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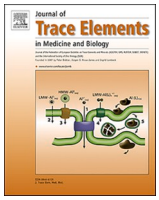

Arsenic speciation and arsenic feed-to-fish transfer in Atlantic salmon fed marine low trophic feeds based blue mussel and kelp

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ABSTRACT

Background: Aquaculture aims to reduce the environmental and climate footprints of feed production. Consequently, low trophic marine (LTM) resources such as blue mussels and kelp are potential candidates to be used as ingredients in salmon feed. It is relevant to study potential undesirables associated with their use, as well as assessing food safety by investigating their transfer from feed-to-fish. The marine biota is well known to contain relatively high levels of arsenic (As), which may be present in different organic forms depending on marine biota type and trophic position. Thus, it is important to not only obtain data on the concentrations of As, but also on the As species present in the raw materials, feed and farmed salmon when being fed novel LTM feed resources.
Methods: Atlantic salmon were fed experimental diets for 70 days. A total of nine diets were prepared: four diets containing up to 4 % fermented kelp, three diets containing up to 11 % blue mussel silage, and one diet containing 12 % blue mussel meal, in addition to a standard reference diet containing 25 % fish meal. Concentrations of As and As species in feeds, faeces, liver and fillet of Atlantic salmon were determined by inductively coupled plasma mass spectrometry (ICP-MS) and high-performance liquid chromatography coupled to ICP-MS (HPLC-ICP-MS), respectively.
Results: The use of kelp or blue mussel-based feed ingredients increased the concentration of total As, but maximum level as defined in Directive 2002/32 EC and amendments was not exceeded. The concentrations found in the experimental feeds ranged from 3.4 mg kg^{-1} to 4.6 mg kg^{-1} ww. Arsenic speciation in the feed varied based on the ingredient, with arsenobetaine dominating in all feed samples (36–60 % of the total As), while arsenosugars (5.2–8.9 % of the total As) were abundant in kelp-included feed. The intestinal uptake of total As ranged from 67 % to 83 %, but retention in fillet only ranged from 2 % to 22 % and in liver from 0.3 % to 0.6 %, depending on the marine source used. Fish fed feeds containing blue mussel showed higher intestinal uptake of total As when compared with fish fed feeds containing fermented kelp. Fish fed fermented kelp-based feeds had higher retained concentrations of total As when comparing with fish fed feeds containing blue mussel. Despite relatively high intestinal uptake of total As, inorganic and organic As, the retained concentrations of As did not reflect the same trend.
Conclusion: Although the use of LTM feed ingredients increased the level of total As in this feeds, salmon reared on these diets did not show increased total As levels. The well-known toxic inorganic As forms were not detected in salmon muscle reared on LTM diets, and the non-toxic organic AsB was the dominant As species that was

Abbreviations: As, Arsenic; AsIII, Arsenite; AsV, Arsenate; AsB, Arsenobetaine; AsC, Arsenocholine; AsLipids, Arsenolipids; AsSug, Arsenosugar; AsSug-328, Arsenosugar 328 (glycerol arsenosugar); AsSug-392, Arsenosugar 392 (sulfonate arsenosugar); AsSug-482, Arsenosugar 482 (phosphate arsenosugar); BMM, Blue mussel meal; BMS, Blue mussel silage; CRM, Certified reference material; DMA, Dimethylarsinate; DMAA, Dimethylarsinoyl acetate; DMAE, Dimethylarsinoyl ethanol; DMAP, Dimethylarsinoyl propionate; Dw, Dry weight; EC, European Commission; EFSA, European Food Safety Authority; FK, Fermented kelp; HPLC, High-performance liquid chromatography; iAs, Inorganic arsenic; ICP, Inductively coupled plasma; LOD, Limit of detection; LOQ, Limit of quantification; LTM, Low trophic marine; MA, Methylarsonate; MLs, Maximum levels/limits; MS, Mass spectrometry; Sum AsSug, Sum of arsenosugars; tAs, Total arsenic; TETRA, Tetramethyl arsonium ion; TMAO, Trimethylarsine oxide; TMAP, Trimethylarsoniopropionate; Ww, Wet weight; Dw, Dry weight..

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retained in salmon muscle, while the organic AsSug forms were not. This study shows that speciation analysis of the LTM resources provides valuable information of the feed-to-fish transfer of As, needed to assess the food safety of farmed Atlantic salmon reared on novel low trophic feeds.

1. Introduction

Fishmeal and fish oil have been the most important ingredients in commercial feed formulations. However, availability, sustainable use of marine fish stocks, and decreasing carbon footprint have led to find alternative ingredients. Plant-based ingredients and oils have been used to replace fish oil and meal in aquafeeds. As a result, the use of marine ingredients from pelagic fish stocks has declined in the last decades [1]. At the same time, increased consumer awareness on sustainability and food safety puts pressure on the aquaculture industry to document that the production is safe for consumers and environmentally sustainable [2]. Consequently, the aquaculture industry aims to significantly reduce the environmental and climate footprints of feed production [3]. Goal 12 and goal 14 of the United Nations sustainable development goals (SDGs) call for sustainable consumption and production patterns, and for conservation and sustainable use of oceans, seas, and marine resources, respectively. The use of locally harvested ingredient such as low trophic marine (LTM) resources is in line with these goals [4]. Most recently, several low-trophic resources have been considered good candidates to replace fishmeal in salmonid diets while maintaining optimum growth performance. These include mesopelagic fish, marine macroalgae, bacterial meal, microalgae and insect meal [5].

When using new feed ingredients in food production, mapping of risks associated with the use of novel feed ingredients is of importance [EFSA, [6], OECD, [7]]. Fish feed and feed ingredients can contain undesirable substances, which may derive from the production process and/or the environment [8]. If a farmed animal is fed on contaminated feed, there is a risk that the undesirable substances are transferred to the edible parts such as fillet and liver [9]. In the European Union, legislation is in place to control the presence of undesirable substances in feed and feed ingredients (Directive 2002/32/EC). Heavy metals are a relevant group of undesirables to monitor in feed materials and fish feeds [10]. Heavy metals such as mercury (Hg), cadmium (Cd), lead (Pb), and the metalloid arsenic (As) pose a significant risk as they can easily transfer through food chains, lack any known essential biological functions, and exhibit toxic properties [11].

The regulation (EU) 1881/2006 establishes maximum levels (MLs) in certain types of seafood for Cd, Pb, Hg, but not for As [EC, [12]]. In many marine fish and shellfish, the As concentrations can exceed the concentrations found in most terrestrial foods [13]. Consequently, seafood is a significant source of As for humans, with varying concentrations of different chemical forms found in fish and other marine organisms. The toxicity of As is known to be dependent on its chemical form [14]. One can conclude from this that there is a crucial need for data on the speciation of As. There are different As species present in seafood, e.g. arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MA) and dimethylarsinic acid (DMA). Arsenobetaine (AsB) is the major As species in most fish and seafood. Other As species such as As(III), As (V), MA, DMA, arsenocholine (AsC), trimethylarsine oxide (TMAO) and arsenolipids (AsLipids) are also present in aquatic organisms. Furthermore, arsenosugars (AsSug) were also found in marine algae [15].

