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Blue mussel (*Mytilus edulis*) silage, a possible low trophic marine protein source for Atlantic salmon (*Salmo salar* L.)

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ABSTRACT

Blue mussel (*Mytilus edulis*) could be a promising marine protein source in fish feeds and is of great interest since it can be cultivated along the Norwegian coastline. However, the use of blue mussels in feeds is dependent on developing suitable preservation and processing methods to produce a feed grade raw material. The present studies were conducted to investigate whether blue mussel silage could be used in the feed for Atlantic salmon post-smolt. Two feeding experiments were conducted using the same reference diet with FM inclusion of 25%, giving a mix of ~59–63% plant-based ingredients vs ~34–36% marine ingredients to simulate a standard grower feed for salmon post-smolts in SW. In experiment 1, fish were fed diets containing three different inclusion levels of blue mussel silage (BMS 3, 7, and 11%), a diet containing blue mussel meal (BMM) (12%) as well as the reference feed. In this experiment, the fish that were fed a diet containing BMS had a decline in both weight gain and condition factor when compared to the fish given the reference and BMM. The daily feed intake was similar in all groups, but the feed conversion ratio (FCR) increased in the fish fed BMS. The inclusion of BMS and BMM did not affect the digestibility of nutrients, but reduced retention of whole-body lipid and protein retention was observed. Salmon given BMS in the diet also had lower iron (Fe) concentrations in liver and whole body, indicating lower Fe uptake, irrespective of inclusion level. These findings were followed up in a second feeding experiment aiming to investigate whether different processing methods of blue mussel silage could influence the bioavailability of iron, as well as feed utilization and growth. The reference feed was formulated similar to the feed in exp. 1. Additionally, fish were fed diets containing BMM (9%) and the same batch of BMS (9%) used in exp. 1 as well as two diets containing new productions of BMS (9%) using either a lower acid level or only formic acid at the same level. In experiment 2, no differences were seen in weight gain, feed intake, FCR, nutrient retention or body composition between fish given BMS and reference diet. The lower Fe status observed in experiment 1 was not seen in the second study. In both experiments, there were no differences in fish welfare indicators between the group of fish fed with BMS, BMM and the reference group.

The present results show that blue mussel silage can be used in the diet for Atlantic salmon, however, the different processing and preservation methods to produce BMS influence the nutritional properties and consequently growth performance and feed utilization of Atlantic salmon post-smolts.

1. Introduction

In 2020, over one million tons of Atlantic salmon, accounting for 53% of the global salmon production, was produced in Norway (Fiskeridirektoratet, 2023a). The annual use of feed ingredients in farming of salmon in Norway is almost 2 mill tons (as is) (Aas et al., 2022). Today >90% of the ingredients used in fish feeds in Norway is imported (Aas et al., 2022), contributing to >70% of the greenhouse gas

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emissions from the industry. Thus, new local resources with a low climate footprint are needed.

The past decades, the protein used in aquafeeds has shifted from a high level of fish meal (FM) to plant proteins (Albrektsen et al., 2022; Aas et al., 2019), and today only 12% of the protein and 10% of the lipids in the salmon feed comes from the sea. The traditional sources for marine ingredients, industrially fished species (reduction fisheries) and offal's, cannot be increased, thus, new marine resources will have to come from lower trophic levels. Blue mussels (*Mytilus edulis*) are of high interest as a potential marine protein source (Bellona, 2021; Filgueira et al., 2019; Gjøsund et al., 2020; Kiessling, 2009). The production of blue mussels for use in food in Norway was only 2647 tons in 2022 (Fiskeridirektoratet, 2023b), but the possibilities for increasing the production has recently been simulated (Gatti et al., 2023) which was shown the Hardangerfjord could host large-scale mussel farming for aquafeed and human consumption (Gatti et al., 2023).

The nutritional profile of blue mussels makes them suitable for use in fish feeds (Berge and Austreng, 1989; Kikuchi and Furuta, 2009b; Kikuchi and Sakaguchi, 1997). Several studies have shown that blue mussel processed into a meal can be used in fish feeds (Kikuchi and Furuta, 2009a, 2009b; Langeland et al., 2016; Nagel et al., 2014; Vidakovic et al., 2016; Weiss and Buck, 2017), also improving the palatability of plant protein-diets and growth of fish (Kikuchi and Furuta, 2009a; Nagel et al., 2014). Previous studies have also shown that using the whole shell is challenging due to increased ash level and reduced energy density of the feeds (Berge and Austreng, 1989). Blue mussel meat has a high moisture content (> 95%), neutral pH (6.7–7.1), and hydrolytic enzymes that result in fast degradation, despite using refrigerated storage (Bhunia et al., 2017; Ovissipour et al., 2013; Zhou et al., 2019). Additionally, blue mussels have a large seasonal variation in nutrient composition. For instance, peak carbohydrate accumulation (glycogen) occurs in spring and summer, with subsequent depletion during autumn and winter. Conversely, lipid and protein trends displayed smoother variations and depended mostly on the reproductive cycle of the mussels. It has been shown that the highest nutritional quality accrues before the gametogenesis phase of maturation in mussels which usually is in late spring, while it can vary in different regions (Petes et al., 2008; Fernández et al., 2015). Therefore, efficient preservation methods are also necessary to produce a feed raw material with high nutritional quality throughout the year (Fernández et al., 2015). To minimize the deterioration of fresh by-products like fish offal or meat of blue mussels, preservation by acid silage is a simple and inexpensive alternative (Olsen and Toppe, 2017). Low manufacturing cost, preserving the nutrients with high quality in addition to being an environmentally friendly process (low waste, low carbon footprint) are the main advantages of acid silage (Fagbenro and Jauncey, 1993; Vidotti et al., 2003). Through silage technology using short-chain organic acids, the proteins are hydrolyzed, resulting in the formation of small peptides and free amino acids (Espe et al., 2015). These peptides are quickly digested and absorbed by the gastrointestinal tract, which could impact the overall digestibility of the products (Gilbert et al., 2008) and enhance the availability of nutrients in the feed (Espe et al., 1999). For instance; replacing up to 15% of the FM protein by fish protein hydrolysate (FPH) in Atlantic salmon diet resulted in increased growth, feed utilization, and digestibility (Espe et al., 1999; Refstie et al., 2004). Similarly, the replacement of 18–24% of the FM with FPH in post-smolt salmon diets resulted in increased feed intake, specific growth rate, feed conversion ratio, and protein digestibility (Hevrøy et al., 2005). In the diet of rainbow trout (*Oncorhynchus mykiss*), FM could also be substituted with 20% FPH without adverse effects on growth performance, fatty acid composition and serum biochemical variables (Güllü et al., 2014). However, a high amount of water in the silage products can be a drawback in terms of transportation and commercialization (Barreto-Curiel et al., 2016). It also make it difficult to be used directly in dry or moist feed (Madage et al., 2015). To address this issue, silage can be dried together with dry ingredients, such as soybean-, feather- or poultry by products meals or cereal brans or drum drying have been suggested as a solution (Dong et al., 1993; Goddard and Perret, 2005; Hardy et al., 1984; Madage et al., 2015; Nwanna et al., 2004). Drying techniques that use heat to remove water may affect the nutritional value of the end products (Goddard and Perret, 2005). Therefore, it is important to choose an appropriate drying method that preserves the quality of the final product, while also minimizing the climate footprint of the processing methods.

Blue mussel silage (BMS) has been tested as a dietary ingredient in pigs, resulting in higher ileal crude protein digestibility compared to FM (Nørgaard et al., 2015). However, up to date, there has been no prior study on its use in fish diets, particularly salmonids. It is important to determine the effect of raw material processing as well as the availability of nutrients from the raw material to avoid the occurrence of nutritional deficiencies, imbalances or the effect of potential antinutrients that historically have been related to malnutrition, reduced welfare and occurrence of production-related disorders when introducing new raw materials in the feed (reviewed by Waagbø and Remø, 2020). The present study was conducted to investigate whether blue mussel silage and blue mussel meal can be used in feed for Atlantic salmon post-smolts, based on growth, welfare, nutrient digestibility and retention.

2. Material and method

2.1. Ethical statement

Both feeding experiments were conducted at Matre Research station, Norway, according to the Norwegian regulations on Animal Experimentation (FOTS ID # 25202 for experiment 1).

2.2. Blue mussel silage

The blue mussel silage was provided by Ocean Forest AS (Bergen, Norway). To mitigate the impact of seasonal and geographical variations on the nutritional composition of BM in both experiments, undersized blue mussels were collected from commercial blue mussel farming operated by Blå Biomass A/S in Limfjorden, Denmark in spring season. The first blue mussel silage product was made by adding soft acid (aqua M, produced by Borregaard) to the meat part of blue mussel after a mechanical crushing step and separating the blue mussels into three parts: shell, byssus threads and meat. According to the safety data sheet (revision date 28.12.2022, version 2.4.0), the substance mixture of soft acid is 75–85% formic acid, 15–25% sodium lignosulfonate (lignosulfonic acid, sodium salt, as antioxidant) and liquid.

For experiment 2, a new blue mussel silage batch was produced using a lower soft acid content (aqua M, produced by Borregaard) and one with only formic acid. The acid level was added, and consequently pH level was lower in the second BMS production. The pH level and proximate composition of BMS products are given in Table 1 and Table A and B of supplementary.

2.3. Experimental diets

2.3.1. Experiment 1

The first feeding experiment was designed as a dose-response study using three different inclusion levels of BMS (3, 7, and 11% of diet, substituting 12%, 28%, 44% of the fish meal (FM) in the diet), and in addition one diet contained blue mussel meal (BMM) (12% of diet, substituting 48% of FM). Each experimental diet was tested in triplicate tanks. The reference diet was formulated as a commercially relevant diet for post-smolt in seawater with 25% FM. In this experiment, the BMS used had a dry matter content of 10% and was therefore co-dried with soy protein concentration (SPC) before feed production to obtain the target levels of BMS in the feed by Cargill (Dirdal, Norway). The proximate and amino acids composition of the control and experimental diets of the first experiment are given in Tables 2 and 4, respectively.

Table 1
Macro-nutrient and mineral proximate composition of blue mussel silage (BMS) products.

	BMS High soft acid[1]	BMS Low soft acid	BMS Low formic acid
Macro-nutrients proximate composition (g 100 g^{-1} WW)			
Protein	23	22	21
Lipid	0.6	0.3	0.4
Ash	5.3	6.6	6.4
Dry matter	48	47	45
Macro-mineral composition (mg kg^{-1} WW)			
Ca	2976	4042	4140
Na	11,520	15,040	14,400
K	3024	3337	3015
Mg	1680	2115	1980
P	1968	1692	1395
Micro-mineral composition (mg kg^{-1} WW)			
Mn	14	66	99
Cu	3.7	1.9	1.7
Fe	274	409	387
Se	1.1	0.8	0.7
Zn	22	24	26
TBARS	79	106	73
pH	2.5	3.7	3.5
Histamine	<5	<5	<5

WW refers to wet weight.
[1] BMS High soft acid group is the same product that was used in both experiment1 and 2. A new blue mussel batch and different types and amount of acid was used for producing the other two groups (BMS with lower soft acid and BMS with only formic acid).

2.3.2. Experiment 2

In experiment 2, the fish were given five different diets. The reference diet was formulated to be similar to the diet used in experiment 1, with 25% FM. Four experimental diets were produced, two containing the same blue mussel meal (BMM9) and BMS (BMS9) that was used in the first trial, and two using new productions of BMS, with lower concentration of soft acid and formic acid content (BMSS9 and BMSF9 respectively). All diets were added a similar inclusion level of blue mussel products of 9% of the diet, substituting 36% of the FM. The new blue mussel products were dried to 50% (EPCON technology), and the batch of BMS used in experiment 1 was dried at Hordafôr. The proximate and amino acids composition of the control and experimental diets of the second experiment are given in Tables 3 and 4 respectively.

The diets were produced by Cargill (Dirdal, Norway) and stored at 4 °C until the feeding trial started in both experiments. The BMM used in both experiments were provided by Triple nine (Esbjerg, Denmark). Yttrium oxide (0.02% ≈ 200 mg kg^{-1}) was added as an inert marker to all diets to determine apparent digestibility/availability of nutrients in both experiments.

