Mn/kg ww and 0.6 ± 0.01 mg Mn/kg ww for the whole-body samples; liver, 1.1 ± 0.1, 1.0 ± 0.1, and 1.1 ± 0.03 mg Mn/kg ww; and in the plasma as 0.4 ± 0.1, 0.3 ± 0.1 and 0.3 ± 0.01 μmol Mn/L.

Whole-body and liver group means of the silage groups were not significantly different from those of the reference ($p > 0.05$, ANOVA), but both indicator tissues showed a dose-dependent decrease (whole body: $p < 0.05$, $R^2 = 0.36$, LR; liver: $p < 0.05$, $R^2 = 0.44$, LR). Plasma Mn concentration in the BMS3, BMS7 and BMS11 groups were all significantly lower than that of the reference ($p < 0.0001$, ANOVA), and a dose-dependent decline in plasma Mn status was also observed in the silage groups ($p < 0.01$, $R^2 = 0.60$, LR). The Mn AACs of the silage groups were not significantly different from that of the reference ($p > 0.05$, ANOVA) and no dose-dependent effect was observed ($p > 0.05$, LR). An illustration of the results can be viewed below (figure 2).

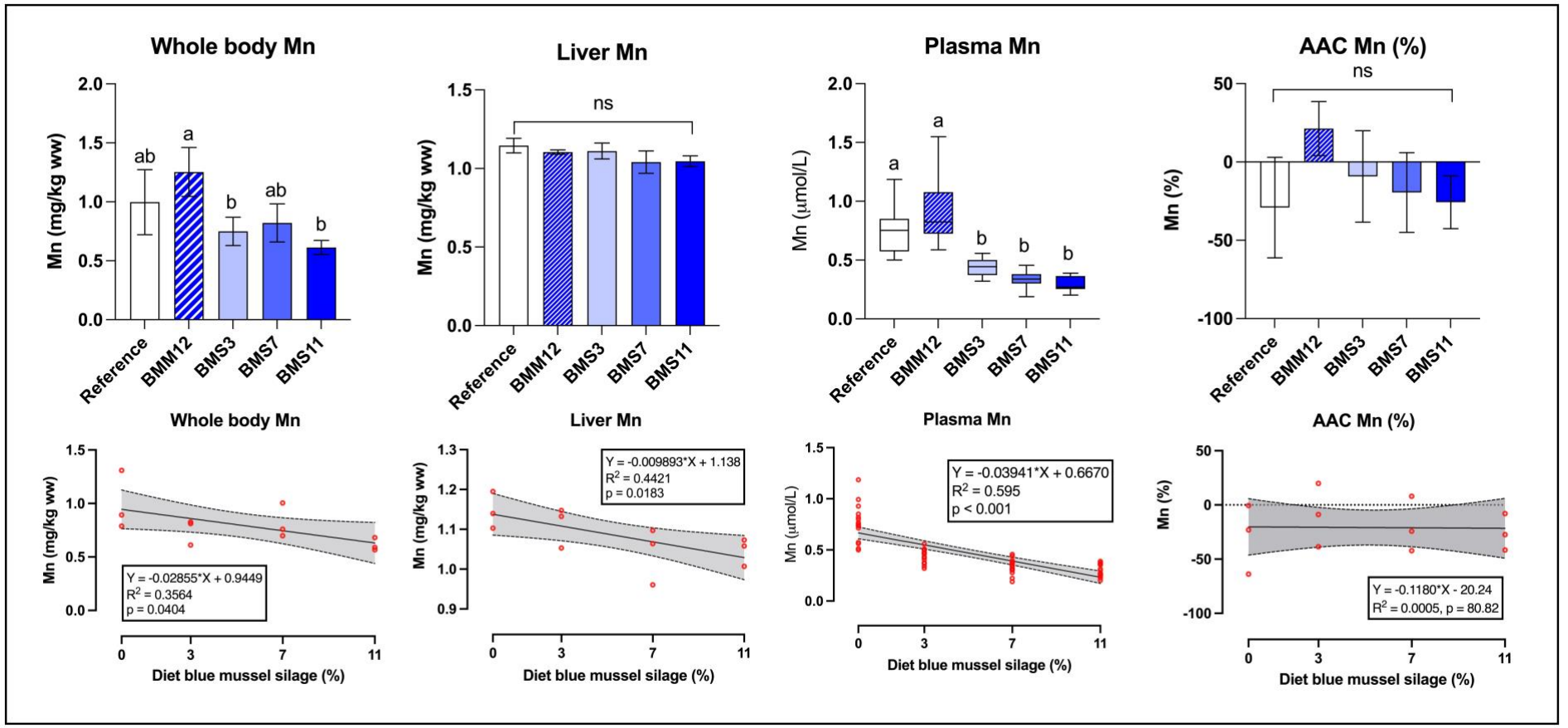

Figure 2. **Whole body, liver, and plasma manganese (Mn) status and apparent availability coefficients (AAC) of Mn**. First row (bar graph): Whole body, liver and AAC data is presented as mean ± SD of pooled samples of 5 fish per diet group. Plasma data is boxplot of n = 15 observations from 3 tanks per diet group. Groups annotated with different letters are significantly different after ANOVA and Tukey HSD tests, $p < 0.05$ was accepted as significant. Second row (regression): linear regression with 95% confidence interval of whole body-, liver-, plasma data and AAC. For whole body, liver and AAC, data points are means of n = 5 fish per tank, for the plasma graph data points are values from n = 15 individual fish from each group.

Copper (Cu)

Copper concentrations in the reference group were 1.6 ± 0.03 mg/kg ww, 132 ± 12 mg/kg ww and 6.5 ± 1.8 μmol/L in whole body, liver, and plasma samples, respectively. In the BMM12 group the whole-body Cu concentration was 1.6 ± 0.1 mg/kg ww whereas the liver and plasma Cu concentrations were 117 ± 18 mg/kg ww and 6.1 ± 2.1 μmol/L, respectively. None of the analyzed tissues in the BMM12 group were significantly different from those of the reference group ($p > 0.05$, ANOVA).

Copper concentration in the BMS3, BMS7 and BMS11 groups were as follows, 1.8 ± 0.1 mg/kg ww, 1.9 ± 0.2 mg/kg ww and 2.1 ± 0.2 mg/kg ww for the whole-body samples; liver, 134 ± 9 mg/kg ww, 169 ± 8 mg/kg ww and 165 ± 18 mg/kg ww; and in plasma, 7.2 ± 1.8 μmol/L, 6.4 ± 1.4 μmol/L and 5.6 ± 1.6 μmol/L, respectively.

Whole body Cu concentration in the BMS11 group was significantly higher than the reference ($p < 0.05$, ANOVA), in the liver samples, only the BMS7 group was significantly higher than the reference ($p < 0.05$, ANOVA). Significant dose-dependent increase was observed in both whole body and liver samples of the silage groups (whole body: $p < 0.001$, $R^2 = 0.71$, LR; liver: $p < 0.05$, $R^2 = 0.58$, LR). Plasma Cu status did not differ significantly between the dietary groups ($p > 0.05$, ANOVA) or show a dose-dependent response in the silage groups ($p > 0.05$, LR). The Cu AAC did not vary significantly between the dietary groups ($p > 0.05$, ANOVA) and no dose-dependent effect was observed in the silage groups ($p > 0.05$, LR). An illustration of the results can be viewed below (figure 3).