Fish feed is composed of terrestrial and marine-derived feed ingredients which can contain different levels of undesirable substances. Feed surveillance includes only measurement of total amounts of undesirables. However, the biological activity, mobility, bioavailability, and toxicity of an element are also dependent on the chemical form in which the element exists [16]. Consequently, to attain detailed information, speciation analyses are needed, providing valuable information on the different chemical forms of an element present in a sample. Legislators are aware of the importance of the element species, even though implementation of MLs for chemical species are not defined for all sample types. A footnote is included in the directive 2002/32/EC stating that "Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic As is lower than 2 ppm". In March 2022, the European food safety authority (EFSA) published a call for continuous collection of chemical contaminants occurrence data both in food and feed where organic As (i.e. thiolated compounds (DMMTA and MMMTA) and others (i.e. MA, DMA, AsLipids, AsSug, among others)) have been included. Data on organic As species are meant to support scientific opinions in progress.

This study aims: (i) to determine concentrations of total As and its different chemical species present in novel salmon feeds partly based on blue mussel and kelp; (ii) to evaluate the intestinal uptake of total As from these novel feeds and further retention in salmon liver and fillet tissue; and (iii) to assess the chemical forms of As in the novel feeds and their specific feed-to-fillet transfer and fillet retention.

2. Material and methods

2.1. Chemicals and reagents

Ultrapure water (18.2 MΩ-cm) was produced in-house using a Milli-Q water purification system (Merck Millipore, Burlington, MA, USA) and was used throughout the study. All reagents used were analytical grade and of high purity. Methanol (MeOH, ≥ 99.97 %), pyridine (C_5H_5N, ≥ 99.5 %), formic acid (HCOOH, ≥ 98 %), nitric acid (HNO_3, 65 %), hydrogen peroxide (H_2O_2, 30 %), ammonia solution (NH_3, 25 %), and ammonium carbonate ($(NH_4)_2CO_3$, reagent grade) were purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN, ≥ 99.95 %) was obtained from VWR Chemicals BDH (Fontenay-sous-Bois, France). Arsenite [As(III)] and arsenate [As(V)] solutions (1000 mg/L) were produced by Spectrascan Teknolab (Ski, Norway). Arsenobetaine (AsB, ≥ 95 %) and a sodium salt of dimethylarsinic acid (DMA, ≥ 98 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tetramethyl arsonium iodide (TETRA, 97 %) and trimethylarsine oxide (TMAO, 95 %) were supplied by Toronto Research Chemicals (Toronto, Ontario, Canada). Arsenocholine (AsC, 19.77 mg kg^{-1}) was produced by the National Institute of Standards and Technology (Gaithersburg, MD, USA), while monomethylarsonic acid (MA, 99.5 %) was procured from Chem Service (West Chester, PA, USA). Other arsenic species such as trimethylarsoniopropionate (TMAP), dimethylarsinoyl acetate (DMAA), dimethylarsinoyl ethanol (DMAE), and dimethylarsinoyl propionate (DMAP), as well as glycerol arsenosugar (AsSug-328), sulfonate arsenosugar (AsSug-392), and phosphate arsenosugar (AsSug-482), were synthesized (purity >99.5 %) using published procedures and obtained from the University of Graz (Graz, Austria). Stock solutions were prepared by dissolving or diluting appropriate amounts of the standards in water. Concentration of As in stock solutions were verified by inductively coupled plasma mass spectrometry (ICP-MS).

2.2. Experimental diets, fish and experimental conditions

The materials used in the present work were obtained from a feeding experiment conducted at Matre Research station, Institute of Marine Research, Norway. Information regarding the experimental conditions of the trial can be seen elsewhere [17–20]. The feeding trial was conducted according to Norwegian regulations on animal experimentation (FOTS approval # 25202). Briefly, Atlantic salmon post-smolt (206 ± 9 g, mean weight±standard deviation) were distributed in 27

experimental tanks and given a standard reference diet or one of eight experimental diets for 70 days. The experimental diets contained either four levels of fermented sugar kelp (FK 1, 2, 3, 4 %), one level of blue mussel meal (BMM 12 %) or three levels of blue mussel silage (BMS 3, 7, 11 %). Fermented sugar kelp (*Saccharina latissima*) and blue mussel (*Mytilus edilus*) silage was provided by Lerøy/Ocean Forest AS. The blue mussel silage was made from undersized mussels from a commercial blue mussel farm in Limfjorden, Denmark. Freshly harvested sugar kelp was obtained from Trollsøy, Austevoll. The blue mussel meal was obtained from Triplenine (999), Denmark. The fish were fed two meals per day and excess feed was collected to estimate feed intake. Yttrium oxide was added (0.02%) as an inert marker to assess intestinal uptake. Feed samples were taken after feed production, homogenised for 10 s at 10, 000 rpm using a knife mill (GM 300, Retsch GmbH, Haan, Germany) and kept at room temperature until further analyses.

2.3. Sampling

Fish were killed by overdose tricaine methane sulfonate (500 mg/L, Finquel MS-222, MSD Animal Health Norge, Bergen, Norway). All fish were individually weighed, and length measured. At the start of the trial, three pooled samples of fillet and liver were made from ten fish (n = 3). At the end of the trial, a pooled sample of fish fillet, liver and faeces (stripped) of five fish were made from each tank (n = 3). Fish were placed with head to the left facing the person handling the samples, top fillet was collected. Samples of fillet and liver were frozen on dry ice after sampling and thereafter stored at −20 °C until analysis. Faeces samples were immediately stored at −20 °C after collection. Pooled samples of the fillet and faeces were freeze-dried and subsequently homogenized. The freeze-dried samples were kept at room temperature and liver samples were kept at −20 °C until further analysis.

2.4. Chemical analyses

2.4.1. Determination of total arsenic and yttrium by ICP-MS

The concentration of total As and yttrium in diets and faeces, and concentration of As in tissues were analysed using a microwave-assisted digestion and an ICP-MS as described elsewhere [21]. In summary, the sample material (approximately 0.2 g) was subjected to digestion using 2 mL of HNO3 (69 % w/w) in an ultra-wave digestion system (UltraWAVE, Milestone, Sorisole, Italy). The tubes were sealed and inserted into the ultra-wave system. After digestion, the samples were diluted to 25 mL with Milli-Q® water. The ICP-MS (iCapQ ICP-MS, Thermo Scientific, Waltham, MA, USA) equipped with an autosampler (FAST SC-4Q DX, Elemental Scientific, Omaha, NE, USA) was used to determine the concentrations of As and yttrium. The ICP-MS was calibrated according to the manufacturer's instructions, and a tuning solution (1 ppb tuning solution B, Thermo Fisher, in 2 % HNO3 and 0.5 % HCl) was utilized prior to analyses. The Qtegra ICP-MS software (version 2.10, 2018, Thermo Scientific, Waltham, MA, USA) was employed for data collection and processing. The method`s accuracy was verified by analysing certified reference materials (i.e., lobster hepatopancreas (TORT-3; National Research Council Canada, Ottawa, ON, Canada) and oyster tissue (SMR1566b; National Institute of Standards and Technology, Gaithersburg, MD, USA)) (data can be seen in Supplementary information, Table S1). For As, the LOQ determined for this method was 0.01 mg kg^{-1} dw. All values reported were compared to LOQ before being reported. For yttrium, the concentration present in feed and faeces are several times above a possible LOQ so this was not determined.