2.4. Fish and rearing condition

2.4.1. Experiment 1

In experiment 1, a total of 975 Atlantic salmon post-smolts originated from Aqua Gen produced at Matre Research Station were randomly distributed among 15 glass fiber square tanks (1.5 m^3). Each tank had 65 post-smolts consisting of 55 fish (mixed population) that were produced from commercially available eggs obtained from Aqua gen in the fall of 2019 and 10 pit-tagged all-male isogenic salmon from a line originally derived from the Aqua Gen strain in 2011 (Fjelldal et al., 2020; Hansen et al., 2020). The mean weight of the mixed population and all-male population was 200 ± 39 g and 203 ± 34 g (Mean ± SD), respectively. The isogenic fish was added as a standard reference to reduce the effect of genetic variation in the growth evaluation. The average biomass per tank at the start of the experiment was 13 ± 0.7 kg (Mean ± SD). The experiment lasted for 10 weeks. The water temperature ranged between 8.8 and 9.2 °C with a mean of 9 ± 0.07 °C (Mean ± SD) and the fish were kept under continuous light (24:0, L:D period). The acclimatization period was three weeks prior to the experimental start. The fish were fed two times per day by automatic feeders (Arvotec TD 2000), between 9:30 to 11:00 and 12:30 to 14:00. The feeding rate was adjusted according to the increase in biomass as the fish grew.

Table 2
Formulation (g $100g^{-1}$) and proximate composition of the experimental diets containing blue mussel meal (BMM) and different levels of blue mussel silage (BMS) in experiment 1.

Experiment 1					
	Control	BMS3	BMS7	BMS11	BMM12
Fish oil	10.2	10.3	10.4	10.4	10.1
Rapeseed oil	13.9	13.3	12.4	11.6	13.2
Fishmeal LT	25.0	20.3	15.4	10.5	13.0
Soy protein concentrate (SPC)	20	21	18.7	17.5	12.3
Raw wheat	11.0	11.0	10.4	10.5	11.0
Other plant proteins[1]	16.8	17.8	21.2	24.9	24.3
Micro-ingredients	3.17	3.30	3.45	3.62	4.11
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
BMM	–	–	–	–	12
BMS High soft acid	–	3	7	11	–
Analyzed proximate composition (g 100 g^{-1} WW)					
Protein	46	45	43	42	44
Lipid	24	24	21	23	23
Ash	7	7	7	6	6
Gross Energy (MJ kg^{-1} WW)	23	23	22	23	23
Digestible energy (MJ kg^{-1} WW)	19	20	19	19	19
Dry matter	95	94	93	94	95
Vit C (mg kg^{-1} WW)	1100	1100	980	990	1000
Vit E (alfa-tocopherol) (mg kg^{-1} WW)	360	360	380	360	350
TBARs (nmol g^{-1} WW)	14	12	15	19	16
Macro-mineral composition (mg kg^{-1} WW)					
Ca	13,300	11,280	11,160	10,340	11,400
Na	3800	4512	5394	5922	4275
K	10,450	10,340	9300	8272	8170
Mg	2185	2068	2139	2068	1900
P	13,300	11,280	11,160	11,280	13,300
Micro-mineral composition (mg kg^{-1} WW)					
Mn	51	54	50	56	56
Cu	10	9	10	10	10
Fe	190	207	244	291	266
Se	0.8	0.8	0.8	0.8	1.0
Zn	162	150	158	158	181

Notes: Ingredients are listed as percentages of whole feed. WW refers to wet weight basis. The sign "-" means no data is available.
[1] Wheat gluten meal. Pea protein concentrate- and guar meal. BMS refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal.

2.4.2. Experiment 2

The second feeding experiment started with randomly distributing 54 Atlantic salmon post-smolts in each of 15 glass fiber square tanks (1 × 1 m) (810 total fish), with an average weight of 119 ± 2 g (Mean ± SD). The fish originated from SalmoBreed and were obtained as parr from Lerøy Sjøtroll Fitjar and smoltified at Matre Research Station prior to the experiment start. The average tank biomass at the start of the experiment was 6 ± 0.12 kg (Mean ± SD). The experiment lasted for 7 weeks. The water temperature ranged between 11.1 and 12.5 °C with a mean of 12 ± 0.2 °C (Mean ± SD) and the fish were kept under continuous light (24:0, L:D period). The acclimatization period was three weeks prior to the experimental start. The fish were fed two times per day by automatic feeders (Arvotec TD 2000), between 09:00 to

Table 3
Formulation (g 100g^{-1}) and proximate composition of the experimental diets containing blue mussel meal (BMM) and different processed blue mussel silage (BMS) in experiment 2.

Experiment 2					
	Control	BMM9	BMS9[2]	BMSS9[3]	BMSF9[4]
Fish oil	10.4	10.7	10.9	10.9	10.9
Rapeseed oil	14.7	14.6	14.1	14.1	14.2
Fishmeal LT	24.9	16	16	16	16
Soy protein concentrate (SPC)	24.6	19.1	23.5	23.5	23.5
Raw wheat	4.4	4.4	6.5	6.5	6.5
Other plant proteins[1]	17	21	21	21	21
Micro-ingredients	3.6	4.9	3.8	3.8	3.8
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
Blue mussel meal	–	9	–	–	–
BMS High soft acid	–	–	9	–	–
BMS Low soft acid	–	–	–	9	–
BMS Low formic acid	–	–	–	–	9
Analyzed proximate composition (g 100 g $^{-1}$ WW)					
Protein	45	45	43	43	41
Lipid	23	23	24	24	24
Ash	7	6	7	8	7
Gross Energy (MJ kg^{-1} WW)	22	22	22	22	21
Digestible energy (MJ kg^{-1} WW)	19	19	20	20	19
Dry matter	93	93	94	93	91
Vit C (mg kg^{-1} WW)	1100	1100	640	670	670
Vit E (alfa-tocopherol) (mg kg^{-1} WW)	210	169	280	330	320
TBARs (nmol g^{-1} WW)	7	14	16	22	16
Macro-mineral composition (mg kg^{-1} WW)					
Ca	13,020	11,160	10,340	10,230	10,010
Na	4185	3813	6486	7626	7280
K	9300	8091	8836	9021	8463
Mg	1953	1860	2162	2352	2184
P	13,020	12,090	11,280	11,160	10,010
Micro-mineral composition (mg kg^{-1} WW)					
Mn	60	66	66	88	100
Cu	12	13	12	12	11
Fe	186	260	282	316	291
Se	0.8	0.8	0.9	0.9	0.8
Zn	158	167	160	158	146

Notes: Ingredients are listed as percentages of whole feed. WW refers to wet weight basis. The sign "-" means no data is available.
[1] Wheat gluten meal. Pea protein concentrate- and guar meal.
[2] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[3] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[4] BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants. BMM refers to blue mussel meal.

10:30 and 12:30 to 14:00. The feeding rate was adjusted according to the increase in biomass as the fish grew.

In both experiments, the environmental conditions of temperature and Oxygen were continuously monitored throughout the experimental period. The tanks had a flow-through system and the flow adjusted to maintain the oxygen saturation as the fish grew. To estimate feed intake according to (Helland et al., 1996), the uneaten feed pellets were collected from the tank outlet 15 min after each meal in both experiments.

2.5. Sampling procedure

All sampled fish were euthanized with an overdose of tricaine methane sulfonate (500 mg L^{-1}, FINQUEL MS-222).

2.5.1. Experiment 1

At the start of experiment 1, the weight and length of all pit-tagged fish from the all-male population (n = 150) were registered to determine the individual specific growth rate (SGR). Also, 30 fish from the mixed population and 15 fish from the all-male population were dissected to determine the organ weight (viscera, liver, and heart) and organ nutrient composition. In addition to that, the same number of fish were pooled (n = 10 fish from mixed population per pool and n = 5 fish from all-male population per pool, n = 3 pools per fish group) to determine the whole-body nutrient composition. At the end of the experiment, the weight and length of all fish in each tank were recorded (n = 65). To determine the whole-body and organ nutrient composition, 10 fish from the mixed population and 10 fish from the all-male population from each tank (In total 20 fish per tank) were sampled for determination of nutrient status. Of these 10 fish, 5 fish whole-body were pooled to determine the whole-body nutrient composition (n = 5 fish per tank, n = 3 per diet), while 5 fish were dissected for individual tissue sampling. Blood samples were collected from the caudal vein by heparinized syringes. The plasma samples were obtained after centrifugation (13,200 RPM, 2 min, 4 °C) of the blood samples and kept on dry ice before transfer to −80 °C. The weight of the viscera, liver and heart was recorded in all sampled fish. The individual liver samples were frozen by liquid nitrogen, transferred on dry ice, and stored in −80 °C for determination of antioxidant responses (GSH-GSSG) (n = 5 fish per tank, n = 3 per diet). The whole fillet and liver samples were pooled per tank, kept on dry ice and stored in −20 °C for determination of mineral composition (n = 5 fish per tank, n = 3 pooled per diet). Feces were collected by gently stripping from 55 fish (45 fish from mixed population and 10 fish from all-male population) per tank and stored at −20 °C for determination of nutrient digestibility.

2.5.2. Experiment 2

At the start of experiment 2, the whole-body of 30 fish were sampled and homogenized to determine the whole-body nutrient composition (n = 10 fish per tank, n = 3 pooled). The organs of 30 fish (viscera, liver, and heart) were individually dissected, and weighed. The tank biomass was recorded at the beginning of the trial and weight and length were measured on all fish at the end. At the conclusion of the experiment, 20 fish were sampled per tank, 10 for collection of blood samples and organs, 10 for whole fish. Of 10 fish for blood and organ samples, blood samples were taken from 5 fish (n = 5 fish per tank, n = 15 per diet) and divided in 2 aliquots, one for plasma samples (as described above) and the other for determining hematocrit (HCT) and blood parameters; muscle samples were obtained from 5 fish (n = 5 fish per tank, n = 3 per diet) and liver samples were collected from 10 fish (n = 10 fish per tank, n = 3 per diet) to determine the nutrient composition. The organ weight of 10 fish per tank was measured to determine the somatic indexes (n = 10 fish per tank, n = 3 per diet). The rest 10 whole-body fish per tank were pooled to determine the whole-body nutrient composition (n = 10 fish per tank, n = 3 pooled per diet). Aliquots of heparinized whole blood were transported on ice and kept in the fridge for 24 h before being analyzed for red blood cell count (RBC) and hemoglobin (Hb) concentration. Feces were collected from all fish and stored at −20 °C before freeze-drying for determination of nutrient and yttrium content.

The HCT measurement was done by filling the capillary tubes with heparinized blood, seal the end of the tubes by wax, centrifuge the tube in a hematocrit centrifuge (12,500 RPM, 3 min, room temp), and read the percentage of packed cells directly by using a HCT ruler.

Individual welfare indicators were evaluated, including visual inspection of the eye, jaw wound and deformity, opercula status, spine deformation, gill condition, skin, and fin damage on the sampled fish from both experiments. According to the standard scoring system (SWIM) (Noble et al., 2018; Stien et al., 2013). A total of 20 fish of each tank (in both experiments) were examined for cataract in darkened conditions using a Heine HSL 150 hand-held slit lamp (HEINE Optotechnik GmbH & Co. KG, Herrsching, Germany), where each lens was

Table 4
Amino acid composition of experimental diets.

(mg g $^{-1}$ WW)	Experiment 1					Experiment 2				
	Control	BMS3	BMS7	BMS11	BMM12	Control	BMM9	BMS9[1]	BMSS9[2]	BMSF9[3]
Hydroxy-Proline	1.9	1.6	1.4	1.2	1.7	2.3	2	1.7	1.6	1.7
Histidine	12.5	11.9	10.9	11.4	11.2	12	11.8	11.6	11.3	11.3
Taurine	1.52	2.0	2.3	2.8	2.1	1.09	1.47	3	2.8	2.7
Serine	19.7	18.9	17.4	18.4	20.1	20.9	20.6	20.5	19.9	19.8
Arginine	28.3	27.5	24.9	25.3	28.0	25.9	24.7	23.9	23.3	23.3
Glycine	21.2	20.2	18.1	18.4	20.8	22.8	21.5	21	20.3	20.2
Aspartic acid	40	40.0	36.0	35.0	39.0	41	39	38	38	38
Glutamic acid	74	76.0	71.0	76.0	81.0	85	89	88	86	87
Threonine	15.8	15.3	13.8	14.1	15.8	16.3	15.9	15.5	15.2	15.2
Alanine	19.7	19.1	16.8	16.3	18.7	20.4	19.2	18.4	18.3	18.3
Proline	21.7	21.6	20.6	23.1	24.0	26.2	28	27.3	26.9	27
Lysine	26.8	26.0	22.0	20.6	25.8	29.1	32	24.3	23.3	24.4
Tyrosine	13.9	13.7	12.8	13.5	14.3	14.4	14.4	14.1	13.3	13.3
Methionine	12	11.2	10.4	10.6	12.0	12.4	12.4	11.5	11	11
Valine	18.8	18.9	17.0	17.4	18.5	19.5	18.8	18.2	18.3	18.4
Isoleucine	17.1	17.4	15.7	16.2	17.0	18.1	17.5	17	16.9	17.1
Leucine	31	29.9	27.1	28.3	30.0	32	32	30	29.8	30
Phenylalanine	19.9	19.3	17.8	19.3	19.7	21	20.5	20.6	19.2	19.4
Tryptophan	4.6	4.5	4.1	4.2	4.6	4.4	4.3	3.8	3.9	4

Notes: WW refers to wet weight basis.
BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.
[1] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[2] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[3] BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants.

given a score between 0 and 4 according to (Wall and Bjerkas, 1999).