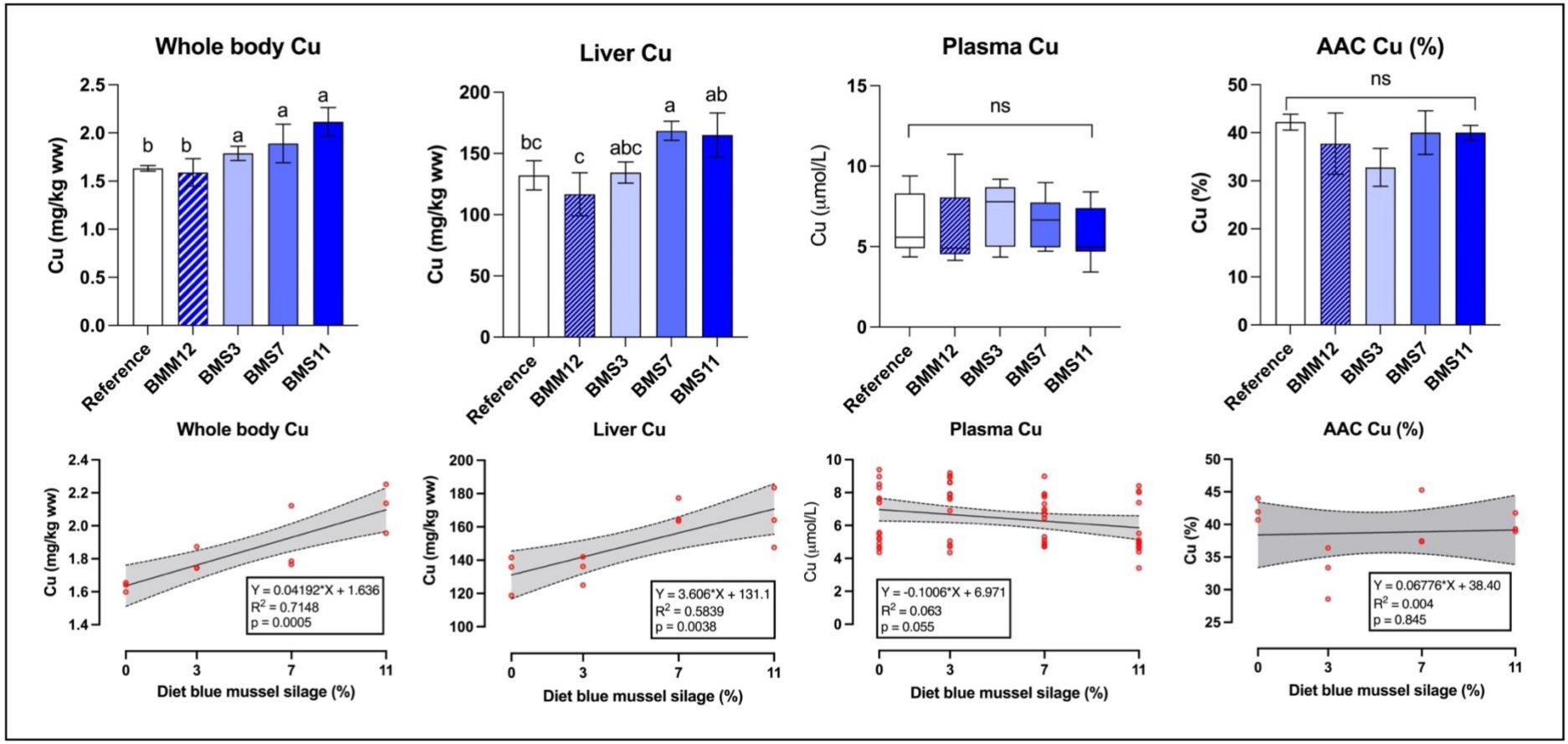

Figure 3. **Whole body, liver, and plasma copper (Cu) status and apparent availability coefficients (AAC) of Cu**. First row: Whole body, liver and ACC data is presented as mean ± SD of pooled samples of 5 fish per diet group. Plasma data is boxplot of n = 15 observations from 3 tanks per diet group. Groups annotated with different letters are significantly different after ANOVA and Tukey HSD tests, $p < 0.05$ was accepted as significant. Second row: linear regression with 95% confidence interval of whole body-, liver-, plasma data and AAC. For whole body, liver and AAC, data points are means of n < 5 fish per tank, for the plasma graph data points are values from n = 15 individual fish from each group.

Iron (Fe)

Iron concentrations in the reference group were 8.4 ± 1.1 mg/kg ww, 64 ± 4 mg/kg ww and 15 ± 11 μmol/L in whole body, liver, and plasma samples, respectively. In the BMM12 group the whole-body Fe concentration was 9.2 ± 0.3 mg/kg ww whereas the liver and plasma Fe concentrations were 80 ± 4 mg/kg ww and 22 ± 13 μmol/L, respectively. In the BMM12 group the liver status was higher than that of the reference ($p < 0.0001$, ANOVA), whereas the whole body and plasma status was not different from the reference.

Iron concentration in the BMS3, BMS7 and BMS11 groups were respectively as follows, 5.9 ± 0.4, 4.6 ± 0.4 and 4.9 ± 0.1 mg Fe/kg ww for the whole-body samples; the same in the liver samples were 21.7 ± 3.2, 16.9 ± 2.8 and 18.0 ± 0.9mg Fe/kg ww; and in in the plasma as 5.8 ± 3.7, 6.2 ± 3.9, and 6.8 ± 4.9 μmol Fe/L.

Whole-body and liver group means of the silage groups were all significantly lower than that of the reference (whole body and liver: $p < 0.0001$, ANOVA) and showed dose-dependent decreases (whole body: $p < 0.01$, $R^2 = 0.64$, LR; liver: $p < 0.01$, $R^2 = 0.59$, LR). The plasma Fe of the BMS3 and BMS7 groups were significantly lower than the reference ($p < 0.0001$, ANOVA), whereas that of the BMS11 was not. A dose-dependent decrease was observed in the plasma status of the silage groups ($p < 0.01$, $R^2 = 0.12$, LR). The Fe AAC of the reference group was -3.7 ± 10.2 and did not differ significantly between the dietary groups ($p > 0.05$, ANOVA) but the silage groups showed dose dependent increase ($p < 0.05$, $R^2 = 0.46$, LR). An illustration of the results can be viewed below (figure 4).