2.4.2. Determination of inorganic arsenic by HPLC-ICP-MS

Inorganic As (iAs) concentration was determined as previously described [22], and based on the European Committee for Standardization method (NS-EN 16802:2016, European Committee for Standardization). A propylene centrifuge tube (13 mL, Sarstedt, Nümbrecht, Germany) was utilized to weigh approximately 0.2 g of the sample, to which 10 mL of extraction solution (0.1 M HNO_3 in 3 % (v/v) H_2O_2) was added. The samples were subjected to shaking in a water bath at 90 °C and 100 rpm for 60 min and subsequently cooled to room temperature before being centrifuged (1780g) for 10 min (Eppendorf® Centrifuge 5702, Hamburg, Germany). The soluble fraction was then collected using a 5 mL disposable needle syringe and filtered through a disposable syringe filter (0.45 μm, Sartorius, Göttingen, Germany) into 1 mL polypropylene HPLC vials. The mobile phase solution was created by dissolving an appropriate amount of $(NH_4)_2CO_3$ in an aqueous 3 % (v/v) MeOH solution (LiChrosolv®, HPLC grade) to attain an ionic strength of 50 mM, followed by pH adjustment to 10.3 with NH_3 (25 % v/v). An HPLC-ICP-MS (1260 HPLC, 7900 ICP-MS, Agilent Technologies, Wilmington, DE, USA) with an anion-exchange column (IonPac AS7, 2 × 250 mm; Dionex, Sunnyvale, CA, USA) and a corresponding guard column (IonPac AG7, 2 × 50 mm; Dionex, Sunnyvale, CA, USA) was employed to determine the iAs concentration. The instrument was tuned according to the manufacturer's instructions, and data processing was carried out using MassHunter 4.5 Workstation Software. Certified rice reference material (ERM-BC211; Institute for Reference Materials and Measurements, Geel, Belgium) and an in-house control sample of tuna fish tissue (BCR-627; Institute for Reference Materials and Measurements of the European Commission's Joint Research Centre, Geel, Belgium) were used to verify the method's accuracy (data can be seen in supplementary information, Table S2). The LOQ of this method was 0.01 mg kg^{-1}. All values reported were compared to LOQ before being reported.

2.4.3. Determination of water-soluble arsenic species by HPLC-ICP-MS

The determination of water-soluble As species in feeds, fillet and liver was carried out by cation- and anion-exchange HPLC-ICP-MS as previously described [23]. To demonstrate the applicability of the developed method, a single-laboratory validation was carried out according to Eurachem's recommendations. Method performance characteristics were evaluated based on selectivity, limits of detection and quantification, linearity, trueness, precision, and measurement uncertainty. Detailed information regarding this analytical had previously been reported, please see [23]. In short, 0.2 g of the sample was added to a 13-mL polypropylene tube with 5 mL of aqueous methanol (MeOH: H2O, 50 % v/v), followed by vortex-mixing and placed in a shaking water bath at 90 °C for 30 min (shaking speed at 100 rpm). After centrifugation (1780g) for 10 min, the supernatant was transferred to a 13-mL tube through a 0.45-μm filter into an HPLC vial and diluted with aqueous methanol (MeOH:H_2O, 50 % v/v). An HPLC-ICP-MS system (1260 Infinity HPLC, 7900 ICP-MS, Agilent Technologies, Wilmington, DE, USA) was used for As speciation. Cationic As species were separated using a Metrosep C6 column (250 × 4.0 mm, 5 μm; Metrohm, Herisau, Switzerland) with pyridine-based mobile phases, and anionic As species were separated with a PRP-X100 column (250 × 4.6 mm, 5 μm; Hamilton, Reno, NV, USA) using carbonate-based mobile phases. Examples of chromatograms are shown in the supplementary information (see Fig. S1 and S2). Quantification was done using external calibration curves with mixed standard solutions of As compounds. Quality control included certified reference materials of tuna fish tissue (BCR-627) and fish protein (DORM-4) (data can be seen in supplementary information, Table S3). Data processing was done with MassHunter 4.5 Workstation Software. The LOQ values ranged from 0.005 to 0.025 mg kg^{-1} for the different As species [23]. All values reported were compared to LOQ before being reported.

2.5. Formulas and statistics

The intestinal uptake (%) was determined using a ratio between the concentration of the inert yttrium marker in diet and faeces and the concentration of total As, iAs or one of the water-soluble As species in diet and in faeces, as described by the following equation:

$$Intestinal\ uptake(\%) = 100 - \left(100\frac{\text{yttrium in } diet}{\text{yttrium in } faeces} * \frac{\text{arsenic in } faeces}{\text{arsenic in } diet}\right) \quad (1)$$

Intestinal uptake was calculated for total As, iAs and water-soluble As species.

The retention (%) is calculated by dividing the difference between the final and initial As concentrations in liver/fillet by the As concentration in diet and feed intake.

$$Retention\ in\ fillet\ (\%) = \frac{(\text{arsenic in } fillet,\ \text{end} * \text{weight } fillet,\ \text{end}) - (\text{arsenic in } fillet,\ \text{start} * \text{weight } fillet,\ \text{start})}{(arsenic\ in\ diet * feed\ intake)} \quad (2)$$

$$\text{Retention in } liver\ (\%) = \frac{(\text{arsenic in } liver,\ \text{end} * \text{weight } liver,\ \text{end}) - (\text{arsenic in } liver,\ \text{start} * \text{weight liver,\ start})}{(\text{arsenic in } diet * \text{feed } intake)} \quad (3)$$

Retention in fillet and liver was calculated for total As, iAs and water-soluble As species.

Statistical analyses were performed in R studio [RStudio Team, [24]] and a 95 % confidence interval was applied in all the statistical analysis. One-way ANOVA and Tukey Honest Significant Difference test were performed to assess statistical significance between diets. Further, the statistical significance between the different feed ingredients was assessed by a linear mixed effects models where ingredient type was included as random factor in all models to avoid pseudo replication. Graphs were made in GraphPad Prism (Version 8.4.3).

3. Results and discussion

3.1. Experimental ingredients and diets

3.1.1. Total arsenic in marine low trophic feed ingredients and salmon feed

In fermented kelp, total As concentration (n = 2) was 35.4 ± 0.5 mg kg^{-1}. Total As concentrations in blue mussel silage and blue mussel meal (n = 2) were 7.0 ± 0.6 mg kg^{-1} and 7.6 ± 0.6 mg kg^{-1}, respectively. Previous values are presented in wet weight and considering 12 % moisture in the samples. Concentration of As in the feed ingredients as received in the laboratory are shown in the Supplementary information (Table S5). The potential future application of fermented kelp and blue mussel as feed ingredients depends on the compliance with existing legislation. In the EU directive 2002/32/EC, blue mussel meal or blue mussel silage lies in the category of "feed materials of fish, other marine animals and products derived thereof" with an EU mL at 25 mg kg^{-1}, and the fermented kelp will be categorized as "feed materials of seaweed meal and feed materials derived from seaweed" with an EU mL at 40 mg kg^{-1} (Directive 2002/32/EC and amendments). Overall, the concentrations of total As in both feed ingredients were below the MLs. Large natural variations have been seen when collecting seaweed or blue mussel in different locations and periods of the year [FAO, [25,26]]. This can lead to different levels in feed ingredients than what is seen in this study.