2.6. Analytical methods

Nutrient composition of raw materials, diets, whole-body, plasma, organ (liver and muscle) and feces samples were determined as described below: The crude protein was determined based on the nitrogen content of the samples by a nitrogen analyzer (Vario Macro Cube, Elementar Analysensysteme GmbH, Germany) (AOAC, 1995). Two different analytical methods were used for determination of crude fat in feed/tissue/feces samples and raw material samples. Ethyl acetate was used for extracting fat from feed, plasma, organs, and feces samples. The fat residue was weighted after filtering the solvent (Lie et al., 1988). However, gravimetry after acid hydrolysis was used for determination of crude fat in raw material samples (EU directive 84/41983). Dry matter was measured after drying the samples to constant weight at 105 °C for 24 h (Hamre and Mangor-Jensen, 2006) and a combustion in a muffle furnace at 550 °C for 16–18 h determined ash content. An IKA calorimeter C7000 was used for measuring the energy content of samples after drying the homogenized samples 48 h at 60 °C. The fatty acid composition in feed and raw materials was analyzed by gas-chromatography (GC) as previously described by(Jordal et al., 2007), modified after (Lie and Lambertsen, 1991). The amino acid composition (except cysteine and tryptophan) in feed and raw materials were determined using ultra-performance liquid chromatography (UPLC, Waters Acquity UPLC system) coupled with a UV detector (Cohen and De Antonis, 1994; Cohen and Michaud, 1993; Espe et al., 2014). Tryptophan was determined after basic hydrolysis with barium hydroxide ($Ba(OH)_2$) as described by (Liaset et al., 2003). Histamine in raw materials was determined by high- pressure liquid chromatography (HPLC) as previously describe by (Eerola et al., 1993; Liaset and Espe, 2008).

Inductively coupled plasma mass spectrometry (ICP-MS) as described by (Julshamn et al., 2001; Long and Martin, 1990) was used for determination of micro-minerals and yttrium oxide in raw materials, diets, whole-body, muscle, liver, plasma and feces samples. In brief, after digesting the 0.2 g freeze-dried sample material in a microwave oven (Milstone-MLS-1200) and diluting to 25 mL with Milli-Q Water, ICP-MS (Agilent 7500c) is used to determine the micro-minerals.

To determine the RBCs and Hb, CellDyn 400 (Sequoia-Turner, California, USA) instrument was used. Para 12 control blood (Streck) was used for calibration. After preparation of the diluted samples, the samples were read in the instrument for determining RBC and Hb. The RBC values were expressed as the value obtained $\times 10^{12}$ cells L^{-1} and the Hb measured is expressed as g 100 mL^{-1}.

The vitamin C and E analysis in feed was determined by HPLC as described by (Hamre et al., 2010; Mæland and Waagbø, 1998), respectively. The concentration of oxidation products in feed and raw materials was assessed using a spectrophotometric method by measuring Thiobarbituric Acid Reactive Substances (TBARS) (Hamre et al., 2001; Schmedes and Hølmer, 1989). To analyze the levels of total (tGSH) and oxidized (GSSG) glutathione in the liver samples, a method described by (Skjærven et al., 2013) and (Hamre et al., 2022) was used. The samples were treated with a commercial kit (Prod. No. GT40, Oxford Biomedical Research, Oxford, UK) to obtain supernatants, which were then subjected to analysis for absorbance at 405 nm using a microplate reader (iEMS Reader Ms., Labsystems, Finland).

Iron speciation was done on raw materials (BMS products) and the experimental feed samples from both experiments using the thiocyanate colorimetry method. The Fe^{3+} standard solutions (4,6,8, and 10 × 10–5 mol L^{-1}) and sample solutions were prepared as described in the protocol. The ammonium thiocyanate solution was added to each sample and standard solution tubes to make a stable red colour which is readable in a colorimeter measuring the absorbance at a wavelength of 490 nm for each colored solution.

2.7. Calculations and statistical analysis

The following variables were calculated:

$$Digestible\ energy\left(DE, \frac{MJ}{kg}\right) = Energy\ in\ diet - \left(\frac{yttrium\ in\ diet}{yttrium\ in\ faeces} \times energy\ in\ faeces\right)$$

(Anderson et al., 1991)

$$Weight\ gain\ (WG, g) = final\ mean\ weight\ (g) – initial\ mean\ weight\ (g)$$

$$Specific\ growth\ rate\ (SGR, \%per\ day) = (\ Ln\ final\ biomass - Ln\ initial\ biomass) \times \frac{100}{t}$$

(Hopkins, 1992)

$$Feed\ conversion\ ratio\ (FCR) = \frac{Feed\ intake}{weight\ gain}$$

$$Total\ feed\ intake\ (TFI, g) = \frac{\left(\frac{A \times ADW}{100}\right) - \left(\frac{W \times WDW}{R}\right)}{\frac{ADW}{100}}$$

$$Recovery\ (R, \%) = 100 \times \frac{W \times WDW}{A \times ADW}$$

(Helland et al., 1996)

$$Condition\ factor\ \left(K, \frac{g}{cm3}\right) = 100 \times \frac{body\ weight\ (g)}{body\ length\ (cm3)}$$

$$Survival\ (\%) = 100 \times \frac{Final\ number\ of\ fish}{initial\ number\ of\ fish}$$

$$Hepatosomatic\ index\ (HSI, \%) = 100 \times \left(\frac{liver\ weight}{whole\ body\ weight}\right)$$

$$Cardiosomatic\ index\ (CSI, \%) = 100 \times \left(\frac{Heart\ weight}{whole\ body\ weight}\right)$$

$$Viscerosomatic\ index\ (VSI, \%) = 100 \times \left(\frac{viscera\ weight}{whole\ body\ weight}\right)$$

$$ADC\ (\%) = 100 - \left(100 \times \frac{yttrium\ in\ diet}{yttrium\ in\ faeces} \times \frac{nutrient\ in\ faeces}{nutrient\ in\ diet}\right)$$

$$AAC\ (\%) = 100 - \left(100 \times \frac{yttrium\ in\ diet}{yttrium\ in\ faeces} \times \frac{Mineral\ in\ faeces}{Mineral\ in\ diet}\right)$$

$$\text{Retention}\ (\%) = 100 \times \frac{(BM\ f \times nutrient\ or\ mineral\ content\ f) - (BM\ i \times \text{nutrient or mineral content I})}{feed\ intake \times nutrient\ or\ mineral\ in\ feed}$$

Where t is sum of feeding days (70 days in the current study), A is weight of air-dry feed (g), A_{DW} is dry matter content of air-dry feed (%), W is weight of waste feed collected (g), W_{DW} is dry matter content of waste feed (%), and R is recovery of dry matter of waste feed (%), BM f *and* i are standing for final and initial biomass, respectively.

To calculate the daily feed intake per kg biomass (DFI, % biomass), the following equation was used for estimating the daily biomass based on SGR and recorded daily feed intake:

$$lnWdayx = \left(\frac{SGR}{100}\right) \times \left(1 + lnWday(x-1)\right)$$

W_{dayx} is the biomass on a given day (Árnason et al., 2015).

Redox potential (E_h) was calculated by the following equation:

$$Eh = \frac{E0 - RT}{nF\ Ln\ \frac{GSH^2}{GSSG}}$$

Where the GSH and GSSG concentrations are in mol and E_h is in volts. E0 was assumed to be −0.240 V and it is the standard reduction potential at pH 7 and 25 °C. Universal gas constant (R), temperature in Kelvin (T), ion charge (n) (moles of electron), and faraday constant (F) are constant data.

In experiment 1, all data from control and BMS inclusion 3,7 and 11% were analyzed using linear regression (LR) to evaluate dose-dependent responses by determining the best-fit line for each data set. Furthermore, a one-way ANOVA was conducted to determine if there were statistically significant differences among the control group, BMM12 group, and BMS11 group. If a statistically significant difference was found, Tukey's multiple comparisons post-hoc analysis was applied to identify the specific groups with significant differences. In experiment 2, a similar approach was followed. One-way ANOVA was performed to examine the statistical differences between the experimental groups and the control group. Subsequently, Tukey's multiple comparisons post-hoc analysis was utilized to identify any statistically significant differences among the groups.

For all data sets the homogeneity of variance and normality of the data was tested by Bartlett's/ Brown-Forsythe test and Shapiro Wilk's test, respectively. Outliers of the growth dataset were identified with the ROUT test in GraphPad Prism. One of the BMM12 tanks was removed as the outlier in experiment 1. "Tank" was considered as the experimental unit ($n = 3$ for all the experimental diets and $n = 2$ for the BMM12 group) and a significant level of $p < 0.05$ was employed in all cases. The results are expressed as mean ± SEM. All the statistical analysis and the graphs were performed in GraphPad Prism (version 8.4.3 (686) San Diego, California USA).

3. Result

3.1. Fish performance indicators

In experiment 1, the fish given diets containing BMS had a linear decrease in weight gain (WG) and SGR ($p < 0.0001$) (Fig.1a and Table 5). The fish given BMS had a lower growth rate, resulting in up to 46% reduced weight gain (WG), from 275 ± 5 g in the control group to 148 ± 13 g in the fish given the diet containing 11% BMS. Also, the SGR decreased from 1.19 ± 0.01% day^{-1} in the control group to 0.77 ± 0.02% day^{-1} in the fish given the diet containing 11% BMS.

The FCR increased from 0.68 ± 0.01 in the control group to 1.08 ± 0.14 in the BMS11 group ($p < 0.005$) (Fig.1b). Daily feed intake was not influenced by BMS inclusion (Fig.1c). Condition factor decreased with a higher BMS inclusion in the diet ($p < 0.0001$) from 1.25 ± 0.01 to 1.12 ± 0.01 (Fig.1d). No differences were seen in the somatic indices with mean levels of 1.12 ± 0.01 for HSI, 10.05 ± 0.14 for VSI, and 0.13 ± 0.00 for CSI (data not shown). Using BMS in diets did not influence cataract development, combined mean score of all fish of 1.30 ± 0.08, or any of the other welfare assessments (data not shown).

Fish fed BMS11 diet had lower WG ($p = 0.001$), and condition factor ($p = 0.001$) compared to both the BMM12 and control groups (Fig.3a, d). However, Fish given BMM diet performed comparably with the reference group, and no differences were observed in WG (Fig.3a), feed utilization (Fig.3b, c), and condition factor (Fig.3d) between fish fed the BMM12 and the reference group.

The final weight of the all-male population was within the same range as that of the fish from mixed population (Fig. A supplementary). The individual SGR of the all-male population decreased as determined by a segmental linear regression with a broken point in BMS3 (R^2 = 0.69).