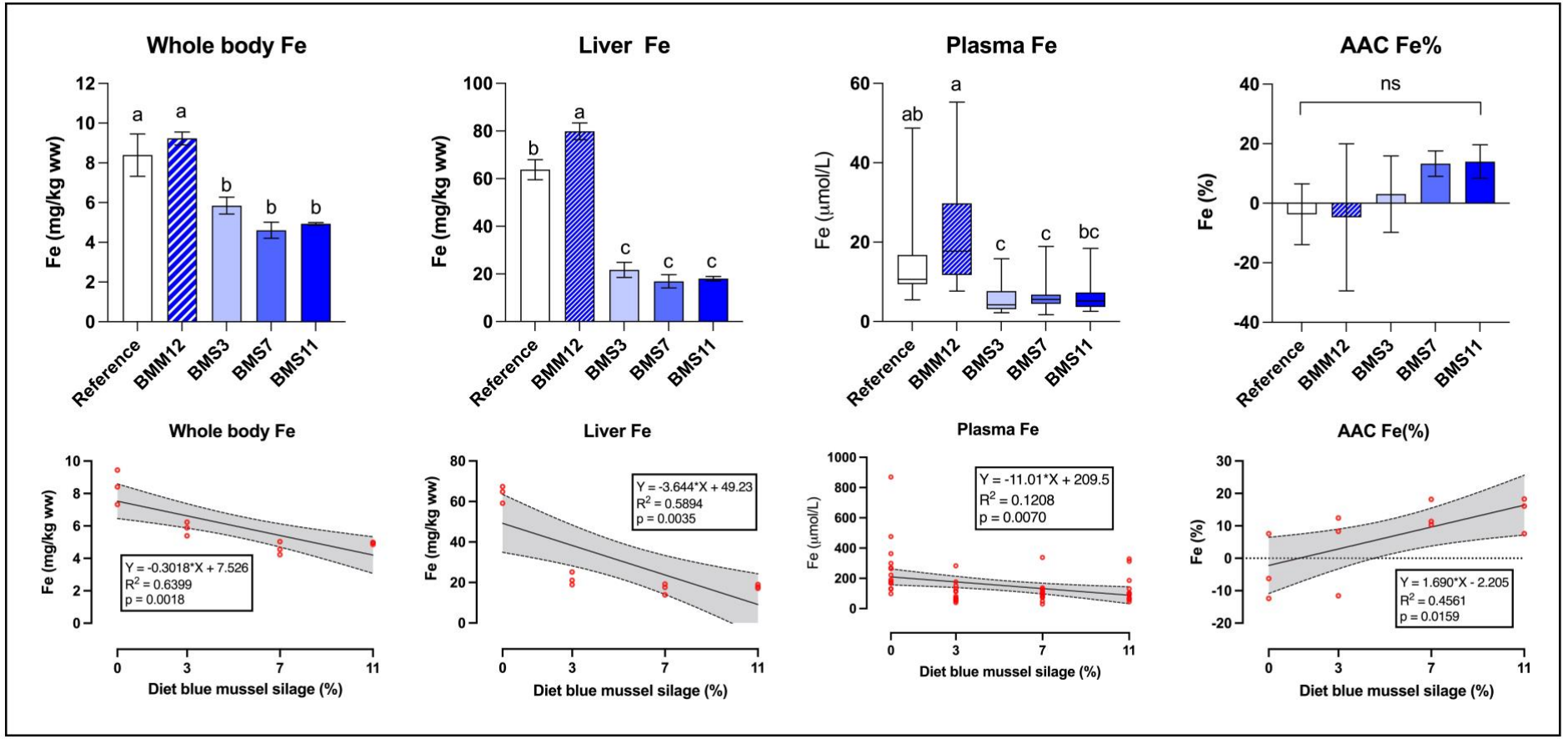

Figure 4. **Whole body, liver, and plasma iron (Fe) status and apparent availability coefficients (AAC) of Fe**. First row: Whole body, liver and ACC data is presented as mean ± SD of pooled samples of 5 fish per diet group. Plasma data is boxplot of n = 15 observations from 3 tanks per diet group. Groups annotated with different letters are significantly different after ANOVA and Tukey HSD tests, $p < 0.05$ was accepted as significant. Second row: linear regression with 95% confidence interval of whole body-, liver-, plasma data and AAC. For whole body, liver and AAC, data points are means of n = 5 fish per tank, for the plasma graph data points are values from n = 15 individual fish from each group

Selenium (Se)

Selenium concentrations in the reference were 0.22 ± 0.01 mg/kg ww, 1.6 ± 0.1 mg/kg ww and 1.8 ± 0.3 μmol/L respectively in the whole-body, liver and plasma. The same in the blue mussel meal (BMM12) group was 0.23 ± 0.003 mg/kg ww, 1.6 ± 0.1 mg/kg ww and 1.7 ± 0.3 μmol/L in the whole-body, liver and plasma, not significantly different from the reference group.

The selenium concentrations in the BMS3, BMS7 and BMS11 groups were respectively as follows 0.21 ± 0.001 mg/kg ww, 0.21 ± 0.005 mg/kg ww and 0.18 ± 0.002 mg/kg ww in the whole body; the same in the liver were 1.5 ± 0.09 mg/kg ww, 1.5 ± 0.1 mg/kg ww, 1.4 ± 0.04 mg/kg ww; and in plasma as 1.6 ± 0.2 μmol/L, 1.4 ± 0.2 μmol/L and 1.2 ± 0.2 μmol/L.

The BMS3 and BMS7 groups were not significantly different from the reference in the whole-body Se status, however, that of BMS11 was significantly lower than the reference ($p < 0.001$, ANOVA). A dose-dependent decrease was observed in whole-body Se status of the silage groups ($p < 0.001$, $R^2 = 0.73$, LR). Liver Se status of BMS groups were not significantly different from the reference or BMM12 groups ($p > 0.05$, ANOVA). A dose-dependent decrease was observed in the liver Se status of the silage groups ($p < 0.05$, $R^2 = 0.48$, LR). Plasma Se concentration in the BMS7 and BMS11 were significantly different from the reference at 1.4 ± 0.2 and 1.2 ± 0.2 μmol/L, respectively ($p < 0.0001$, ANOVA). A dose-dependent decrease was observed in the plasma Se status of the silage groups ($p < 0.001$, $R^2 = 0.51$, LR). Selenium AAC was 55 ± 3 in the reference group. None of the other treatments were significantly different from the reference ($p > 0.05$, ANOVA), but a dose-dependent increase was observed ($p < 0.05$, $R^2 = 0.40$, LR). An illustration of the results can be viewed below (figure 5).