The total As concentration in the reference feed was 3.41 ± 0.02 mg kg^{-1} diet (n = 2). The total As (n = 2) in diets containing 1, 2, 3 or 4 % fermented kelp were 4.1 ± 0.1, 4.22 ± 0.08, 4.59 ± 0.03, and 4.5 ± 0.2 mg kg^{-1} diet, respectively. In the blue mussel meal diet, with only one inclusion level of 12 %, the total concentration of As was 3.5 ± 0.1 mg kg^{-1} diet (n = 2). In the diets containing 3, 7 or 11 % blue mussel silage, the total concentration of As (n = 2) was 3.6 ± 0.002, 3.5 ± 0.03, and 3.7 ± 0.004 mg kg^{-1} diet, respectively. Previous values are presented in wet weight. The total As concentration in the reference feed was statistically different then the total As concentration in fermented kelp-based diets. In contrary, the total As concentration in the reference feed was not statistically different compared to blue mussel-based diets. All concentrations of total As and As species in the different experimental feeds can be seen in Supplementary information, Table S6. The use of kelp or blue mussel-based feed ingredients increased the levels of total As in feed, but all samples were below the EU mL for complete feed for fish at 10 mg kg^{-1} (Directive 2002/32 EC and amendments). Moisture in feed samples was 6 ± 1 % (average±SD) and this means that the values presented in here are higher than if 12 % moisture was taken in consideration.

In commercial fish feed collected in Norway in 2021, the As concentration was 2.2 ± 1.3 mg of kg^{-1} diet ww (n = 82), with concentrations ranging from 0.8 to 6.7 mg kg^{-1} ww [27]. The As concentrations in the commercial fish feeds were in the same concentration range as the experimental diets (Table 1). Fish meal generally contribute with higher levels of As than the terrestrial ingredients [27], and also fish oil can contribute with relatively high levels of As [28].

3.1.2. Arsenic speciation in marine low trophic feed ingredients to salmon feeds

Arsenic species vary in toxicity [15,29], thus, As speciation data can provide complementary information. Arsenic speciation in the feed varied based on the ingredient (Fig. 1A), with AsB being a major fraction in blue mussel-based feeds, while arsenosugars were abundant in kelp-containing feed. The measured AsB concentration corresponded to about 60 % of the total As in the reference diet. The AsB in experimental diets were present in lower proportions, ranging from 36 % to 45 % of total As in fermented kelp diets and 36–52 % in blue mussel meal diets. This decrease in AsB for the experimental diets was expected as the inclusion of the experimental ingredients was done by replacing the content of fish meal in the diet. The main species of As found in marine fish is AsB [[30,31] EFSA[32]]. Fish meal can contain high levels of total As, and generally more than 95 % of As in forms of organic As species [28]. Approximately 2 % of the total As was found to be in inorganic As forms in fish feed samples [27].

In fermented kelp, iAs concentration (n = 2) was 0.211 ± 0.005 mg kg^{-1} ww (12 % moisture), whereas higher concentrations of iAs was detected in blue mussel-based ingredients (n = 4), at 0.5 ± 0.1 mg kg^{-1} ww (12 % moisture). Inorganic As accounted for 1.7–4.0 % of the total As in the experimental diets, with highest proportions in the diet containing the blue mussel meal (Fig. 1A). The reference diet contained a low level of inorganic As, accounting for 1.7 % of total As (Fig. 1A), which was in accordance with previous analysis of commercial fish feed in Norway [27]. If requested by competent authorities, one must be able to demonstrate that the content of iAs is lower than 2 mg kg^{-1} (2002/32/EC). All experimental feeds were well below this limit as concentrations of iAs ranged from 0.06 to 0.14 mg kg^{-1} (Table 1).

As can be seen in Fig. 1A, arsenosugars were one of the main As species detected in fermented kelp diets, with proportions ranging from 5.2 % to 8.9 % of total As. Furthermore, minor concentrations of arsenosugars were found in blue mussel-based diets (1.2–2.3 % of total As). In the reference feed, the concentration of AsSug-328 found is similar to the concentration of AsSug-328 found in blue mussel-based

Table 1
Concentrations of yttrium, total arsenic (tAs), inorganic arsenic (iAs), arsenobetaine (AB), trimethylarsine oxide (TMAO), trimethylarsonio propionate (TMAP), arsenocholine (AsC), tetramethyl arsonium ion (TETRA), dimethylarsinic acid (DMA), methylarsonite (MA) and three arsenosugar species (arsenosugar-328, −392, and −482) in experimental diets containing fermented kelp (FK), blue mussel meal (BMM) or blue mussel silage (BMS). Values sharing the same superscript are not significantly different from each other.

	(mg/kg ww)	Yttrium	tAs	iAs	AsB	TMAO	TMAP	AsC	TETRA	DMA	MA	AsSug-328	AsSug-482	AsSug-392
Reference feed (A)	Average	153	$3.4^{(d)}$	$0.0592^{(g)}$	$2.05^{(a)}$	< LOQ	$0.0177^{(ab)}$	$0.0109^{(cd)}$	< LOQ	$0.01987^{(f)}$	ND	$0.0193^{(e)}$	ND	ND
	Std. Dev.	6	0.02	0.0006	0.01		0.0007	0.0008		0.0007		0.0004		
FK 1 % (B)	Average	157	$4.1^{(c)}$	$0.070^{(f)}$	$1.8148^{(bc)}$	< LOQ	$0.0155^{(bc)}$	$0.00999^{(d)}$	< LOQ	$0.080^{(d)}$	< LOQ	$0.098^{(c)}$	0.039	0.075
	Std. Dev.	4	0.1	0.001	0.0003		0.0003	0.00004		0.002		0.001	0.002	0.002
FK 2 % (C)	Average	152	$4.22^{(bc)}$	$0.0742^{(e)}$	$1.77^{(bcd)}$	< LOQ	$0.0143^{(cd)}$	$0.0096^{(d)}$	< LOQ	$0.095^{(c)}$	< LOQ	$0.130^{(b)}$	0.051	0.103
	Std. Dev.	3	0.08	0.0005	0.03		0.0002	0.0003		0.001		0.003	0.001	0.004
FK 3 % (D)	Average	167	$4.59^{(a)}$	$0.0865^{(d)}$	$1.69^{(cde)}$	< LOQ	$0.0146^{(cd)}$	$0.01025^{(d)}$	< LOQ	$0.137^{(b)}$	< LOQ	$0.17^{(a)}$	0.069	0.137
	Std. Dev.	9	0.03	0.0003	0.05		0.0005	0.00008		0.003		0.01	0.005	0.005
FK 4 % (E)	Average	169	$4.5^{(ab)}$	$0.0936^{(c)}$	$1.60^{(e)}$	< LOQ	$0.0130^{(d)}$	$0.0089^{(de)}$	< LOQ	$0.148^{(a)}$	< LOQ	$0.178^{(a)}$	0.0767	0.144
	Std. Dev.	8	0.2	0.0003	0.07		0.0002	0.0005		0.003		0.008	0.0002	0.002
BMM 12 % (F)	Average	158	$3.38^{(d)}$	$0.1368^{(a)}$	$1.2294^{(g)}$	< LOQ	$0.0140^{(cd)}$	$0.0072^{(e)}$	< LOQ	$0.049^{(e)}$	< LOQ	$0.03054^{(de)}$	0.033	ND
	Std. Dev.	1	0.06	0.0005	0.0004		0.0007	0.0006		0.002		0.00008	0.002	
BMS 3 % (G)	Average	153	$3.570^{(d)}$	$0.0733^{(e)}$	$1.8526^{(b)}$	< LOQ	$0.01767^{(ab)}$	$0.01291^{(bc)}$	< LOQ	$0.028^{(f)}$	ND	$0.0284^{(de)}$	0.013	ND
	Std. Dev.	2	0.002	0.0004	0.0008		0.00008	0.00008		0.003		0.0002	0.001	
BMS 7 % (H)	Average	167	$3.54^{(d)}$	$0.0866^{(d)}$	$1.65^{(de)}$	< LOQ	$0.017^{(ab)}$	$0.014^{(ab)}$	< LOQ	$0.023^{(f)}$	ND	$0.037^{(de)}$	0.025	ND
	Std. Dev.	7	0.03	0.0006	0.05		0.001	0.001		0.001		0.003	0.001	
BMS 11 % (I)	Average	174	$3.661^{(d)}$	$0.1028^{(b)}$	$1.45^{(f)}$	< LOQ	$0.0182^{(a)}$	$0.0154^{(a)}$	< LOQ	$0.027^{(f)}$	ND	$0.0452^{(d)}$	0.039	ND
	Std. Dev.	2	0.004	0.0002	0.02		0.0008	0.0006		0.003		0.0006	0.002	