In experiment 2, no differences were seen in WG and feed utilization

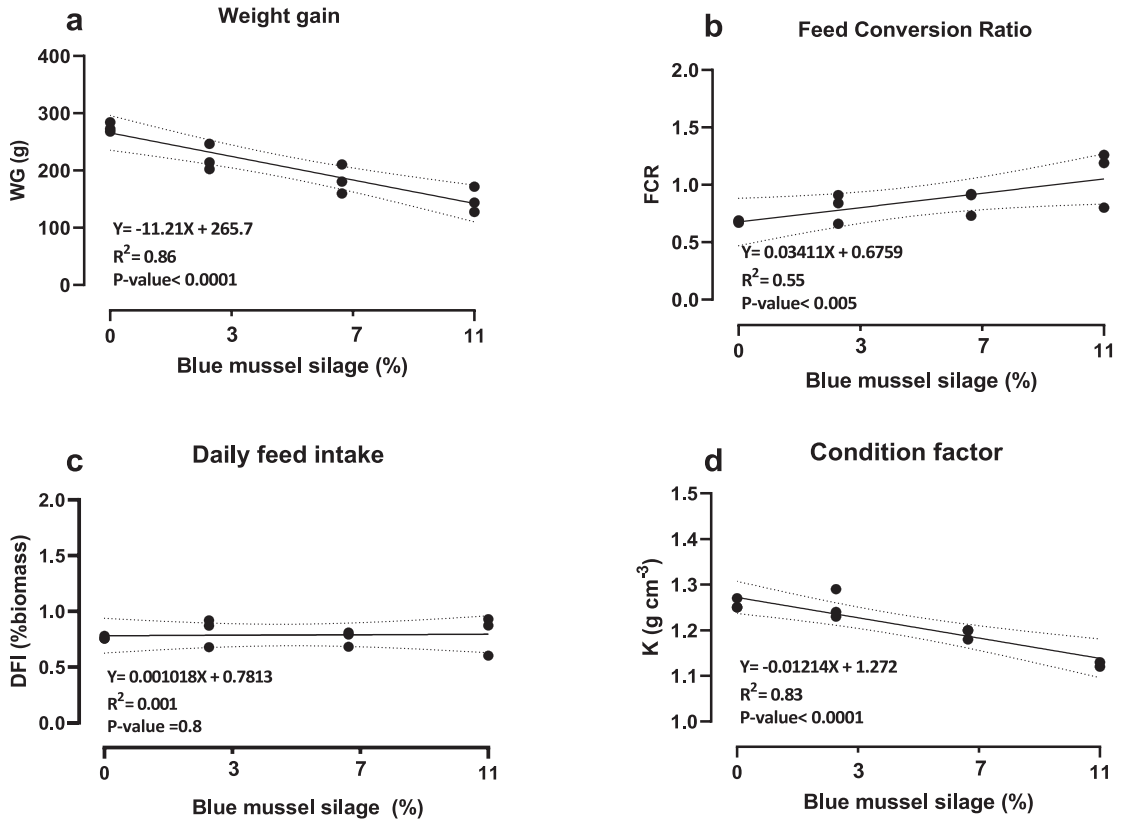

Fig. 1. Growth performance and feed utilization indicators of Atlantic salmon post smolt fed graded inclusion of blue mussel silage (BMS) in experiment 1. The best-fit regression lines for each data set were presented ($n = 15$ fish per diet, each filled circle shows a mean of 5 fish per tank). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig.2a, b, c). The SGR and condition factor was however lower in the fish given the diet containing BMS made with low soft acid (BMSS9) compared to the fish fed the reference feed, but not different from the fish fed the other blue mussel containing feeds (Table 5, Fig.2d).

The HSI was higher in the fish given BMS in the diet compared to fish given the reference feed and BMM9 in the diet, increasing from 1.04 ± 0.03 in the control group and 1.09 ± 0.02 in the BMM9 group to 1.24 ± 0.03 in BMS9, 1.35 ± 0.06 in BMSS9 and 1.25 ± 0.05 in BMSF9 ($p < 0.0001$, data not shown). The CSI and VSI were comparable between experimental groups with a mean of 0.16 ± 0.00 and 8.75 ± 0.11, respectively. The growth performance, feed utilization and somatic indexes were comparable between fish given BMM9 in the diet and fish given the reference feed. No effect was observed on cataract scores with the mean of 2.33 ± 0.07 or other welfare indicators (data not shown).

3.2. Apparent digestibility (ADC), apparent availability (AAC) coefficient

In experiment 1, the inclusion of BMS did not influence the ADC of protein, total fat and energy (Table 6). However, the ADC of dry matter increased from 63.60 ± 1.97% in the control group to 70.13 ± 0.35% in the BMS3 group where the levels appeared to plateau, as determined by a segmental linear regression with a broken point in BMS3 ($R^2 = 0.74$) (Table 6). Additionally, the fish fed BMM12 showed comparable ADC of macro-nutrients with the control and BMS11 groups (Table 6). In experiment 2, the ADC of macro-nutrients was comparable between the fish given control feed, BMM9, and BMS9. Notably, the fish fed BMS with low soft acid (BMSS9) and BMS with only formic acid (BMSF9) had an increase in the ADC of protein, total fat, and energy ($p = 0.006$, $p = 0.01$, $p = 0.005$) compared with the control group. However, ADC of dry matter was not influenced by the experimental diets (Table 6).

In experiment 1, the Fe availability had a linear increase ($p = 0.01$), while the Se availability was expressed by a second-order polynomial equation (quadratic, $R^2 = 0.67$), with increasing from 54.90 ± 1.50% in the control group to 61.93 ± 1.41% in BMS7 and decreased to 60.80 ± 0.66% in the BMS11 group. However, no difference was observed in Fe and Se AAC between the control, BMM12, and BMS11 groups. Moreover, the availability of other micro-minerals was comparable with the control group (Table 6). In experiment 2, The Fe and Se availability were not affected by the experimental diets. However, the Zn availability increased in all experimental groups compared with the control group ($p < 0.0001$). The Zn availability in the fish given the control feed was 19.83 ± 1.08%, while it was higher in the fish fed BMM9 (32.40 ± 1.53%), BMS9 (40.60 ± 1.73%), BMSS9 (46.63 ± 1.78%), and BMSF9 (43.00 ± 0.64%). The availability of Mn and Cu increased in fish fed BMM9, BMSS9 and BMSF9 compared with the fish fed control feed ($p = 0.005$, $p = 0.01$, respectively) (Table 6).

3.3. Whole-body macro-nutrients status

In experiment 1, the fish given diets containing BMS had lower protein ($p = 0.009$), energy ($p = 0.01$), and dry matter ($p = 0.008$) content of whole-body with higher inclusion of BMS in their diets. (Table 7). However, the total lipid level did not change. The whole-body macro-nutrient status of fish fed BMM12 was comparable with fish fed control and BMS11. However, the BMS11 group had a reduction in the levels of energy ($p = 0.01$) and dry matter ($p = 0.03$) compared to the control group (from 9407 ± 104 to 8927 ± 108 j g^{-1} WW in the BMS11 group, and from 33.03 ± 0.40% to 31.35 ± 0.37% in the control group, respectively). In experiment 2, the status of all macronutrients in the whole body was comparable between the experimental groups, and no significant changes were detected (Table 7).

Table 5
Growth performance of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2.

Experiment 1					
	Control	BMS3	BMS7	BMS11	Regression (0, BMS3, 7, 11)
IBW (g)	210 ± 3	211 ± 5	201 ± 7	204 ± 10	n.s.
FBW (g)	485 ± 8	432 ± 14	385 ± 21	351 ± 23	$R^2 = 0.78$, $p = 0.0001$[1]
SGR (% day $^{-1}$)	1.19 ± 0.01	102 ± 0.04	0.92 ± 0.03	0.77 ± 0.02	$R^2 = 0.90$, $p < 0.0001$[2]
TFI (kg)	11.92 ± 0.19	11.91 ± 0.83	10.21 ± 0.23	10.12 ± 0.82	$R^2 = 0.43$, $p < 0.01$[3]

Experiment 2						
	Control	BMM9	BMS9[4]	BMSS9[5]	BMSF9[6]	ANOVA
IBW (g)	119 ± 2	121 ± 1	120 ± 2	119 ± 1	118 ± 1	n.s.
FBW (g)	232 ± 8^{ab}	254 ± 10^{a}	222 ± 4^{ab}	197 ± 8^{b}	211 ± 10^{b}	$p = 0.007$
SGR (% day $^{-1}$)	1.36 ± 0.08^{a}	1.50 ± 0.07^{a}	1.25 ± 0.01^{ab}	1.03 ± 0.07^{b}	1.28 ± 0.01^{ab}	$p = 0.004$
TFI (kg)	5.68 ± 0.37	5.50 ± 0.55	4.93 ± 0.55	4.63 ± 0.48	5.02 ± 0.35	n.s.

Notes: IBW = initial body weight (g). FBW = final body weight (g). SGR = specific growth rate (% day $^{-1}$). TFI = total feed intake (g).
BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.
Data is listed as mean ± SEM. In experiment 1, all diets are triplicate except BMM12%, that is in duplicate. In experiment 2, $n = 3$ tank per diet.
The column labeled "Regression" gives R^2 and p-value for linear regression performed for the control and silage groups with silage inclusion percentage as x-variable (0, 3, 7, and 11). The column labeled "ANOVA" gives a p-value for ANOVA in case of a significant difference between the groups. Means with different superscripts are significantly different ($p < 0.05$) under the Tukey HSD test. n.s stands for not significant.
[1] Simple linear regression: $Y = -11.99\times + 476.2$.
[2] Simple linear regression: $Y = -0.03624\times + 1.169$.
[3] Simple linear regression: $Y = -193.8\times + 12{,}061$.
[4] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[5] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[6] BMSF9 refers to diets containing blue mussel silage with only formic acid and (pH 3.5) without antioxidants.

3.4. Body micro-mineral composition and blood parameters

The mineral compositions of whole-body, liver, plasma, and muscle of both experiments are provided in Table 7 and supplementary tables D and E. In experiment 1, the fish given diets containing BMS has low Fe level in whole body expressed by a second-order polynomial equation ($R^2 = 0.89$) compared to the control group (Fig. 4). The whole-body Fe level decreased from 8.36 ± 0.60 mg kg^{-1} WW in the control group to 4.96 ± 0.03 mg kg^{-1} WW in BMS11 (Fig. 4a). Liver and plasma Fe concentrations also decreased and could be expressed by a segmental linear regression with a broken point in BMS3 ($R^2 = 0.98$ and $R^2 = 0.26$, respectively). Fish fed the diet containing BMS11 had a lower concentration of Fe in the liver (18.01 ± 0.54 mg kg^{-1} WW) and in plasma (6.88 ± 1.26 μmol L^{-1} WW) compared to the control group (liver 63.77 ± 2.43 mg kg^{-1} WW and plasma 15.16 ± 2.82 μmol L^{-1} WW) (Fig.4b, c). Similarly, muscle Fe concentration decreased under a second-order polynomial model ($R^2 = 0.62$), from 2.10 ± 0.05 mg kg^{-1} WW in the control group to 1.30 ± 0.17 mg kg^{-1} WW in the fish given BMS7, and then increased to 1.50 ± 0.20 mg kg^{-1} WW in the fish given BMS11 (Fig.4d). The highest level of Fe in the liver and plasma was observed in the fish given BMM12 (82.00 ± 1.00 mg kg^{-1} WW and 27.11 ± 4.89 μmol L^{-1}, respectively) (supplementary table D and E, ANOVA test). Along with Fe, the whole-body Mn and Se concentration had a decreased dose-response which similarly was observed in plasma as well (Table 7 and supplementary table E). In contrast with that, the Cu status in whole-body and liver increased linearly ($R^2 = 0.73$ – $p = 0.0004$, and $R^2 = 0.57$ - $p = 0.004$, respectively), whereas it decreased in muscle ($R^2 = 0.48$, $p = 0.01$). In the control group, whole-body Cu levels were 1.63 ± 0.03 mg kg^{-1} WW, which increased to 2.13 ± 0.08 mg kg^{-1} WW in the BMS11 group (Table 7). The micro-mineral composition in fish fed BMM12 was comparable with the control group, whereas the whole-body Cu and Se was lower ($p = 0.003$) and plasma Mn concentration was higher ($p < 0.0001$) than BMS11.

In experiment 2, the whole-body Fe level increased ($p < 0.0001$) in fish fed diets containing BMS (13.33 ± 0.16 mg kg^{-1} WW) compared to the control group (11.00 ± − mg kg^{-1} WW) (Fig.4e). The liver Fe level in fish fed diets containing BMS also increased ($p = 0.0002$) to 125.60 ± 5.30 mg kg^{-1} WW, while the control group showed lower levels of 71.00 ± 3.21 mg kg^{-1} WW (Fig.4f). Similarly, the muscle Fe status increased ($p = 0.003$) in fish fed with BMS groups (2.85 ± 0.05 mg kg^{-1} WW) compared to the control group (2.36 ± 0.06 mg kg^{-1} WW) (Fig.4f). Along with that, the whole-body Zn level increased in both BMSS9 (39.33 ± 0.88 mg kg^{-1} WW) and BMSF9 (38.00 ± − mg kg^{-1} WW) groups compared with the control group (31.33 ± 0.33 mg kg^{-1} WW) (Table 7). The plasma Zn concentration also increased in fish fed BMS with high soft acid (BMS9) and BMS with only formic acid (BMSF9) compared with the control group (supplementary table E). No changes were seen in the concentration of other micro minerals experiment 2.