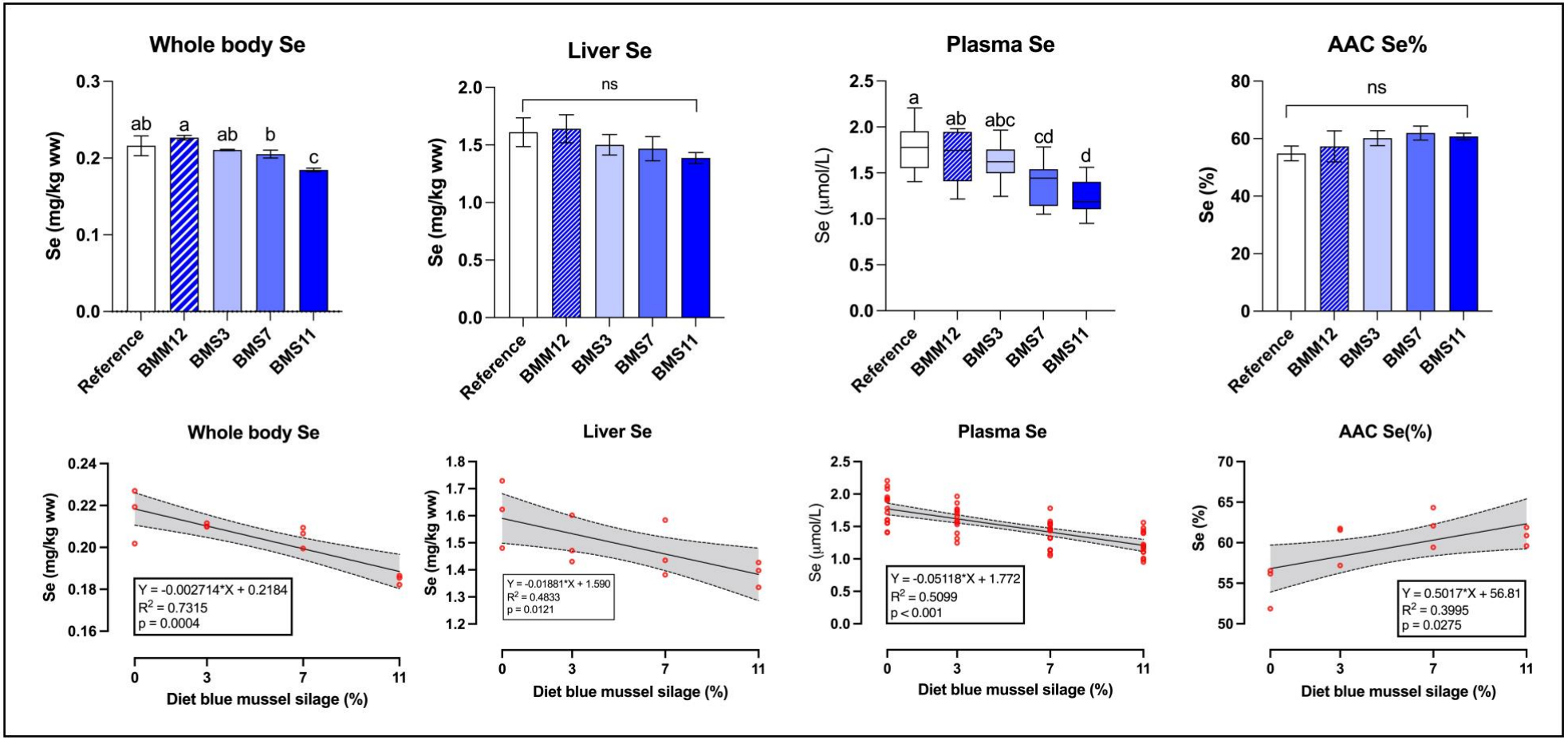

Figure 5. **Whole body, liver, and plasma selenium (Se) status and apparent availability coefficients (AAC) of Se**. First row: Whole body, liver and ACC data is presented as mean ± SD of pooled samples of 5 fish per diet group. Plasma data is boxplot of n = 15 observations from 3 tanks per diet group. Groups annotated with different letters are significantly different after ANOVA and Tukey HSD tests, $p < 0.05$ was accepted as significant. Second row: linear regression with 95% confidence interval of whole body-, liver-, plasma data and AAC. For whole body, liver and AAC, data points are means of n = 5 fish per tank, for the plasma graph data points are values from n = 15 individual fish from each treatment group.

Zinc (Zn)

The zinc concentration in the reference group was 26 ± 1 mg/kg ww, 21 ± 1 mg/kg ww and 209 ± 39 μmol/L in the whole body, liver, and plasma samples, respectively. Neither the BMM12 group nor the silage groups were statistically different from the reference in any of the analyzed tissues ($p > 0.05$, ANOVA). The plasma Zn status in the BMM12 group however was significantly higher than that of the silage groups ($p < 0.005$, ANOVA). No significant dose dependent effects were observed in either whole-body, liver or plasma Zn status ($p > 0.05$, LR). The Zn AAC for the reference group was 30 ± 6% and was not significantly different from any of the other treatment groups ($p > 0.05$, ANOVA). No dose-dependent effect was observed in Zn AAC ($p > 0.05$, LR). An illustration of the results can be viewed below (figure 6).

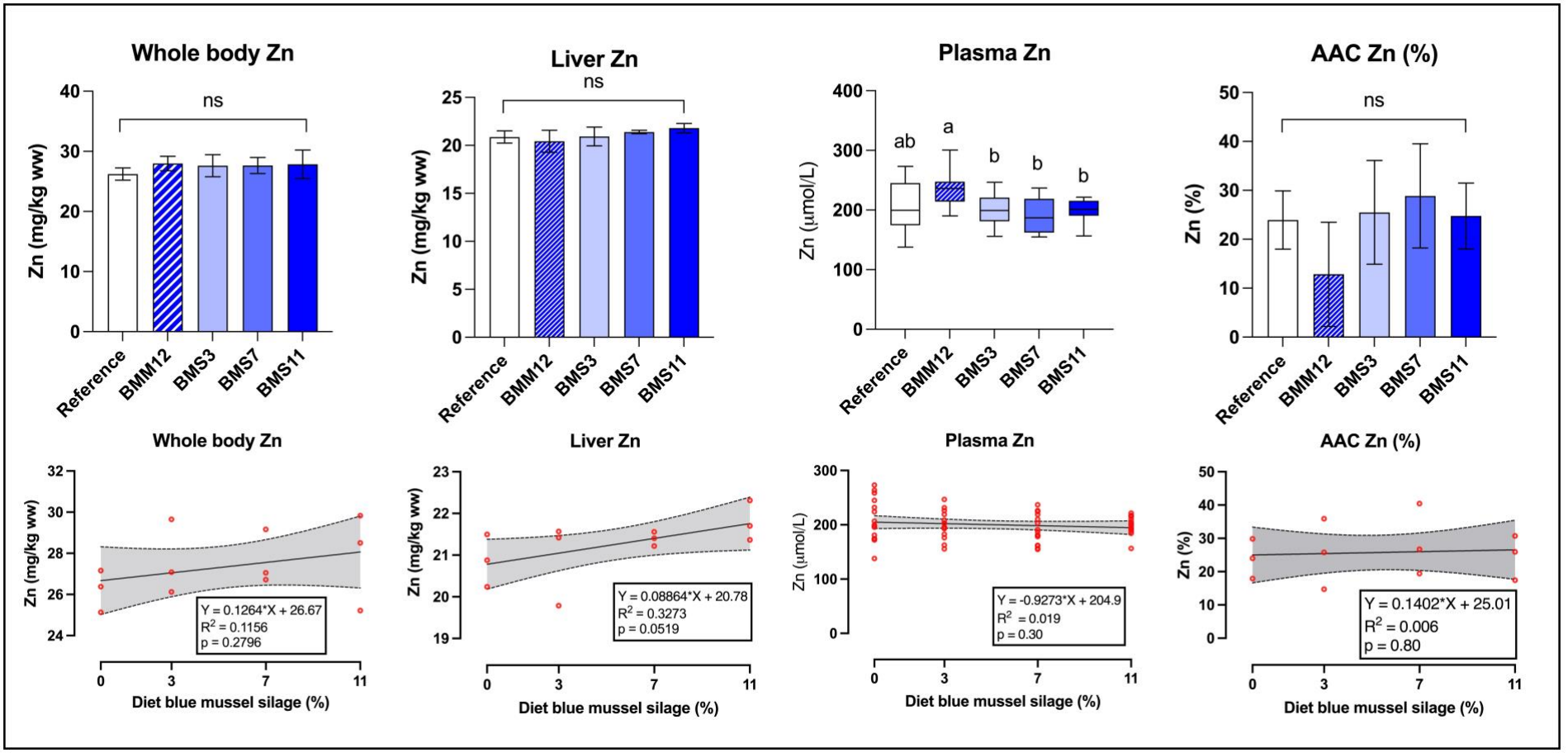

Figure 6. **Whole body, liver, and plasma Zn status and apparent availability coefficients (AAC) of Zn.** First row: Whole body, liver and ACC data is presented as mean ± SD of pooled samples of 5 fish per diet group. Plasma data is boxplot of n = 15 observations from 3 tanks per diet group. Groups annotated with different letters are significantly different after ANOVA and Tukey HSD tests, $p < 0.05$ was accepted as significant. Second row: linear regression with 95% confidence interval of whole body-, liver-, plasma data and AAC. For whole body, liver and AAC, data points are means of n = 5 fish per tank, for the plasma graph data points are values from n = 15 individual fish from each group.