ND = not detected.
< LOQ = below limit of quantification.

diets. A difference in the concentration of AsSug-328 can be seen when comparing the concentration of AsSug-328 in the reference diet and the kelp-based diets (see Table 1). In blue mussel-based diets, AsSug-328 and AsSug-482 were found. In addition to AsSug-328 and AsSug-482, AsSug-392 was also detected in fermented kelp diets (See Table 1). Seaweeds contain As primarily in the form of arsenosugars [33]. Also, the presence of arsenosugars was seen in blue mussels [23]. An *in vitro* toxicological characterization of two arsenosugars (AsSug-328 and AsSug-408) and their metabolites (i.e. dimethylarsinic acid, thio-dimethylarsinic acid, oxo-dimethylarsenoacetic acid, thio-dimethylarsenoacetic acid, oxo-dimethylarsenoethanol and thio-dimethylarsenoethanol) was performed in cultured human bladder cells. Genotoxic or cytotoxic effects were not induced by many of the chemicals evaluated. However, two of the arsenosugar metabolites (i.e. dimethylarsinic acid and thio-dimethylarsinic acid) were found to be toxic [34]. A different study compared the *in vitro* toxicity of a trivalent and a pentavalent arsenosugar in human epidermal keratinocytes. The trivalent arsenosugar was more cytotoxic than the pentavalent arsenosugar. However, both the trivalent and the pentavalent arsenosugars were significantly less toxic than MMA(III), DMA(III), and As(V) [35]. While there are no current MLs for organic As in food and feed, arsenosugars and other organoarsenicals are considered potentially toxic [36].

In the different experimental diets, AsC and TMAP were present as minor fractions (approximately 0.29 ± 0.09 % (n = 2) and 0.42 ± 0.09 % (n = 2) of the total As, respectively), as well as DMA (approximately 1.6 ± 1.0 % (n = 3) of the total As). Arsenic species such as TMAO, TETRA and MA were present in some of the diets but data is not shown as the values were below LOQ. In aquatic animals, AsC is a metabolic precursor for AsB, but it is observed in much lower concentrations when compared to AsB [37]. The same trend is seen in our samples, were the concentration of AsB is much higher than AsC. Other methylated As species exist as minor components in most seafood where DMA is usually the most common [15]. In our study, this was also the case, as the concentration of DMA was higher than TMAP, TMAO, TETRA or MA. The presence of TMAP was reported in crustaceans and some fish species [38,39]. The concentration of TMAP reported in both studies is comparable with the concentration determined in this work, except for the crab samples reported by Wolle and colleagues which have shown elevated concentrations (0.1–0.8 mg kg^{-1}).

Five metalloproteins containing As were detected in seaweed (*Fucus vesiculosus*) [40]. In blue mussel, a residual fraction of As was identified as cysteine-rich peptides [41]. With the growing interest on metallomics, more arsenic-binding proteins are expected to be identified under biological conditions. Unfortunately, there is not much information about the metabolic fate of As compounds which are protein-bounded [42]. This is considered a knowledge gap and speciation studies will benefit from more knowledge in this area.

3.2. Intestinal uptake and arsenic species levels and retention in liver and fillet

As shown in the previous section, several As species were found to be present in different concentrations in the experimental diets (Fig. 1 A). In this section, data regarding the intestinal uptake, levels and retention of As species in liver and fillet, and As retention rates of the different As species in fillet and liver will be described (Table 2 and Fig. 1B). Concentrations of yttrium, arsenic and arsenic species in feaces, fillet and liver is presented in Tables S7, 3 and 4, respectively.

3.2.1. Total arsenic

The intestine was considered the major tissue for As absorption and metabolism in marine medaka (*Oryzias melastigma*) [43]. In addition, dietary absorption by the intestine has been indicated as the main pathway for As bioaccumulation in fish [44,45].

The intestinal uptake of total As ranged from 67 % to 83 % in Atlantic

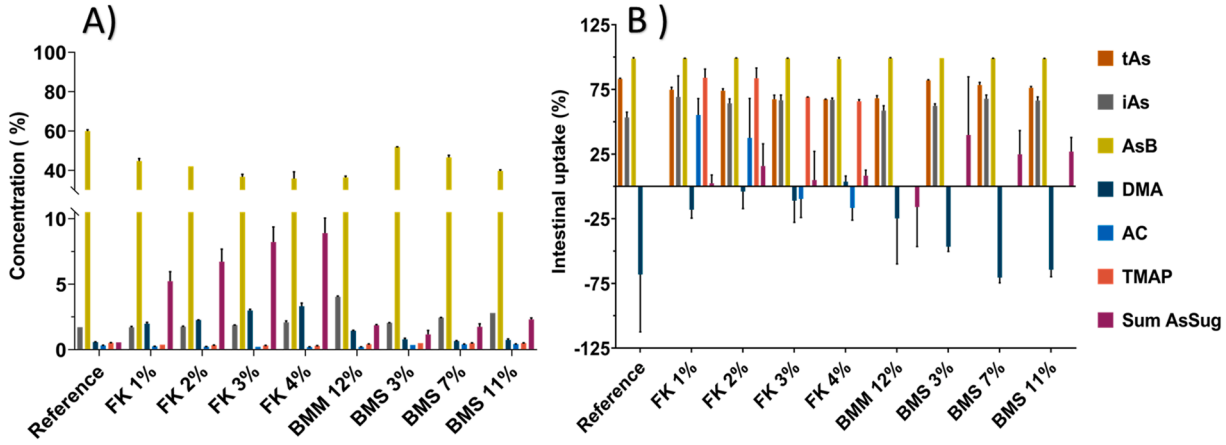

Fig. 1. A) Proportions of the different arsenic species in experimental diets containing fermented kelp (FK), blue mussel meal (BMM) or blue mussel silage (BMS); The concentration (%) of each compound was calculated based on the amount of the different arsenic specie compared to the total arsenic determined in each feed sample. B) Intestinal uptake of total arsenic (tAs), inorganic arsenic (iAs), arsenobetaine (AsB), dimethylarsinic acid (DMA), arsenocholine (AsC), trimethylarsonio propionate (TMAP) and sum of arsenosugars 328, 392, and 482 (sum AsSug) in Atlantic salmon fed experimental diets; The intestinal uptake (%) was determined using a ratio between the concentration of arsenic in diet and in faeces and the concentration of an inert marker (i.e. yttrium oxide, which is not transferred over the intestine) in diet and faeces (see Eq. 1).