No differences were observed in the mean of RBC count 1.32 ± 0.03 × 1012 cells L^{-1}, Hb 9.73 ± 0.07 g 100 mL^{-1}, and HCT 43.20 ± 0.52% in experiment 2 (supplementary table E).

3.5. Nutrient retention

In experiment 1, the retention of all macronutrients decreased linearly in fish fed with a higher inclusion of BMS ($p_{protein} = 0.003$, $p_{total\ fat} = 0.01$, $p_{energy} = 0.004$, $p_{dry\ matter} = 0.004$, and $p_{ash} = 0.04$) (Table 8). The fish fed BMM12 had a comparable retention of macronutrients with control group in their body, while it was lower in the BMS11 ($p_{protein} = 0.04$, $p_{energy} = 0.04$, $p_{dry\ matter} = 0.03$, respectively) (Table 8). In experiment 2, the retention of macronutrients was not affected by the experimental diets, and all were comparable to the control group (Table 8).

The retention of Fe decreased in fish fed with a higher inclusion of BMS, as determined by a segmental linear regression with a broken point in BMS3 ($R^2 = 0.92$) (Table 8). The fish fed BMM12 had a comparable Fe retention with the control group (6.15 ± 0.69% and 4.52 ± 0.40%, respectively), whereas it was found lower ($p < 0.0001$) in the BMS11 group (0.31 ± 0.09%). Moreover, the retention of Zn ($p = 0.003$), Mn ($p = 0.01$), and Se ($p < 0.0001$) decreased linearly. Fish fed with BMS11 had lower levels of Mn ($p = 0.05$), Se ($p = 0.001$) and Zn ($p = 0.02$) compared with the control and BMM12 groups (Table 8). In experiment 2, the retention of microminerals was not influenced by the experimental diets (Table 8).

3.6. Liver antioxidant status

In experiment 1, the fish fed BMS did not have any dose-dependent responses in the levels of GSH and GSSG, or in the ratio of GSH/GSSG in the liver, as well as in the redox potential. The GSH level in liver of the control, BMM12, and BMS groups were 878 ± 138, 1037 ± 89 μmol kg^{-1}, and 943 ± 58 μmol kg^{-1}, respectively. Similarly, the GSSG level in liver of control, BMM12, and BMS groups were 2.52 ± 0.13 μmol kg^{-1}, 2.78 ± 0.21 and 3.1 ± 0.31, respectively. The GSH/GSSG ratio was 304 ± 47 and 346 ± 41 μmol kg^{-1} in the control and BMM12 groups,

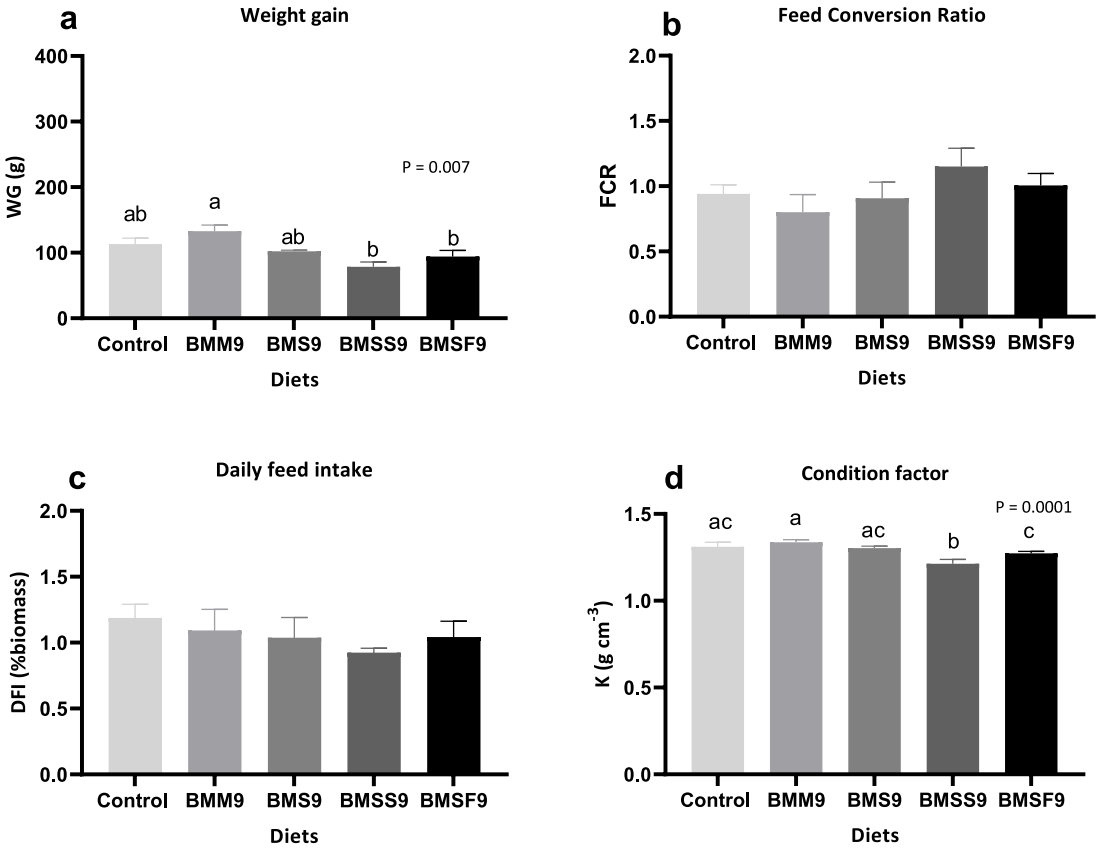

Fig. 2. Growth performance and feed utilization indicators of Atlantic salmon post smolt fed blue mussel silage (BMS) and blue mussel meal (BMM) in experiment 2. Statistically significant differences between the experimental groups were represented with different letters above the bars ($p < 0.05$) under the Tukey HSD test (mean ± SEM, n = 15 fish per diet). BMS9 refers to diets containing blue mussel silage with a high amount of soft acid (pH 2.5) and antioxidants, BMSS9 refers to diets containing blue mussel silage with a lower amount of soft acid (pH 3.7) and antioxidants, and BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

respectively, while it was 380 ± 24 μmol kg^{-1} in BMS groups. The redox potential in all experimental groups was in a similar range with an average of −0.22 ± 0.002 V (data not shown).

3.7. Iron speciation

The BMS product before drying had the lowest Fe^{3+} to total Fe ratio (18% of total Fe), which increased to 39% of total Fe after drying by SPC. However, heat drying increased Fe^{3+} to total Fe ratio almost 2-folds in BMS High soft acid, BMS Low soft acid (35 and 37% of total Fe, respectively) and BMS only formic acid group (43% of total Fe) (Fig.5a).

Furthermore, the inclusion of BMS in the diet resulted in an overall increase in Fe^{3+} to total Fe ratio in both experiments. In experiment 1, the ratio in the BMS diets (3, 7, and 11) was 11, 16, and 24% of total Fe, respectively (Fig.5b). Similarly, the ratio in BMS9, BMSS9, and BMSF9 diets in experiment 2 was 15, 49, and 53% of total Fe), respectively (Fig.5c). The diets contain BMS with low levels of acid (BMSS9 and BMSF9) had the highest ratio (49 and 53% of total Fe, respectively). The BMM group had the lowest ratio in both experiments (6% of total Fe).

4. Discussion

One of the key challenges in salmon production is identifying appropriate alternative feed ingredients for sustainable future salmon production (Albrektsen et al., 2022). Future feed resources are expected to include low-trophic species produced or cultivated in the ocean (Albrektsen et al., 2022). However, the utilization of marine-based organisms as feed materials comes with certain challenges, such as seasonal availability, variation in the nutritional composition and preservation and processing methods, which can limit their use in aquafeed. To address these challenges, two studies were done to investigate the potential use of blue mussel silage (BMS) and blue mussel meal (BMM) as a marine protein ingredient in the Atlantic salmon diet.

In both experiments, no differences were seen in growth or feed conversion ratio between fish given reference feed and BMM. The findings are in line with previous studies that showed Juvenile Ussuri catfish (*Pseudobagrus ussuriensis*) had no negative effects on growth and nutrient utilization when 50% of the FM (28% of control diet – 48% crude protein) was replaced by BMM (Luo et al., 2019). The reference diets used in the present studies were based on a commercially relevant post-smolt diet regarding the protein: lipid ratio, as well as FM inclusion of 25%, giving a mix of ~59–63% plant-based ingredients vs ~34–36% marine ingredients in all the diets. In our study, the experimental feeds contained 9 and 12% BMM, replacing 36 and 48% of the FM, respectively. Therefore, findings from both studies are comparable based on the dietary FM content (25–28% in diet), dietary protein level (45–46%) and the inclusion level of BMM in diet (around 50% of FM). However, it has been shown that replacing 50 or 100% of FM (30% of control diet) led to reduced growth in turbot (Weiss and Buck, 2017).

Contrary to the results shown in the fish given BMM, the fish given BMS in experiment 1 had a dose-dependent reduction in weight gain, SGR and condition factor and an increased FCR. The highest level of BMS was close to the level of blue mussel meal used, however it resulted in a 46% reduction in weight gain, 35% reduction in SGR, 10% reduction in condition factor and 37% increase in FCR. While one study is available on the use of blue mussel silage in animal nutrition (Nørgaard

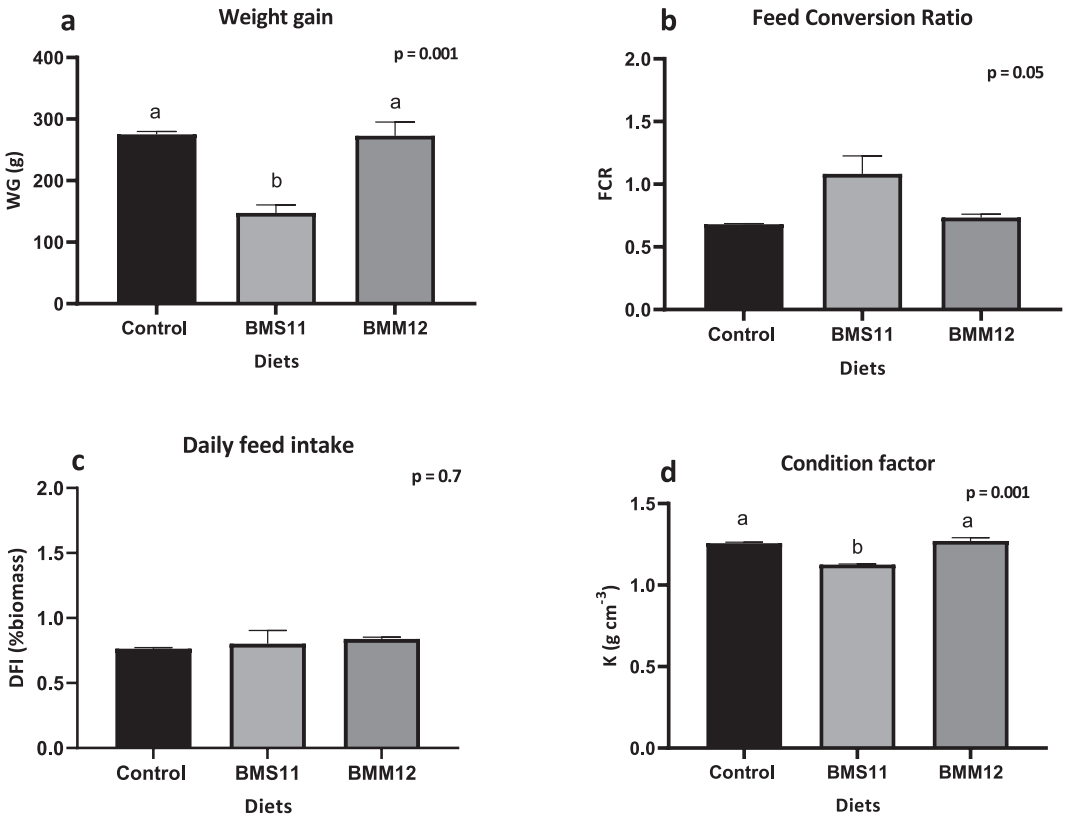

Fig. 3. Comparing growth and feed utilization of Atlantic salmon post-smolt fed control and blue mussel silage 11 (BMS11) versus fed blue mussel meal (BMM12) in experiment 1. Statistically significant differences between the experimental groups were represented with different letters above the bars ($p < 0.05$) under the Tukey HSD test (mean ± SEM, n = 15 fish for control and BMS11, $n = 10$ fish for BMM12). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2015), several studies have shown that fish silage or fish protein concentrates in similar inclusion levels (5–15% of FM) can be used in fish feed such as Atlantic salmon feed (Berge and Storebakken, 1996; Espe et al., 1999; Liang et al., 2006; Olsen and Toppe, 2017; Refstie et al., 2004; Ridwanudin and Sheen, 2014). Early studies on the use of silage-based diets has resulted in either no effect on growth or even marginal enhancements (Heras et al., 1994; Lall, 1991; Parrish et al., 1991), while some have also indicated a significant reduction in growth (Hardy et al., 1984; Stone et al., 1989). For example; replacing whole FM (50% of diet) with 12.5 and 25% fish silage co-dried with soybean meal and feather meal and 50% fish silage dried by vacuum dryer reduced the average weight of rainbow trout, while FCR was comparable between the experimental diets (Hardy et al., 1984). However, similar to the results in experiment 1, it was shown that replacing whole FM (50% of diet) with fish silage made from fresh or frozen ingredients decreased the final mean weight and increased the feed conversion ratio of rainbow trout (Stone et al., 1989).