Iodine (I)

For the reference group, the whole body I status was 0.2 ± 0.02 mg/kg ww, the liver status was 0.5 ± 0.1 mg/kg ww and the AAC was 70 ± 6%. Iodine status in neither whole fish, liver, nor AAC was affected by feed type as there were no significant differences between the treatment groups ($p > 0.05$, ANOVA) and no dose-dependent effects were observed in any of the analyzed tissues or AAC ($p > 0.05$, LR). An illustration of the results can be viewed below (figure 7).

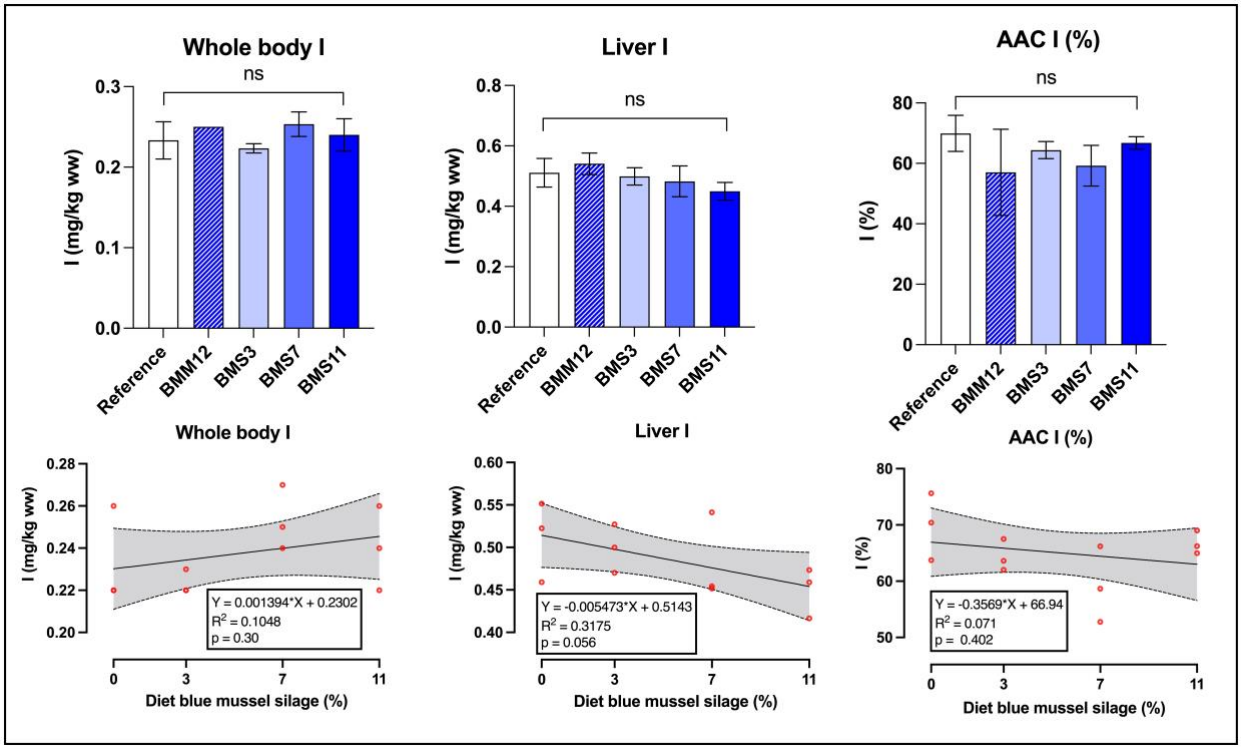

Figure 7. **Whole body, and liver iodine (I) status and apparent availability coefficients (AAC) of I.** First row: Whole body, liver and AAC data is presented as mean ± SD of pooled samples of 5 fish per diet group. Groups annotated with different letters are significantly different after ANOVA and Tukey HSD tests, $p < 0.05$ was accepted as significant. Second row: linear regression with 95% confidence interval of whole body- and liver data, and AAC. For whole body, liver and AAC, data points are means of n = 5 fish per tank.

5. Discussion

The aim of this experiment was to assess the potential for blue mussel silage in feeds for Atlantic salmon. Although there have been studies on the use of blue mussel meal in fish feeds, no literature is available on using blue mussel silage in diets for Atlantic salmon.

Growth performance and feed utilization

In summary, the fish fed blue mussel meal performed comparably to the reference, whereas the silage groups exhibited reduced growth. The reference grew as expected for fish at this size and temperature with a mean weight gain of 275 ± 8g. There was slight decrease in growth in the BMM12 group, but it was not significant. The variation in the final body weight and weight gain of the BMM12 group, however, was very large with a mean FBW of 426 ± 98g and mean WG of 220 ± 94g. The large variation growth was also manifested in other growth performance indicators of the BMM12 group such as the SGR and TFI (Table 7). The reason behind this large variation is possibly due to random tank effects.

All the silage groups had significantly lower growth (WG) than the reference, with the lowest seen in the BMS11 group. Similarly, the dose-dependent declines in SGR and K indicate the inclusion of BMS caused poorer growth performance. The dose-dependent decrease in total feed intake, is expected as the feed intake increases with increasing size (in absolute terms). The non-significant results in the feed intake adjusted for biomass (DFI) however, suggests the fish ate relatively equal amounts of feed thus ruling out decreased feed intake caused by e.g., palatability as the cause of reduced growth in the silage groups. The FCR showed a dose-dependent increase in the silage groups, indicating a poorer utilization of the silage feed compared to the reference. The reduced growth and poorer feed utilization observed in the BMS group contrasts with the results of previous studies on fish or blue mussel silage in fish or animal feeds (Espe, Haaland, & Njaa, 1992; Nørgaard et al., 2015).

Fish silage made using acid hydrolysis of bycatch or byproducts from the fish processing industry is not a new phenomenon and its use in countries like Denmark, Poland and Norway goes back to the 1940s as a protein source in feed for pigs, poultry and mink (Arason, 1994; Jackson, Kerr, & Cowey, 1984). Silage can typically replace 5-15% of formulated feeds for fish and can replace added water before extrusion of feed pellets (Toppe et al., 2018). Adding silage can also make the finished pellet stronger, thus reducing losses during transport and feeding

(Toppe et al., 2018). Espe et al. (1992) found that substituting about 20% of dietary protein with fish silage for Atlantic salmon resulted in the same protein utilization as fish fed non-hydrolyzed protein, but lower fat deposition in the filet. Heras et al. (1994) also found no significant changes in weight gain, feed efficiency or protein efficiency ratio for Atlantic salmon fed dogfish and herring silage. Other studies have found that low or moderate inclusions of fish protein concentrate (concentrated silage) results in better growth than with high or no inclusion at all (Espe, Sveier, Høgøy, & Lied, 1999; Hevroy et al., 2005), and that inclusion of fish protein concentrate may lower the visceral mass relative to body weight in Atlantic salmon (Espe, Ruohonen, & El-Mowafi, 2012). In one study done on pigs, feeds containing blue mussel silage yielded similar growth to a commercially relevant diet and increased ileal digestibility of protein (Nørgaard et al., 2015). As previously mentioned, several studies have shown successful results substituting FM with BMM up to as much as 40 % in salmonids without impairing growth (Table 1). Based on these findings from literature there was no reason to assume that blue mussel silage would not result in acceptable growth in Atlantic salmon.