Table 2
Retention (%) of several arsenic compounds found in fillet and liver of Atlantic salmon fed kelp and blue mussel-based diets: FK = fermented kelp; BMM = blue mussel meal; BMS = blue mussel silage; tAs = total arsenic; AsB = arsenobetaine; AsSug 328 = arsenosugar 328; DMA = dimethylarsinic acid; TMAP = trimethylarsoniopropionate. In fillet samples, arsenocholine (AsC) and trimethylarsine oxide (TMAO) were <LOQ. In liver samples, DMA, TMAO, AsC were <LOQ and TMAP was below <LOD. The retention (%) is expressed as the ratio of the difference between the final and initial As concentrations in liver/fillet, and the concentration of As in diet and feed intake (see Eqs. 2 and 3). Values sharing the same superscript are not significantly different from each other.

Retention in fillet (%)																				
Diet name	**tAs**				**AsB**				**AsSug 328**				**DMA**				**TMAP**			
Reference feed	22	±	7	a	33	±	8	ab	23	±	2	a	30	±	11	a	9	±	4	a
FK 1 %	20	±	5	a	39	±	13	a	5	±	1	cd	9	±	1	b	13	±	3	a
FK 2 %	16	±	6	ab	32	±	11	ab	3	±	0	cd	8	±	2	b	14	±	4	a
FK 3 %	11	±	5	ab	26	±	15	ab	2	±	1	d	5	±	1	b	13	±	3	a
FK 4 %	8	±	6	ab	18	±	13	ab	2	±	1	d	4	±	2	b	10	±	6	a
BMM 12 %	10	±	8	ab	24	±	23	ab	10	±	5	bc	17	±	10	ab	7	±	3	a
BMS 3 %	13	±	5	ab	23	±	8	ab	13	±	2	b	19	±	3	ab	13	±	4	a
BMS 7 %	7	±	6	ab	9	±	12	ab	8	±	3	bcd	14	±	6	ab	9	±	6	a
BMS 11 %	2	±	4	b	0.3	±	6.9	b	4	±	0	cd	9	±	2	b	7	±	3	a
Retention in liver (%)																				
Diet name	**tAs**				**AsB**				**AsSug 328**											
Reference feed	0.6	±	0.2	a	0.8	±	0.4	a	1.3	±	0.39	a								
FK 1 %	0.5	±	0.2	a	1.0	±	0.3	a	0.2	±	0.04	b								
FK 2 %	0.5	±	0.1	a	1.1	±	0.2	a	0.2	±	0.05	b								
FK 3 %	0.4	±	0.1	a	0.7	±	0.4	a	0.1	±	0.03	b								
FK 4 %	0.3	±	0.2	a	0.6	±	0.5	a	0.1	±	0.03	b								
BMM 12 %	0.3	±	0.2	a	0.4	±	0.5	a	0.4	±	0.18	b								
BMS 3 %	0.6	±	0.1	a	0.9	±	0.4	a	0.6	±	0.10	b								
BMS 7 %	0.6	±	0.2	a	0.8	±	0.9	a	0.4	±	0.31	b								
BMS 11 %	0.4	±	0.1	a	0.6	±	0.4	a	0.3	±	0.19	b								

salmon given feeds containing both sugar kelp and blue mussel. However, the retention in tissues was considerably lower, ranging from only 2–22 % in the fillet and in liver from 0.3 % to 0.6 %. This shows that even though a considerable amount of As is crossing the intestinal barrier, much less is retained in fish fillet or liver, no matter the dietary source. This is in agreement with data from other studies as described shortly after. For instance, a previous study on quantification and feed-to-food transfer of total and iAs was studied on livestock fed on brown seaweed (*Ascophyllum nodosum*) based feed, suggested that brown seaweed-based feed does not contribute considerably to the final As concentration in chicken meat [46]. Further, Vreman and co-workers performed two experiments to evaluate the transfer of potentially toxic elements (i.e. Cd, Pb, Hg and As) from feed into milk and various edible tissues of dairy cows [47]. None of the trials showed an increased concentration of Cd, Pb, Hg, and As in milk or blood. However, administration of soluble As (i.e. As_2O_3) resulted in higher concentrations of As in muscle samples. Few studies focusing on accumulation of As in different tissues of fish found that higher accumulation of As can occur in liver and gills when compared with muscle in tilapia (*Oreochromis spp*) [48] or other tissues of sea mullet (*Mugil cephalus*) [49]. In the present study, in general muscle had slightly higher total As levels than liver (Tables 3 and 4). The dominant As species in muscle was AsB (~>90 % of total As), which is the main organ retaining AsB thus explaining relative high As levels in fish muscle [50].

Metabolism encompasses all chemical reactions that take place within living organisms. These reactions take place in cells that transform one molecule into another that can be more or less complex than the initial molecule [51]. Arsenic is actively metabolised in fish liver, and it can therefore also be accumulated there. However, in this case, a higher retention rate was seen in muscle when compared to liver. This can be related to differences in the metabolism of As compounds in different fish species [52,53]. Another hypothesis can be the fact that

Table 3
Concentrations of total arsenic (tAs), inorganic arsenic (iAs), arsenobetaine (AsB), trimethylarsine oxide (TMAO), trimethylarsonio propionate (TMAP), arsenocholine (AsC), tetramethyl arsonium ion (TETRA), dimethylarsinic acid (DMA), methylarsonite (MA) and three arsenosugar species (arsenosugar-328, −392, and −482) in fillet from fish fed experimental diets containing fermented kelp (FK), blue mussel meal (BMM) or blue mussel silage (BMS).