One reason for the reduced growth was suggested to be a lower availability of lysine and other essential amino acids (Hardy et al., 1984). It has also been shown that the level of acid-sensitive amino acids, especially tryptophan, in fish silage may decrease due to the processing methods (Arason, 1994). The level of acid-sensitive amino acids is influenced more by the acid amount and pH level than the duration of storage (Espe et al., 1999; Gildberg and Raa, 1977; Haaland and Njaa, 1989; Jackson et al., 1984; Mach and Nortvedt, 2009; Nørgaard et al., 2015). Previous studies have also reported reductions in amino acids such as arginine (Haaland and Njaa, 1989; Stone and Hardy, 1986), phenylalanine, glutamic acid (Stone and Hardy, 1986), lysine (Vidotti et al., 2003), tyrosine (Haaland and Njaa, 1989), methionine (Shahidi et al., 1995), leucine, and isoleucine (Vidotti et al., 2003) in fish silage. In the present studies, no effect was seen on tryptophan level in reference vs silage products, while variations were seen in several amino acid levels in BMS products that could also be explained by different blue mussel productions (Table A of the supplementary material), however, the dietary levels were above the amino acid requirements for Atlantic salmon (NRC, 2011). Although no differences were seen in protein digestibility, the fish fed higher BMS had lower protein retention and whole-body protein composition. This may mean that nutrients from diets containing BMS were not efficiently used for growth, despite being easily digested.

Reduced growth might also be attributed to the bitter taste of the feed or the presence of bitter-tasting peptides (Adler-Nissen, 1984; Hevrøy et al., 2005). This bitterness can occur when formic acid, sulfuric acid, or propionic acid is used during the fish silage process, leading to decreased feed intake and growth in fish (Adler-Nissen, 1984; Hevrøy et al., 2005). The presence of rancid lipid compounds in feed can be another factor in the reduced growth and feed utilization (Hevrøy et al., 2005). Lipid rancidity can be a major concern to determine the feed stability and cellular antioxidant homeostasis (Aklakur, 2018). In larger-scale operations, it has been suggested to remove oil from fish silage if it exceeds 4% (Tatterson and Windsor, 1974). In our current study however, the TBARS levels in the diets showed variations, while feed intake remained constant and the redox potential and GSH/GSSG ratio in the liver of fish fed BMS diets remained stable.

The status of almost all the essential micro-minerals in whole-body, liver, and plasma were affected by the lower growth and higher FCR in BMS groups in experiment 1. Notably, lower levels of Fe were observed in the BMS groups which were not dose dependent. Dietary Fe is the primary source of Fe for fish (Bury and Grosell, 2003). It has been reported that dietary Fe deficiency impaired the growth performance of stinging catfish (*Heteropneustes fossilis*) (Zafar and Khan, 2020), bighead carp (*Aristichthys nobilis*) (Feng et al., 2020), and yellow catfish

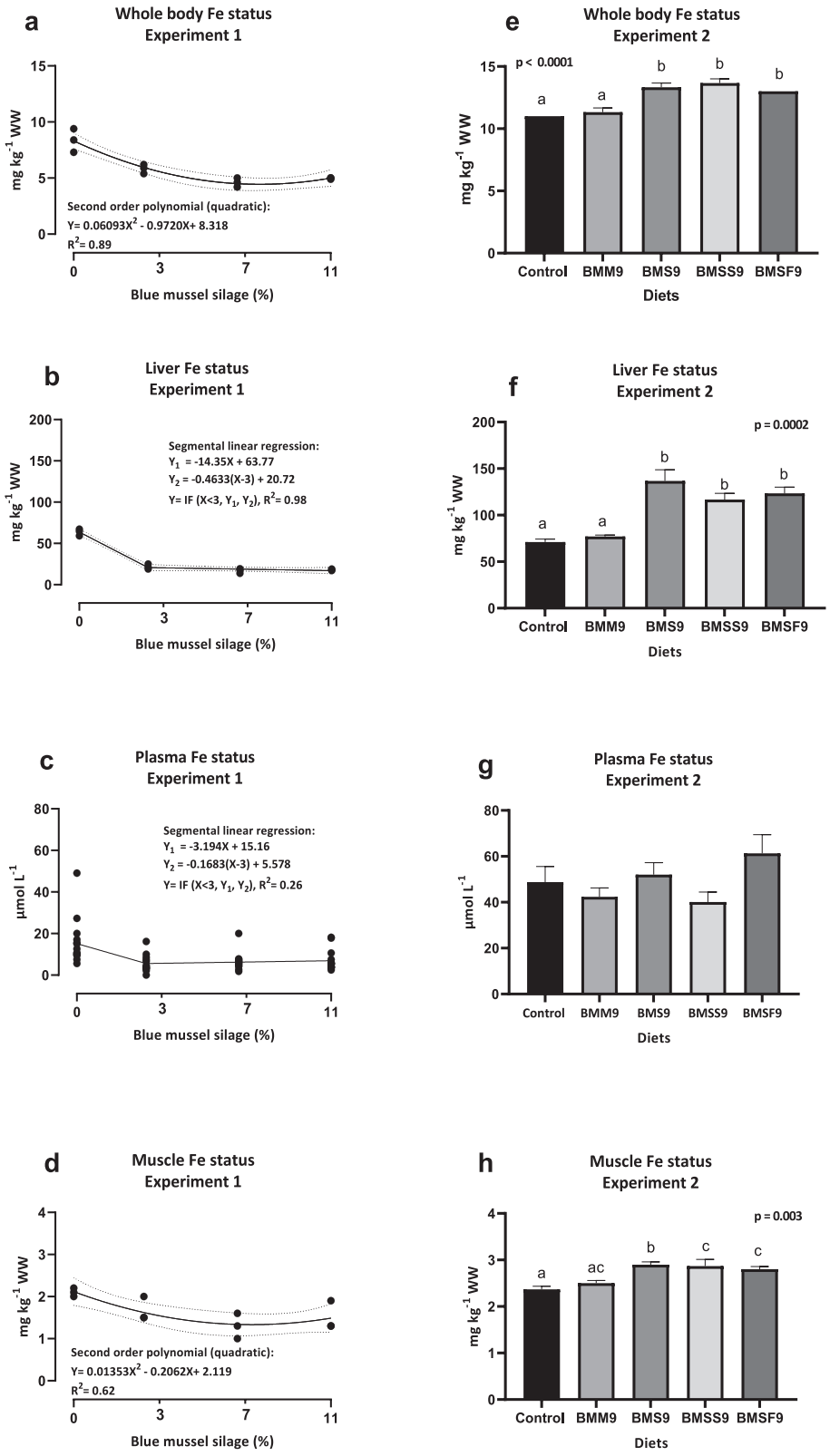

Fig. 4. Whole body, liver, and muscle Fe status of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2. In experiment 1 (a, b, c, and d), the best-fit regression lines for each data set were presented (n = 15 fish per diet, in a, b, and d. Each filled circle shows a mean of 5 fish per tank, while each filled circle is an individual fish in graph c (n = 15 per diet). In experiment 2 (e, f, g and h), statistically significant differences between the experimental groups were represented with different letters above the bars ($p < 0.05$) under the Tukey HSD test (mean ± SEM, n = 15 per diet). BMS9 refers to diets containing blue mussel silage with a high amount of soft acid (pH 2.5) and antioxidants, BMSS9 refers to diets containing blue mussel silage with a lower amount of soft acid (pH 3.7) and antioxidants, and BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

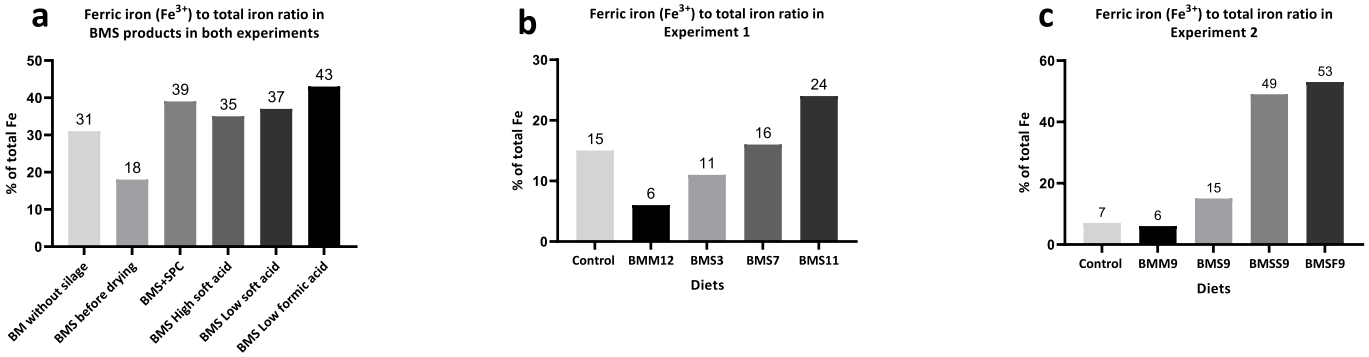

Fig. 5. Ferric iron (Fe^{3+}) to total iron ratio in blue mussel silage (BMS) products and the experimental diets of experiments 1 and 2. In experiment 1, the BMS was mixed with SPC before feed production, and this sample is indicated by the label BMS + SPC in graph (a). In graph (b), BMS 3, 7 and 11 refer to diets containing 3, 7, and 11% blue mussel silage. In graph (c), BMS9 refers to diets containing blue mussel silage with a high amount of soft acid (pH 2.5) and antioxidants, BMSS9 refers to diets containing blue mussel silage with a lower amount of soft acid (pH 3.7) and antioxidants, and BMSF9 refers to diets containing blue mussel silage with only formic acid (pH 3.5) and without antioxidants. BMM refers to blue mussel meal in both experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Apparent digestibility/availability coefficients (ADC/AAC) of nutrients of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2.

Experiment 1							
	Control	BMS3	BMS7	BMS11	BMM12	Regression (0, BMS3, 7, 11)	Comparison (0, BMS11, BMM12)
Macro-nutrients (%)							
Protein	86.93 ± 0.63	88.93 ± 0.27	88.07 ± 0.53	87.53 ± 0.38	87.25 ± 1.05	n.s.	n.s.
Total fat	94.40 ± 0.35	96.43 ± 0.38	95.93 ± 0.95	95.77 ± 0.49	93.85 ± 1.25	n.s.	n.s.
Energy	77.93 ± 1.15	82.07 ± 0.42	81.50 ± 0.46	80.97 ± 0.43	79.40 ± 1.60	n.s.	n.s.
Dry matter	63.60 ± 1.97	70.13 ± 0.35	70.43 ± 1.10	70.47 ± 0.78	66.20 ± 2.00	$R^2 = 0.74$[1]	n.s
Micro-minerals (%)							
Mn	−29.13 ± 18.52	−9.20 ± 16.83	−19.47 ± 14.68	−25.67 ± 9.73	13.75 ± 11.15	n.s.	n.s.
Cu	42.20 ± 0.96	32.80 ± 2.27	40.07 ± 2.61	40.00 ± 0.90	35.95 ± 5.55	n.s.	n.s.
Fe	−3.66 ± 5.91	3.06 ± 7.37	13.30 ± 2.47	14.00 ± 3.26	−17.15 ± 12.05	$R^2 = 0.45$, $p = 0.01$[2]	n.s.
Se	54.90 ± 1.50	60.17 ± 1.48	61.93 ± 1.41	60.80 ± 0.66	54.30 ± 1.20	$R^2 = 0.67$[3]	n.s
Zn	23.93 ± 3.46	25.47 ± 6.12	28.83 ± 6.15	24.70 ± 3.89	16.50 ± 8.60	n.s.	n.s.