The apparent digestibility coefficient (ADC) of DM showed a slight increase in the silage groups, whereas the ADCs for fat and protein were not significantly affected. Judging from the ADCs alone, the fish were able to digest BMM and BMS relatively well. In one study done with pigs, using blue mussel silage improved ileal digestibility of protein compared to when using blue mussel meal (Nørgaard et al., 2015). In that study the mussels were boiled and deshelled prior to the making of silage. This is in contrast with the production method chosen in this experiment where the mussels were crushed and mechanically separated without any heat treatment prior to silage processing. How the different approaches to producing BMS affects its nutritional properties is an open question.

Of the somatic indices only the cardio somatic index showed a slight dose-dependent increase (Table 8), however the fit of the model used in this analysis was so poor ($R^2 = 0.084$) that the result should be interpreted with caution. In this trial the HSI was unaffected by dietary inclusion of blue mussels. Berge and Austreng (1989) however, observed enlarged livers in rainbow trout diets made from whole crushed mussels and suspected that the high degree of crushed shells in the diet caused physiological stress on the liver. In the trial of Berge and Austreng (1989), the diet with the most BMM (45%) had twice as much ash content than that of the control (20 vs. 10% of the diet). In this experiment however, the BMM and BMS were produced from de-shelled mussels and thus had much lower ash contents. The ash content of

the diets containing blue mussel were marginally lower than the reference, with the lowest in the BMM12 at 66 g/kg. This is in accordance with other trials using deshelled mussels as a protein source, where effects on liver size have not been observed. Berge and Austreng (1989) hypothesized that the tendency towards poorer growth in the fish fed 45% BMM could be caused by the lower energy density of the feed. In this study however, the energy densities of the experimental diets were similar (Table 4) and would therefore not explain the reduced growth in the BMM and BMS groups alone.

Another possible explanation for poorer growth could be the presence of glycogen in the feed as carbohydrates are generally not easily digested by salmonids. Glycogen serves as a nutrient storage for mussels that can be drawn upon during spawning or other energy demanding processes. The glycogen content of blue mussels varies considerably throughout the year and is heavily influenced by the feed availability and spawning behavior (Okumuş & Stirling, 1998). By calculation, the carbohydrate contents in the formulated diets were slightly higher in the groups with the highest silage content (Table 4). The dry matter ADC however showed a dose dependent increase in the silage groups, suggesting that the feeds with BMS were more easily digested than the reference diet. Furthermore, the VSI and HSI indices showed no signs of an effect, which could have been indicative of increased visceral mass, fat deposition etc. that is associated with high carbohydrate contents in salmonid feeds (Hemre, Mommsen, & Krogdahl, 2002; Vangen & Hemre, 2003). Therefore, it is less likely that the growth differences observed in this study were due to limitations in macro-nutrient digestibility and utilization of the feeds.

Mineral status and utilization

The macro-mineral status did not vary significantly between the experimental groups or show any dose-dependent responses. Whole body Ca, Na and P levels in all the groups were slightly lower than whole body mineral data as reported in literature while Mg and K levels were within the ranges described in Table 2. The discrepancies between the observed concentrations in the reference group to those listed in Table 2 could be due to due to differences in life stage or fish size. Consequently, the feeds containing both blue mussel meal and blue mussel silage at the inclusion levels used in this experiment appears to not affect the macro-mineral supply for Atlantic salmon post smolt.

On the contrary, the micro-mineral status of Atlantic salmon fed BMM and BMS feeds were differentially affected. In the case of manganese, the dose-dependent decrease in whole-body Mn and the significantly lower plasma Mn status in the silage groups indicate that the Mn homeostasis of fish fed BMS was impaired. Of the microminerals studied, copper was the only one to increase in the silage groups as copper status and AAC of Cu increased in the BMS-fed fish despite lower Cu-contents in the feed compared to that of the reference.

The most striking results of this experiment was the low iron status in fish fed blue mussel silage. Despite an increasing trend in dietary iron (Table 5), whole body, liver and plasma Fe concentrations of the fish fed BMS were almost a third of that of the reference and BMM12 group (Figure 3). The highest hepatic iron concentration was observed in the BMM12 group which reflects the high Fe content in the feed (Table 5). Iron is present in all cells and plays a vital role in oxygen transport as a component of hemoglobin and myoglobin, as well as being part of enzymes involved in DNA synthesis and amino acid-, catecholamine- and serotonin catalysis (Lall, 2003). The diet is the main source of iron (Bury & Grosell, 2003) and tissues that can be used as indicators for iron status include whole body, head kidney, and liver concentrations as well as hematological parameters like blood hemoglobin or hematocrit levels (Andersen et al., 1996; Bjørnevik & Maage, 1993; Naser, 2000). Most of the iron contents of fish occurs as heme-iron as parts of hemoglobin, myoglobin, or cytochromes, whereas non-heme iron is part of many enzymes and constitutes most of the iron reserves in the liver, spleen, and hematopoietic tissue where it is bound to hemosiderin or ferritin (Walker & Fromm, 1976).

In this study, the analyzed feeds contained iron at concentrations ranging from just under 200 to over 300 mg Fe/kg, well above the 60-100mg Fe/kg which is considered necessary for Atlantic salmon to maintain appropriate body stores according to Andersen et al. (1996). Nevertheless, all the BMS groups displayed markedly lower iron status than that of the reference and BMM12 group. The dietary supply of minerals needs to be sufficient to maintain mineral homeostasis as mineral body stores of fish decrease over time when fed sub-optimal levels (Maage & Julshamn, 1993; Watanabe, Kiron, & Satoh, 1997). Furthermore, Atlantic salmon fed a diet of low iron content may develop microcytic anemia (Andersen et al., 1996; Bjørnevik & Maage, 1993). While the whole-body and plasma Fe status in the silage groups showed significant decrease, the most pronounced effect was observed in the liver samples. This is to be expected as the liver Fe status is the main storage of Fe (Walker & Fromm, 1976). The normal range of hepatic Fe concentration for post smolt Atlantic salmon is 56 - 102 mg/kg,

the liver Fe concentrations in the reference and BMM12 group were in this range, however, the concentrations observed in BMS3, BMS7, and BMS11 groups were significantly lower ranging from 18 to 21 mg Fe/kg ww, respectively. Thereby, indicating that the iron homeostasis in the BMS groups was impaired. Considering neither the reference nor the BMM12 group showed signs of decreased iron status and that all diets were added the same mineral premix which included iron as $FeSO_4$, there is little reason to believe the iron supplementation of the BMS feeds varied sufficiently to produce the observed effects. Furthermore, as fish can absorb iron over the gills at least 22 weeks is considered necessary for fish to develop iron deficiency when fed a low iron diet (Naser, 2000). The fact that the iron stores were depleted to such an extent in this experiment which lasted only about 10 weeks suggests that dietary availability or utilization of iron was hampered severely.

The major site of iron absorption in fish is the gastrointestinal tract (Whitehead, Thompson, & Powell, 1996) whereas small amounts of iron are excreted by the liver through bile, by the kidney through urine, or feces by sloughing of epithelial cells in the intestinal wall. In the present study, despite the decline in whole body and tissue Fe levels, the AAC of Fe was found to increase with increasing BMS inclusion. Intestinal absorption and homeostasis of iron is regulated by the iron status of the fish, with increased absorption and incorporation when iron stores are low (Standal, Dehli, Rørvik, & Andersen, 1999). As the feces samples were taken at the end of the experiment, the increase seen in the AAC of Fe for the silage groups (Figure 3) reflects this and indicates that the BMS fed fish were attempting to replenish its iron stores. The low iron and manganese status observed in the silage groups could also explain the increase in copper status as divalent ions such as Fe^{2+}, Mn^{2+} and Cu^{2+} may compete for the same route of uptake (Bury et al., 2003) and dietary antagonistic interactions between these trace elements in fish are well known (Antony Jesu Prabhu, Silva, et al., 2019; Lorentzen & Maage, 1999; Ogino & Yang, 1980).