	(mg/kg ww)	tAs	iAs	AsB	TMAO	TMAP	AsC	TETRA	DMA	MA	AsSug 328	AsSug 482	AsSug 392
Starting fish	Average	2.27	<LOQ	2.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Std. Dev.	0.06		0.1									
Reference feed	Average	1.63	<LOQ	1.539	<LOQ	0.0026	<LOQ	<LOQ	0.008	<LOQ	0.0044	<LOQ	<LOQ
	Std. Dev.	0.06		0.007		0.0006			0.002		0.0001		
FK 1 %	Average	1.57	<LOQ	1.46	<LOQ	0.002	<LOQ	<LOQ	0.0079	<LOQ	0.0045	<LOQ	<LOQ
	Std. Dev.	0.06		0.02		0.00005			0.0007		0.0003		
FK 2 %	Average	1.47	<LOQ	1.36	<LOQ	0.0023	<LOQ	<LOQ	0.0083	<LOQ	0.0038	<LOQ	<LOQ
	Std. Dev.	0.06		0.02		0.0004			0.0007		0.0002		
FK 3 %	Average	1.4	<LOQ	1.31	<LOQ	0.002	<LOQ	<LOQ	0.0076	<LOQ	0.00383	<LOQ	<LOQ
	Std. Dev.	0.0		0.06		0.0004			0.0007		0.00002		
FK 4 %	Average	1.4	<LOQ	1.4	<LOQ	0.00213	<LOQ	<LOQ	0.0081	<LOQ	0.0036	<LOQ	<LOQ
	Std. Dev.	0.2		0.2		0.00005			0.0007		0.0010		
BMM 12 %	Average	1.3	<LOQ	1.2	<LOQ	0.0020	<LOQ	<LOQ	0.0093	<LOQ	0.0030	<LOQ	<LOQ
	Std. Dev.	0.1		0.1		0.0004			0.0008		0.0002		
BMS 3 %	Average	1.43	<LOQ	1.37	<LOQ	0.003	<LOQ	<LOQ	0.0070	<LOQ	0.0038	<LOQ	<LOQ
	Std. Dev.	0.06		0.04		0.0001			0.0007		0.0004		
BMS 7 %	Average	1.37	<LOQ	1.21	<LOQ	0.003	<LOQ	<LOQ	0.0062	<LOQ	0.0038	<LOQ	<LOQ
	Std. Dev.	0.06		0.03		0.0007			0.0005		0.0006		
BMS 11 %	Average	1.37	<LOQ	1.24	<LOQ	0.003	<LOQ	<LOQ	0.0065	<LOQ	0.0029	<LOQ	<LOQ
	Std. Dev.	0.06		0.02		0.0006			0.0006		0.0002		

< LOQ = below limit of quantification.

Table 4
Concentrations of total arsenic (tAs), inorganic arsenic (iAs), arsenobetaine (AsB), trimethylarsine oxide (TMAO), trimethylarsonio propionate (TMAP), arsenocholine (AsC), tetramethyl arsonium ion (TETRA), dimethylarsinic acid (DMA), methylarsonite (MA) and three arsenosugar species (arsenosugar-328, −392, and −482) in liver from fish fed experimental diets containing fermented kelp (FK), blue mussel meal (BMM) or blue mussel silage (BMS).

	(mg/kg ww)	tAs	iAs	AsB	TMAO	TMAP	AsC	TETRA	DMA	MA	AsSug 328	AsSug 482	AsSug 392
Starting fish	Average	1.07	<LOQ	1.04	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Std. Dev.	0.06		0.07									
Reference feed	Average	1.43	<LOQ	1.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.012	<LOQ	<LOQ
	Std. Dev.	0.06		0.2							0.002		
FK 1 %	Average	1.3	<LOQ	1.20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.011	<LOQ	<LOQ
	Std. Dev.	0.1		0.04							0.002		
FK 2 %	Average	1.33	<LOQ	1.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.013	<LOQ	<LOQ
	Std. Dev.	0.06		0.06							0.002		
FK 3 %	Average	1.33	<LOQ	1.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.012	<LOQ	<LOQ
	Std. Dev.	0.06		0.3							(n = 1)		
FK 4 %	Average	1.3	<LOQ	1.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.009	<LOQ	<LOQ
	Std. Dev.	0.2		0.2							0.001		
BMM 12 %	Average	0.96	<LOQ	0.67	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.010	<LOQ	<LOQ
	Std. Dev.	0.04		0.08							(n = 1)		
BMS 3 %	Average	1.5	<LOQ	1.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0090	<LOQ	<LOQ
	Std. Dev.	0.1		0.3							0.0009		
BMS 7 %	Average	1.67	<LOQ	1.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.010	<LOQ	<LOQ
	Std. Dev.	0.06		0.5							0.004		
BMS 11 %	Average	1.4	<LOQ	1.0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.011	<LOQ	<LOQ
	Std. Dev.	0.0		0.4							0.003		

< LOQ = below limit of quantification.

previous studies have included iAs salts or organic As [54] in their feeding trials whereas in this study, the experimental diets contained As naturally occurring in the feed ingredients. As can be seen in Fig. 1B, the intestinal uptake of total As was higher in fish fed feeds containing blue mussel silage when comparing with fish fed feeds containing fermented kelp ($p = 0.03$). Conversely, fish fed fermented kelp-based feeds had higher retained concentrations of total As when comparing with fish fed feeds containing blue mussel silage. The retention of As in fish tissues decreased with increased inclusion levels of both kelp or blue mussel-based feed ingredients (Table 2). A study regarding As bio-accessibility observed that As bioaccessibility was not affected by the fibre content but it was affected by the presence of fat and bile salts [55]. Therefore, the differences seen in our study might be related with the use of different marine low trophic ingredients. The assumed reduced As bioaccessibility with increased used of the LTM ingredient caused total fillet As levels to rather decrease than increase with higher inclusion levels of for example fermented kelp (range 1.4–1.57 mg kg^{-1} ww total As in fish fed 1–4 % FK, respectively) (Table 3), although the total As levels in feed increase with increased use of fermented kelp (4.1–4.6 mg kg^{-1} total As in feed with 1–4 % FK, respectively) (Table 1). In fact, although all LTM feeds had higher total As levels than the reference feed (Table 1), the muscle total As level in all fish fed low marine trophic feed resources had lower total As levels compared to fish fed the reference feed (Table 3).

3.2.2. *Arsenic speciation*

For iAs, the intestinal uptake ranged from 54 % to 69 %, but iAs was not retained in fillet nor liver as no quantifiable levels were found (Table 1 and Fig. 1B). This suggested that iAs was either transformed into other As species or eliminated from the body after intestinal

absorption. The renal pathway is the primary elimination route in marine organisms [56]. A study has given focus on the biotransformation of dietary iAs in crucian carp (*Carassius auratus*) [57]. The previous study conducted a dietary exposure trial using iAs (i.e As(III) and As(V)). At the end of exposure trial, the levels of total As in fish muscle were similar between the As(III) and As(V) groups. However, Cui and colleagues noted an increase in the proportions of AsB in the fish body after iAs dietary exposure, suggesting biotransformation of iAs. In our study, we did not see an increase of retained AsB when fish was fed diets with higher concentrations of iAs arising from increased inclusion of the ingredients. The reason can be due to the levels present in our experimental diets being much lower compared to the concentration in experimental diets from Cui and co-workers' study (50 and 100 mg kg^{-1}). The toxic form of arsenic, iAs, is typically found in marine organisms in amounts less than 1 % of the total As [22]. Thus, most marine organisms, including fish, bivalves, and crustaceans, predominantly contain As in the form of organic species, with the relatively non-toxic chemical species AsB being the prevalent form. In the present trial, AsB contributed to around 35–44 % of the total in the kelp diets (Table 1), however in salmon muscle AsB contributed to around 93–99 % of total muscle As levels (Table 3). Other minor organic As species such as the AsSugs in kelp feed contributing to 5–8 % of kelp made feed (Table 1), however besides AsSug328, these were not detected in salmon muscle fed on fermented kelp (Table 3). Relatively increase in total AsB levels from feed-to-fillet and relatively decreased AsSug levels is reflected by the high muscle retention for AsB and lower retention for AsSugs (Table 2).