Experiment 2						
	Control	BMM9	BMS9[4]	BMSS9[5]	BMSF9[6]	ANOVA
Macro-nutrients (%)						
Protein	87.77 ± 0.64^{a}	89.90 ± 0.15^{b}	88.97 ± 0.08^{ab}	89.77 ± 0.32^{b}	89.60 ± 0.11^{b}	$p = 0.006$
Total fat	93.07 ± 0.03^{a}	93.87 ± 0.54^{ab}	93.73 ± 0.17^{ab}	94.83 ± 0.18^{b}	94.70 ± 0.37^{b}	$p = 0.01$
Energy	81.30 ± 0.60^{a}	83.50 ± 0.50^{ab}	82.77 ± 0.12^{ab}	84.57 ± 0.68^{b}	84.20 ± 0.20^{b}	$p = 0.005$
Dry matter	67.43 ± 1.88	70.57 ± 0.58	70.50 ± 0.37	71.10 ± 2.04	71.07 ± 0.43	n.s.
Micro-minerals (%)						
Mn	−18.47 ± 4.61^{a}	2.86 ± 0.48^{b}	−1.43 ± 5.99^{ab}	11.33 ± 0.98^{b}	9.70 ± 6.2^{b}	$p = 0.005$
Cu	15.47 ± 2.06^{a}	31.33 ± 1.19^{b}	26.00 ± 2.78^{ab}	27.70 ± 3.95^{b}	25.93 ± 1.80^{ab}	$p = 0.01$
Fe	−16.50 ± 2.89	6.93 ± 12.18	−8.73 ± 3.57	−0.53 ± 2.38	−11.53 ± 1.84	n.s.
Se	52.67 ± 1.44	54.33 ± 1.12	54.60 ± 2.26	57.97 ± 2.55	51.77 ± 1.38	n.s.
Zn	19.83 ± 1.08^{a}	32.40 ± 1.53^{b}	40.60 ± 1.73^{c}	46.63 ± 1.78^{c}	43.00 ± 0.64^{c}	$p < 0.0001$

Notes: Data is listed as mean ± SEM. The mean is from $n = 3$ pooled feces sample per diet. In experiment 1, all diets are triplicate except BMM12, that is in duplicate. In experiment 2, n = 3 tank per diet.
The column labeled "Regression" gives R^2 and p-value for linear regression performed for the control and silage groups with silage inclusion percentage as x-variable (0, 3, 7, and 11). The column labeled "comparison" under experiment 1 gives a p-value for ANOVA in case of a significant difference between control, BMS11 and BMM12. The column labeled "ANOVA" under experiment 2 gives a p-value for ANOVA in case of a significant difference between all experimental groups. Means with different superscripts are significantly different ($p < 0.05$) under the Tukey HSD test. n.s stands for not significant.

[1] Segmental linear regression: $Y_1 = 2.193\times + 63.60$. $Y_2 = 0.04167$ (X-3) + 70.179. Y=IF (X < 3. Y_1. Y_2). $X_0 = 3$.

[2] Simple linear regression: $Y = 1.690\times - 2.205$.

[3] Second order polynomial (quadratic): $Y = -0.1317X^2 + 1.960\times + 55.04$. BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.

[4] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.

[5] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.

[6] BMSF9 refers to diets containing blue mussel silage with only formic acid and (pH 3.5) without antioxidants.

Table 7
Whole body nutrient composition of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2.

Experiment 1

	Control	BMS3	BMS7	BMS11	BMM12	Regression (0, BMS3, 7, 11)	Comparison (0, BMS11, BMM12)
Whole- body macro-nutrients (g 100 g $^{-1}$ WW)							
Protein	18.33 ± 0.33	17.67 ± 0.33	17.67 ± 0.33	17.00 ± –	17.50 ± 0.50	$R^2 = 0.50$, $p = 0.009$[1]	n.s.
Total fat	13.47 ± 0.42	12.73 ± 0.23	13.13 ± 0.20	12.53 ± 0.33	13.20 ± 0.17	n.s.	n.s.
Energy (J g^{-1} WW)	9407 ± 104^{a}	9190 ± 67	9297 ± 61	8927 ± 108^{b}	9330 ± 35^{ab}	$R^2 = 0.47$, $p = 0.01$[2]	$p = 0.9$
Dry matter	33.03 ± 0.40^{a}	32.29 ± 0.20	32.48 ± 0.20	31.35 ± 0.37^{b}	32.89 ± 0.35^{ab}	$R^2 = 0.52$, $p = 0.008$[3]	$p = 0.03$
Whole-body micro-minerals (mg kg^{-1} WW)							
Mn	0.99 ± 0.15	0.75 ± 0.07	0.82 ± 0.09	0.61 ± 0.03	1.15 ± 0.15	R^2= 0.36, $p = 0.03$[4]	n.s.
Cu	1.63 ± 0.03^{a}	1.76 ± 0.06	1.90 ± 0.10	2.13 ± 0.08^{b}	1.50 ± 0.10^{a}	R^2= 0.73, $p = 0.0004$[5]	$p = 0.003$
Fe	8.36 ± 0.60^{a}	5.83 ± 0.23	4.60 ± 0.23	4.96 ± 0.03^{b}	9.30 ± 0.30^{a}	R^2= 0.89	$P < 0.0001$
Se	0.21 ± 0.01^{a}	0.21 ± –	0.20 ± 0.00	0.18 ± 0.00^{b}	0.23 ± –^{a}	R^2= 0.64, $p = 0.002$[6]	$p = 0.003$
Zn	26.00 ± 0.57	27.67 ± 1.20	27.67 ± 0.66	28.00 ± 1.52	27.50 ± 0.50	n.s.	n.s.

Experiment 2

	Control	BMM9	BMS9[7]	BMSS9[8]	BMSF9[9]	ANOVA
Whole-body macro-nutrients (g 100 g $^{-1}$ WW)						
Protein	18 ± –	18 ± –	17 ± –	17 ± –	17 ± –	n.s.
Total fat	11.13 ± 0.22	11.23 ± 0.28	11.63 ± 0.43	11.67 ± 0.08	11.70 ± 0.23	n.s.
Energy (J g^{-1} WW)	8163 ± 67	8280 ± 156	8260 ± 196	8230 ± 112	8250 ± 155	n.s.
Dry matter	29.99 ± 0.30	30.30 ± 0.40	30.23 ± 0.40	30.13 ± 0.29	29.95 ± 0.46	n.s.
Whole-body micro-mineral (mg kg^{-1} WW)						
Mn	1.80 ± 0.05	1.53 ± 0.17	1.50 ± 0.10	1.30 ± 0.11	1.46 ± 0.16	n.s.
Cu	1.23 ± 0.08	1.26 ± 0.03	1.40 ± 0.05	1.26 ± 0.03	1.26 ± 0.03	n.s.
Fe	11.00 ± –^{a}	11.33 ± 0.33^{a}	13.33 ± 0.33^{b}	13.67 ± 0.33^{b}	13.00 ± –^{b}	$P < 0.0001$
Se	0.19 ± 0.01	0.19 ± 0.00	0.20 ± 0.00	0.18 ± 0.00	0.18 ± 0.00	n.s.
Zn	31.33 ± 0.33^{a}	33.67 ± 0.66^{a}	35.00 ± 1.52^{ab}	39.33 ± 0.88^{c}	38.00 ± –^{b}	$p = 0.0004$

Notes: Data is listed as mean ± SEM. The mean is from n = 3 pooled whole- body sample per diet (n = 5 fish per tank). In experiment 1, all diets are triplicate except BMM12, that is in duplicate. In experiment 2, n = 3 tank per diet.
The column labeled "Regression" gives R^2 and p-value for linear regression performed for the control and silage groups with silage inclusion percentage as x-variable (0, 3, 7, and 11). The column labeled "comparison" under experiment 1 gives a p-value for ANOVA in case of a significant difference between control, BMS11 and BMM12. The column labeled "ANOVA" under experiment 2 gives a p-value for ANOVA in case of a significant difference between all experimental groups. Means with different superscripts are significantly different ($p < 0.05$) under the Tukey HSD test. n.s stands for not significant.

[1] Simple linear regression: Y = −0.1067× + 18.23.
[2] Simple linear regression: Y = −35.85× + 9393.
[3] Simple linear regression: Y = −0.1303× + 32.97.
[4] Simple linear regression: Y = −0.02823× + 0.9424.
[5] Simple linear regression: Y = 0.04424× + 1.626.
[6] Simple linear regression: Y = −0.002545× + 0.2184. BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.
[7] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[8] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[9] BMSF9 refers to diets containing blue mussel silage with only formic acid and (pH 3.5) without antioxidants.

(*Pelteobagrus fulvidraco*) (Luo et al., 2017). Iron concentration in whole-body, head kidney, and liver in addition to hemoglobin and hematocrit levels are commonly used as indicators of the Fe status (Andersen et al., 1996; Bjørnevik and Maage, 1993; Naser, 2000). In experiment 1, the diets containing BMS had increasing dietary Fe levels, and all diets were above the minimum requirement (60–100 mg Fe kg^{-1}) for Atlantic salmon (Andersen et al., 1996). Furthermore, the same mineral premix containing $FeSO_4$ was added to all experimental diets. Despite this, Fe homeostasis was disrupted in BMS groups, resulting in significantly lower Fe levels in targeted tissues, particularly the liver, which is the main storage site for Fe (Walker and Fromm, 1976), compared to both control and BMM groups. The post-smolt Atlantic salmon normal range of Fe is considered between 10 and 20 mg kg^{-1} WW in whole-body, 96 ± 45 (56–102) mg kg^{-1} WW in liver (Andersen et al., 1996), and 11 ± 0.5 μmol L^{-1} in plasma (Antony Jesu Prabhu et al., 2016). The mean Fe concentrations whole-body, liver and plasma of BMS groups was lower than the mentioned range (Andersen et al., 1996; Antony Jesu Prabhu et al., 2016). It has been shown that it takes at least 22 weeks for fish to develop Fe deficiency when fed a low-Fe diet and uptake Fe from water through the gills (Naser, 2000). Since the gastrointestinal tract is the main site of Fe absorption in fish (Whitehead et al., 1996), the severe reduction of Fe stores after only 10 weeks in the current study may be caused by both weakness in dietary availability and the utilization of Fe. However, the findings from the present study showed the availability of Fe increased with a higher inclusion level of BMS, which can be explained by a relatively increased uptake when the iron status is low (Standal, 1999). It should be mentioned that fecal samples were collected at the end of the experiment when the fish already had low Fe level in the body. Consequently, the enhanced availability and absorption of Fe towards the end of the experiment appear plausible, as the fish's bodily iron status governs the intestinal uptake of iron.

Mineral availability can be affected by various factors, such as the antagonistic interactions between divalent ions like Fe, Mn, and Cu that compete for the same uptake route (Bury and Grosell, 2003; Lorentzen and Maage, 1999; Ogino and Yang, 1980; Prabhu et al., 2019). This finding is consistent with the current study, which revealed lower Mn

Table 8
Macro-nutrients, and mineral retention of Atlantic salmon post smolt fed blue mussel meal (BMM) and graded inclusion of blue mussel silage (BMS) in experiment 1 and 2.