Several anti-nutritional factors present in plant-derived ingredients are known to affect mineral availability, as they interfere with the entero-hepato-pancreatic system of fish (Antony Jesu Prabhu et al., 2016). As the diets used in this experiment contained equal amounts of plant-derived ingredients, possible ANFs such as phytate should affect the diets alike judging from their possible concentrations alone. One problem that occurred during the manufacturing of the experimental feed for this trial was that the water content of the blue mussel silage was too high for it to be directly incorporated into pellets. This was solved by co-drying the BMS with soy

protein concentrate. Whether this affected the mineral availability of the feed is an open question.

As with other minerals, the availability of iron is affected by several factors such as the amount and chemical form of the mineral and interaction with other minerals or nutrients. As mentioned earlier, the absence of calcium-rich shells present during silage process could cause the chemical conditions to differ from those during production of conventional fish silage as bone present in the mixture can increase the buffering capacity of the silage (Arason, 1994). For instance, the in-vitro availability of iron is shown to be affected in feed for ruminants after silage fermentation, a process similar to the method used in this trial as it is characterized by a decrease in pH (Hansen & Spears, 2009). Iron naturally occurs in different redox states and can switch between ferric (Fe^{3+}) and ferrous (Fe^{2+}) forms in response to e.g., pH. which in turn may affect its bioavailability. Only ferrous iron is transported through the epithelium of the cells in the intestinal wall, thus ferric iron needs to be reduced for it to be taken up by the cell (Bury & Grosell, 2003; Bury et al., 2003). This process is mediated by enzymes known as ferric reductases, present in the apical membrane of intestinal epithelial cells (figure 2).

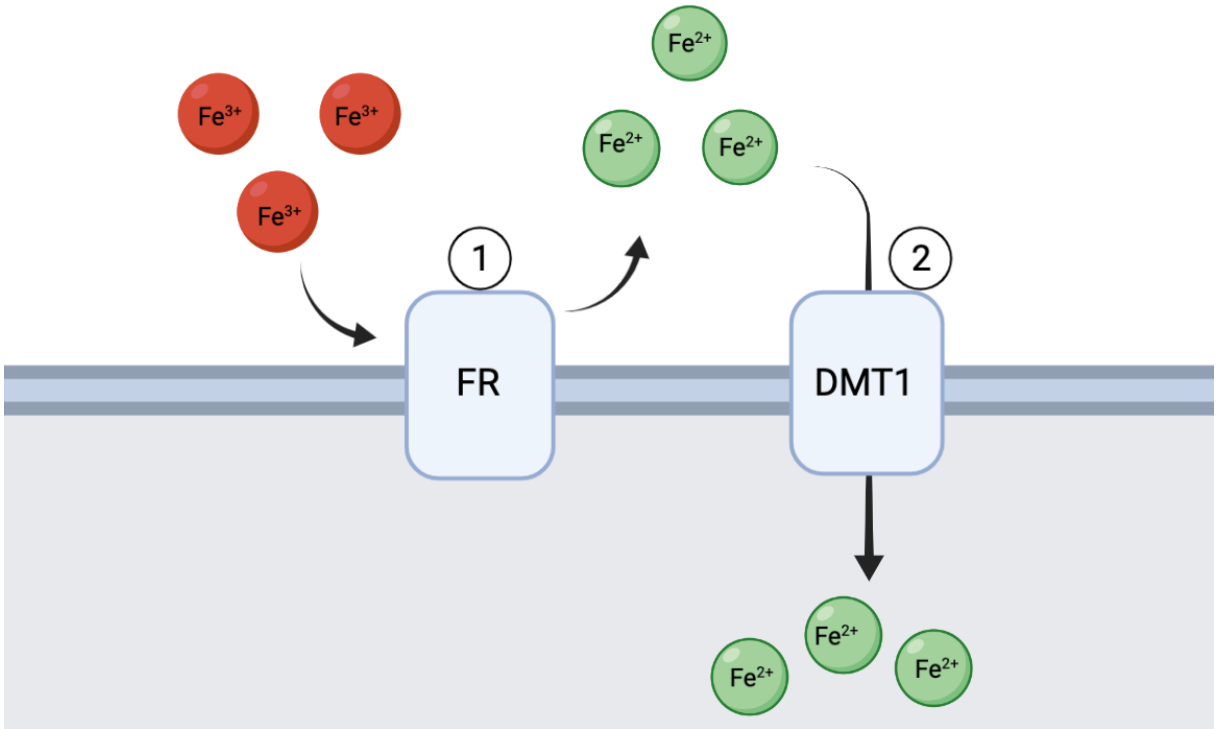

Figure 2: Schematic outline of Fe absorption from the lumen to the interior of the epithelial cell. (1) Apical ferric reductase reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}); (2) apical Fe uptake of ferrous iron via the divalent metal transporter 1 (DMT1). Adapted from Antony Jesu Prabhu (2015).

This process is a limiting factor in intestinal iron uptake physiology which causes the availability of ferric iron to be lower than that of ferrous iron. For instance, the Fe availability to juvenile tilapia (*Oreochromis niloticus* × *O. aureus*) from ferric citrate ($FeC_6H_5O_7 \cdot H_2O$) was only half (~50%) of that of ferrous sulphate ($FeSO_4 \cdot 7H_2O$) (Shiau & Su, 2003), which was the form of supplemented iron used in this trial. One hypothesis for this could be that in the production of the BMS feeds, the formic acid caused the dietary iron to form ferric formate or related ferric compounds resulting in a relative abundance of ferric iron over ferrous iron. The capacity of the ferric reductases would not have been sufficient to convert enough ferrous iron, thus reducing the overall availability and utilization of dietary iron, hence a net loss of iron occurred in the animal resulting in the observed phenotype.

In the BMS groups a decrease in selenium status was also observed. Selenium is an integral part of selenoproteins that have a range of biological functions and is important for immune responses in fish (Brigelius-Flohé, 2015). In this experiment, the reference group whole body and liver Se concentrations were within what is considered a normal range for Atlantic salmon whereas the plasma Se concentration was slightly lower (1.8 ± 0.3 μmol/l in the reference group versus 3.3 ± 0.7 μmol/l, Table 2). The selenium concentrations of the analyzed feeds were all ~0.9 mg/kg as is, which was enough to meet the dietary requirement of Se in Atlantic salmon post-smolts (Table 3). The similar levels of Se in the experimental diets, but lower Se status suggests a sub-optimal utilization of the dietary selenium in the silage groups. Although the whole body Se status seen in the silage groups was not dramatically lower than that of the reference at the time of the sampling, the significantly lower plasma Se status and increased AAC of Se indicate that the fish in the silage groups were not able to maintain body stores of Se. If this trend were to continue, there is reason to believe the fish would develop symptoms of sub-optimal Se supply over long run. Examples of Se deficiency symptoms in fish include reduced growth in rainbow trout and muscular dystrophy in Atlantic salmon when also deficient in vitamin E (Hilton, Hodson, & Slinger, 1980; Poston, Combs, & Leibovitz, 1976).

Neither zinc nor iodine status was affected by the addition of blue mussel meal or blue mussel silage indicating that the inclusion of BMM or BMS was unproblematic with respect to these minerals.