Experiment 1							
	Control	BMS3	BMS7	BMS11	BMM12	Regression (0, BMS3, 7, 11)	Comparison (0, BMS11, BMM12)
Macro-nutrients (%)							
Protein	54.07 ± 2.06[a]	43.30 ± 512	41.38 ± 4.21	31.10 ± 4.90[b]	48.06 ± 0.43[ab]	R^2= 0.60, $p = 0.003$[1]	$p = 0.04$
Total fat	79.00 ± 4.50	62.00 ± 6.55	66.00 ± 4.93	47.67 ± 10.67	75.50 ± 4.50	R^2= 0.44, $p = 0.01$[2]	n.s.
Energy	57.63 ± 1.20[a]	47.61 ± 5.12	46.28 ± 2.91	34.84 ± 6.49[b]	51.59 ± 1.65[ab]	R^2= 0.57, $p = 0.004$[3]	$p = 0.04$
Dry matter	52.00 ± 1.14[a]	43.81 ± 4.57	41.90 ± 2.59	31.36 ± 5.85[b]	47.74 ± 1.09[ab]	R^2= 0.59, $p = 0.004$[4]	$p = 0.03$
Ash	27.63 ± 2.36	22.88 ± 3.05	22.68 ± 4.60	15.98 ± 4.60	31.26 ± 0.71	R^2= 0.33, $p = 0.04$[5]	n.s.
Micro-minerals (%)							
Mn	2.82 ± 0.73[a]	1.36 ± 0.33	1.60 ± 0.53	0.37 ± 0.08[b]	1.79 ± 0.28[ab]	R^2= 0.50, $p = 0.01$[6]	$p = 0.05$
Cu	23.05 ± 0.90	25.35 ± 4.01	24.26 ± 1.85	25.62 ± 3.74	18.60 ± 2.83	n.s.	n.s.
Fe	6.15 ± 0.69[a]	2.21 ± 0.31	0.51 ± 0.29	0.31 ± 0.09[b]	4.52 ± 0.40[a]	R^2= 0.92[7]	$p < 0.0001$
Se	32.28 ± 2.28[a]	26.00 ± 2.70	22.52 ± 0.93	12.70 ± 2.55[b]	29.75 ± 1.25[a]	R^2= 0.81, $p < 0.0001$[8]	$p = 0.001$
Zn	20.67 ± 0.66[a]	20.67 ± 2.02	18.33 ± 1.20	14.67 ± 0.33[b]	21.50 ± 1.50[a]	R^2= 0.60, $p = 0.003$[9]	$p = 0.02$

Experiment 2						
	Control	BMM9	BMS9[10]	BMSS9[11]	BMSF9[12]	ANOVA
Macro-nutrients (%)						
Protein	37.67 ± 2.33	45.67 ± 6.36	36.67 ± 5.54	26.67 ± 5.54	31.67 ± 3.48	n.s.
Total fat	46.00 ± 5.00	56.33 ± 9.33	50.67 ± 5.69	39.67 ± 7.31	44.33 ± 4.97	n.s.
Energy	35.67 ± 2.84	44.33 ± 7.31	37.67 ± 3.71	28.67 ± 5.78	33.33 ± 4.63	n.s.
Dry matter	34.67 ± 2.84	43.33 ± 6.76	36.67 ± 4.80	27.67 ± 5.92	32.33 ± 2.84	n.s.
Micro-minerals (%)						
Mn	4.17 ± 0.44	358 ± 0.95	3.25 ± 0.72	1.53 ± 0.48	1.68 ± 0.24	n.s.
Cu	9.62 ± 1.88	11.42 ± 2.06	12.91 ± 0.85	8.15 ± 1.90	9.97 ± 1.47	n.s.
Fe	5.72 ± 0.39	5.21 ± 0.86	5.83 ± 0.67	4.37 ± 0.78	4.54 ± 0.37	n.s.
Se	20.46 ± 3.14	22.83 ± 3.76	19.49 ± 2.41	11.10 ± 2.17	15.63 ± 2.83	n.s.
Zn	18.00 ± 1.52	23.33 ± 3.71	24.00 ± 4.93	23.67 ± 2.66	26.00 ± 2.08	n.s.

Notes: Data is listed as mean ± SEM. The mean is from n = 3 pooled whole- body sample per diet (n = 5 fish per tank). In experiment 1, all diets are triplicate except BMM12, that is in duplicate. In experiment 2, n = 3 tank per diet.
The column labeled "Regression" gives R^2 and *p*-value for linear regression performed for the control and silage groups with silage inclusion percentage as x-variable (0, 3, 7, and 11). The column labeled "comparison" under experiment 1 gives a *p*-value for ANOVA in case of a significant difference between control, BMS11 and BMM12. The column labeled "ANOVA" under experiment 2 gives a *p*-value for ANOVA in case of a significant difference between all experimental groups. Means with different superscripts are significantly different ($p < 0.05$) under the Tukey HSD test. n.s stands for not significant.
[1] Simple linear regression. Y = −1.892× + 52.40.
[2] Simple linear regression. Y = −2.395× + 76.24.
[3] Simple linear regression. Y = −1.867× + 56.40.
[4] Simple linear regression. Y = −1.715× + 51.27.
[5] Simple linear regression. Y = −0.9447× + 27.25.
[6] Simple linear regression. Y = −0.1883× + 2.528.
[7] Segmental linear regression. Y1 = −1.394× +6.150. Y2 = −0.2383 (X-3) +1.968. Y=IF (X < 3. Y1. Y2). X0 = 3.
[8] Simple linear regression. Y = −1.681× + 32.20.
[9] Simple linear regression. Y = −0.5612× + 21.53. BMS in experiment 1 refers to diets containing blue mussel silage with different inclusion levels (3, 7, and 11). BMM refers to blue mussel meal in both experiments.
[10] BMS9 refers to diets containing blue mussel silage produced with a higher amount of soft acid (pH 2.5) and antioxidants from the same batch of silage used in experiment 1.
[11] BMSS9 refers to diets containing blue mussel silage with lower amount of soft acid (pH 3.7) and antioxidants.
[12] BMSF9 refers to diets containing blue mussel silage with only formic acid and (pH 3.5) without antioxidants.

and Fe status alongside a higher Cu status in the whole-body of fish fed BMS. The transport of Fe^{2+} into the absorptive enterocyte of the small intestine requires not only the action of divalent metal protein I (DMT1) but is also dependent on the chemical form of Fe (Fe^{3+} and Fe^{2+}), as previously discussed (Hansen and Spears, 2009). According to a study by (Hansen and Spears, 2009), an acidic environment like silage fermentation may reduce Fe^{3+} to the more soluble Fe^{2+} which is in line with the current result that Fe^{3+} to total Fe ratio in BM raw materials (without acid silage) (31% of total Fe) was 1.7-times higher than BMS product before drying (18% of total Fe) (Fig. 5a). However, co-drying BMS with SPC (BMS + SPC) increased the ratio in the product (39% of total Fe) which was reflected in the diets contained BMS + SPC in experiment 1 (Fig. 5b). Therefore, a high level of dietary Fe^{3+} may be assumed as one reason for impairing the activity of ferric reductase enzyme, in the apical membrane of intestinal epithelial cells, and Fe availability.

Ascorbic acid plays a crucial role in Fe metabolism in animals, including fish, as documented by various studies (Harper et al., 1979; Hilton, 1989; Monsen, 1982; NRC, 1993). It enhances the absorption of Fe from the intestine by converting ferric iron (Fe^{3+}) into a more soluble and absorbable ferrous state (Fe^{2+}) (El-Hawary et al., 1975; Harper et al., 1979; Monsen, 1982). The chemical forms of Fe (Fe^{3+} and Fe^{2+}) are important in transporting the Fe through the epithelium of the cells in the intestinal wall (Bury and Grosell, 2003; Bury et al., 2003). The soluble form of iron is Fe^{2+} which is more absorbable by the intestinal cells in fish than the Fe^{3+} form (Bury and Grosell, 2003; Bury et al., 2003). Ascorbic acid also collaborates with adenosine triphosphate (ATP) in the release and reduction of Fe^{3+} from ferritin. This reduced Fe is then incorporated into Fe-binding proteins, apoferritin, and

transferrin, facilitating its storage in bodily tissues (Harper et al., 1979; Mazur et al., 1960). The dietary vitamin C levels were high and similar in all diets, but the decreased Fe status was only observed for the fish given diets containing BMS in experiment 1, thus it is not likely that the differences are caused by the vitamin C content in the feed.

Based on the results from experiment 1, it was hypothesized that either the amount of acid and thus the low pH in the blue mussel silage used, or the use of antioxidant in the silage could modulate the bioavailability of iron. Thus, experiment 2 was designed to both repeat the reference diet and the blue mussel meal diets as positive control, as well as repeating the same batch of blue mussels used in experiment 1. In addition, two new productions of BMS were tested, using a lower acid level and higher pH (3.5) as well as only formic acid at the same level.

In this study, no differences were seen in feed intake, FCR, and weight gain between the experimental groups. A significant reduction was however seen in SGR and condition factor in fish fed BMSS9 (made with silage containing lower level of soft acid) compared with the control group. A lower daily and total feed intake was observed in this group, although not significantly different from other groups. This could be attributed to the higher TBARs level in this diet compared to other diets, potentially reducing the palatability of the diet and growth performance. Although HSI was significantly higher in the BMS groups compared to the control group, it remained within the normal range for Atlantic salmon (1–2%) (Arnesen and Krogdahl, 1993).

Despite variations in the levels of several amino acids in BMS products, likely caused by variations in seasons and productions, the amino acid composition was balanced in the experimental diets. Further, the inclusion of BMS in the diets did not influence the whole-body composition and retention of macro-nutrients. No sign of Fe depletion was observed despite a higher Fe^{3+} to total Fe ratio in BMS products and experimental diets compared to the control diet in experiment 2 (Fig. 5a and c). The fish fed diets containing BMS also had significantly better Fe status in whole-body and targeted tissues, and the Fe availability was comparable between the experimental groups. The blood parameters such as RBC, Hb, and HCT were not influenced by BMS and were comparable between all experimental groups. No differences were seen in Fe availability or body status in this experiment irrespective of the silage being made with or without antioxidants. In addition to Fe, fish fed both BMSS9 and BMSF9 groups had a higher Zn level in the whole-body which was in line with the Zn availability results that got doubled in these groups. The availability of Mn and Cu also increased significantly in these groups; however, this was not reflected in the body composition. The general welfare of the fish was not compromised, which means the nutrition and environment requirements of fish was fulfilled by the experimental diets (Dawkins, 1990; Noble et al., 2018; Stien et al., 2013).

Different outcomes in experiments 1 and 2 may therefore be due to the difference in the production methodology, particularly in the steps to increase the dry matter content in the feed. This discrepancy is likely due to these differences, rather than variations in pH levels, which are associated with the acid used during production, and the addition of antioxidants. In experiment 1, the BMS with 10% DM was mixed with SPC and dried before being added to the feed to reach the target levels of BMS in the finished extruded feeds. In experiment 2, the same batch of blue mussel silage was used, however dried using a falling film evaporator (heat drying) to reach 50% DM allowing direct inclusion in the feed production. Overall, the findings indicate that the processing method can significantly influence the availability of nutrients and the body composition of fish, as demonstrated by the changes in mineral levels in the different experimental groups.

5. Conclusion

According to the findings from both experiments, Atlantic salmon fed a partial inclusion of blue mussel meal as a FM replacement have comparable growth, feed utilization, digestibility, and retention. In experiment 1, a growth reduction was seen already with the inclusion of 3% BMS, which could be explained by production methodology, the co-drying process with SPC, and interactions that likely caused problems with iron uptake.

In experiment 2, no differences were seen in the iron status as well as growth performance and feed utilization by using different drying method; however, somewhat lower growth was seen in the fish given BMS with lower soft acid compared with other BMS groups.

In conclusion, the limiting step for using blue mussel silage in fish feeds appears to be related to the processing of the raw material, as well as the choice of drying methods to facilitate incorporation into extruded feeds.

The use of blue mussel silage as a marine protein resource should be further elucidated, focusing on optimizing methods with a low carbon footprint, as well as focusing on interactions that may reduce the bioavailability of minerals.

CRediT authorship contribution statement

Sahar Sartipiyarahmadi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Investigation, Formal analysis, Conceptualization. **Antony J. Prabhu Philip:** Writing – review & editing, Visualization, Validation, Project administration, Investigation, Funding acquisition, Conceptualization. **Aksel N. Forshei:** Writing – review & editing, Visualization, Investigation, Formal analysis, Conceptualization. **Harald Sveier:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Silje Steinsund:** Writing – review & editing, Resources, Investigation. **Malin Kleppe:** Writing – review & editing, Resources, Investigation. **Erik-Jan Lock:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Angelico Madaro:** Writing – review & editing, Investigation. **Tom Johnny Hansen:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Formal analysis. **Øivind Strand:** Writing – review & editing, Investigation. **Martin Wiech:** Writing – review & editing, Investigation. **Jan Vidar Jakobsen:** Writing – review & editing, Resources, Investigation. **Sofie C. Remø:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.740829.

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