6. Conclusion

The results of this trial indicate that both the fish fed BMM and BMS were able to utilize the feeds well with regards to macro-nutrients and macro-minerals, but that the micro-minerals were the limiting factors in the BMS groups. The growth of the fish fed BMM was comparable to the reference, in line with previous findings. No significant differences were seen in the macro-mineral status in any of the experimental groups, indicating blue mussels processed in either way are satisfactory replacements of FM in this respect. For the micro-minerals, the only significant difference between the reference and BMM12 group was observed for hepatic Fe, reflecting the levels in the feed. The main finding of this experiment was the impaired status of Mn, Se and Fe the BMS groups, with the most pronounced effect on Fe status, which could be caused by the processing method used to produce BMS.

$H0_1$: Replacement of fish meal by blue mussel silage in the feed for Atlantic salmon post-smolt does not affect growth performance and feed utilization, **is rejected. $H1_1$**: Replacement of fish meal by blue mussel silage in the feed for Atlantic salmon post-smolt does affect growth performance, feed utilization, **is accepted.**

$H0_2$: The processing method of blue mussel used in feed for Atlantic salmon post-smolt does not affect growth performance and feed utilization, **is rejected. $H1_2$**: The processing method of blue mussel used in feed for Atlantic salmon post-smolt does affect growth performance and feed utilization, **is accepted.**

7. Future perspectives

Feed and resource utilization is of great importance in salmon farming as it is a large contributor to the environmental footprint of the industry. The introduction of plant-derived ingredients to feeds for farmed salmon has facilitated industry growth, however competing interests with both people and wildlife emphasizes the need to look elsewhere for the feed ingredients of the future. Similarly, as wild fisheries are not likely to grow and are increasingly used for direct human consumption, alternative marine resources should be in focus. While blue mussels have potential, the results of this experiment highlight the need to study effects of both raw materials and processing methods when assessing new feed ingredients. Therefore, the effects of silage processing on micro-mineral availability needs better understanding if it is to be considered as a viable method of preparing blue mussels for use in fish feed.

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Appendix I: Mineral status overview

Whole body, liver, and plasma mineral composition of Atlantic salmon post smolt fed blue mussel meal and graded inclusion of blue mussel silage.

	Reference	BMM12	BMS3	BMS7	BMS11	ANOVA	Regression
Whole body							
Ca	2.1 ± 0.6	2.1 ± 0.4	1.7 ± 0.4	2.0 ± 0.6	1.7 ± 0.1	n.s.	n.s.
Na	0.9 ± 0.03	0.9 ± 0.01	0.9 ± 0.03	0.9 ± 0.04	1.0 ± 0.1	n.s.	n.s.
K	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.4	n.s.	n.s.
Mg	0.4 ± 0.02	0.3 ± 0.1	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.04	n.s.	n.s.
P	3.4 ± 0.3	3.5 ± 0.2	3.2 ± 0.2	3.3 ± 0.3	3.2 ± 0.2	n.s.	n.s.
Mn	1.0 ± 0.3^{ab}	1.3 ± 0.2^{a}	0.7 ± 0.1^{b}	0.8 ± 0.2^{ab}	0.6 ± 0.01^{b}	R^2 = 0.69, p = 0.01	R^2 = 0.36, p = 0.04
Cu	1.6 ± 0.03^{b}	1.6 ± 0.1^{b}	1.8 ± 0.1^{a}	1.9 ± 0.2^{a}	2.1 ± 0.1^{a}	R^2 = 0.75, p = 0.004	R^2 = 0.71, p < 0.001
Fe	8.4 ± 1.1^{a}	9.2 ± 0.3^{a}	5.8 ± 0.4^{b}	4.6 ± 0.4^{b}	4.9 ± 0.1^{b}	R^2 = 0.94, p < 0.0001	R^2 = 0.64, p < 0.01
Se	0.22 ± 0.013^{ab}	0.23 ± 0.003^{a}	0.21 ± 0.001^{ab}	0.21 ± 0.01^{b}	0.18 ± 0.002^{c}	R^2 = 0.87, p = 0.0002	R^2 = 0.73, p < 0.001
Zn	26 ± 1	28 ± 1	28 ± 2	28 ± 1	28 ± 2	n.s.	n.s.
I	0.23 ± 0.02	0.25 ± 0.00	0.22 ± 0.01	0.25 ± 0.02	0.24 ± 0.02	n.s.	n.s.
Liver							
Mn	1.1 ± 0.05	1.1 ± 0.01	1.1 ± 0.05	1.0 ± 0.07	1.0 ± 0.03	n.s.	R^2 = 0.44, p = 0.018
Cu	132 ± 12^{bc}	117 ± 18^{c}	134 ± 8.9^{abc}	169 ± 8^{a}	165 ± 18^{ab}	R^2 = 0.78, p = 0.003	R^2 = 0.58, p < 0.01
Fe	64 ± 4^{b}	80 ± 4^{a}	22 ± 3^{c}	17 ± 3^{c}	18 ± 1^{c}	R^2 = 0.99, p < 0.0001	R^2 = 0.58, p < 0.01
Se	1.6 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.05	n.s.	R^2 = 0.48, p = 0.012
Zn	20.9 ± 0.6	20.4 ± 1.1	20.9 ± 0.9	21.4 ± 0.2	21.8 ± 0.5	n.s.	n.s.
I	0.51 ± 0.05	0.54 ± 0.04	0.50 ± 0.03	0.48 ± 0.05	0.45 ± 0.03	n.s.	n.s.
Plasma							
Mn	0.8 ± 0.2^{a}	0.9 ± 0.3^{a}	0.4 ± 0.1^{b}	0.3 ± 0.1^{b}	0.3 ±0.01^{b}	R^2 = 0.72, p < 0.0001	R^2 = 0.595, p<0.0001
Cu	6.5 ± 1.8	6.1 ± 2.1	7.2 ± 1.8	6.4 ± 1.4	5.6 ± 1.6	n.s.	n.s.
Fe	15 ± 11^{ab}	22 ± 13^{a}	5.8 ± 3.7^{c}	6.2 ± 3.9^{c}	6.8 ± 4.9^{bc}	R^2 = 0.38, p < 0.0001	R^2 = 0.1208, p<0.01
Se	1.8 ± 0.3^{a}	1.7 ± 0.3^{ab}	1.6 ± 0.2^{abc}	1.4 ± 0.2^{cd}	1.2 ± 0.2^{d}	R^2 = 0.47, p < 0.0001	R^2 = 0.51, p<0.0001
Zn	13.7 ± 2.6^{ab}	15.5 ± 2.1^{a}	13.1 ± 1.7^{b}	12.5 ± 1.8^{b}	13.1 ± 1.1^{b}	R^2 = 0.23, p = 0.0001	n.s.

Notes: Whole body- and liver data are mean ± SD of pooled samples of 5 fish with 3 tanks per diet group. Plasma data are mean ± SD of 15 individual samples from 3 tanks per diet group. The column ANOVA displays results of One-Way ANOVA performed with feed type as predictor variable. Means with different superscripts are significantly different ($p < 0.05$) after the Tukey HSD post-hoc test. The column labeled "Regression" shows R^2 and p-value of simple linear regression performed for the reference and silage groups with silage inclusion percentage as x-variable (0, 3, 7 and 11). Macro-mineral concentrations (Ca, Na, K, Mg and P) are listed as g/kg ww. Micro-mineral concentrations (Mn, Cu, Fe, Zn, Se, I) are listed as mg/kg ww from whole body and liver whereas plasma concentrations are µmol/L.