The influence of seawater adaptation on intestinal uptake and muscle accumulation of AsB was investigated in Atlantic salmon (*Salmo salar* L.) [58]. After receiving a single oral dose of AsB, fish in freshwater (FW) and seawater (SW) were observed after 6 h. It was found that Atlantic salmon in SW had significantly higher levels of accumulated As in blood compared to FW salmon. However, the adaptation to seawater did not affect the levels of As accumulated in the fillet. The distribution and excretion of AsB in two marine fish species was performed by administration of a single oral dose of C14-labeled AsB [50]. Disposition of AsB in Atlantic salmon (*Salmo salar L.*) and Atlantic cod (*Gadus morhua L.*) was different. Arsenobetaine was highest in Atlantic salmon muscle tissue, whereas both Atlantic cod muscle and liver showed high levels of AsB. The findings indicate that urine was the primary excretion pathway, which was more significant in Atlantic cod than in Atlantic salmon. Zhang and colleagues have done a study on biotransportation of As in Marine Medaka (*Oryzias melastigma*) [43]. It was suggested that AsB was found to have a greater tendency to accumulate in tissues rather than being eliminated, whereas inorganic and methylated As were observed to be more readily transferred from tissues to the bloodstream for elimination. Our results showed similar information, where AsB was the As species with higher retention rates in fillet when compared to AsSug-328, DMA and TMAP (Table 1), leading to AsB as the main As species salmon tissue, and muscle in particular.

In addition, iAs was not retained in tissues even though it was found present in feed and it crossed the intestinal barrier. In a study conducted by Lescord and co-workers, across the 300 freshwater fish analysed herein, inorganic As species (i.e. As (III) nor As(V)) were not detected in any fish; only AsB and DMA were detected in muscle samples [59]. Data regarding retention of AsB, AsSug-328, DMA and TMAP is available in Table 2. For AsB, the intestinal uptake was approximately 99 %, but retention in fillet ranged from 0.3 % to 39 % and in liver from 0.4 % to 1.1 % (Table 2 and Fig. 1B). In addition, intestinal uptake was evaluated for AsB, DMA, AsC, TMAP and sum of arsenosugars (Fig. 1B). Some are reported as negative values (e.g. DMA and AsC) and this is due to having a higher amount of a certain arsenic species in feaces than in feed. In humans, arsenosugars and arsenolipids are metabolized to several species including DMA [15]. Thus, this can explain the presence of more DMA in the faeces.

Salmon fillet is the most consumed portion of fish and comprises ~75 % the body weight of fish (Norwegian Seafood Council, 2022). Salmon is among the top five species consumed in the EU [60], showing the relevance of this study. Different studies have shown that the speciation of arsenic can change based on the cooking method used [61–64]. Salmon can be consumed both raw and cooked. Thus, future food regulations for arsenic should consider this. In Australia and New Zealand, there is a mL for iAs in seaweed and molluscs (1 mg kg^{-1}), and in fish and crustaceans (2 mg kg^{-1}) (Food Standards Australia and New Zealand, FSANZ). Currently, there are no MLs of As in seafood at EU level but most likely MLs will be set in a near future.

A description of the presence of AsLipids in these samples was not in focus at this time, but it is considered highly relevant. In this study, we have included feeds where kelp and blue mussel were included. Both in kelp, blue mussel and fatty fish like salmon, lipid-soluble As species are likely present [64–66], and it would hence be relevant to assess the transfer of these As species. Recent studies based on *in vitro* experiments using human cell lines (i.e. human bladder, liver and brain cells) and model animals (i.e. fruit flies (*Drosophila melanogaster*) and nematodes (*Caenorhabditis elegans*)) have reported AsLipids as potentially toxic compounds, with studies citing neurotoxic and cytotoxic effects [67–70]. Thus, studying AsLipids is topic of relevance, both in terms of method development and generating toxicological data.

3.2.3. Arsenic speciation at the fish gastro-intestinal tract

An important aspect to take into consideration is the fact that the chemical form of As may change during passage of the gastro-intestinal tract intestine. Thus, regardless of the chemical form by which As is ingested, its absorption will depend on the solubility and chemical form at the point of contact with the absorbing membranes [16]. Weerasinghe et *al.* have demonstrated that the *in vivo* availability of phosphorus in different feed ingredients is strongly correlated with their soluble phosphorus content [71]. This indicates a positive relationship between the aqueous solubility of a chemical compound and its availability. It is possible to predict solubility of the inorganic As species in aqueous conditions by using Visual MINTEQ [72]. At pH 8 and 15 °C, in a solution of As(III) (0.1 ug/L), arsenous acid (H_3AsO_3, 93.7 %) and conjugate base of arsenous acid ($H_2AsO_3^-$, 6.3 %) will be present in solution. Moreover, in the same conditions, in a solution of As(V), arsenate ion (AsO_4^{-3}, 0.01 %), hydrogen arsenate ($HAsO_4^{-2}$, 90.75 %) dihydrogen arsenate (H_2AsO^{4-}, 9.24 %) will be present in solution. Unfortunately, it is not possible to make predictions using organic As compounds. Furthermore, the absorption of As through the intestinal epithelium can also be affected by compounds that either inhibit or enhance its uptake at the site of contact with the absorbing membranes [73]. More research is needed to fully understand the events that take place during the passage of As through the gastrointestinal tract.

4. Conclusions

The inclusion of kelp or blue mussel-derived feed ingredients raised the overall levels of total As in the feed, yet did not surpass the maximum limits. The arsenic composition in the feed was dependent on the ingredient, with AsB being the primary species in all feed samples, while kelp-based feed had greater amounts of arsenosugars. The use of novel marine feed ingredients requires further monitoring since their inclusion resulted in varying As species distribution, which was dependent on the type of material added as feed component. Fish fed blue mussel silage-based feed had higher intestinal uptake of total As when comparing with fish fed fermented kelp-based feed. In contrast total As was more retained in fish fed fermented kelp-based feeds when comparing fish fed blue mussel silage-based feeds. Despite relatively high intestinal uptake of arsenic, the retained concentrations of As did not reflect the same trend. The findings of this study indicate that speciation analysis is a valuable tool for understanding the transfer of As from feed to fish, as differences in As speciation in the diet can result in variations in the As speciation profiles in fish tissues. Despite LTM ingredients will increase

total As and As species such as AsB and AsSug in salmon feeds, the total muscle As levels did not increase in salmon fed on these diets. The lack of As transfer from LTM feeds to the fillet of farmed fish is likely due to a decreased bioaccessibility and/or low As species specific muscle retention.

CRediT authorship contribution statement

Marta S Silva: Conceptualization; Data curation; Formal analysis; Methodology; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. **Jojo Tibon:** Formal analysis; Methodology; Validation; Roles/Writing - original draft; Writing - review & editing. **Sahar Sartipiyarahmadi:** Data curation; Methodology; Writing - review & editing. **Sofie C. Remø:** Conceptualization; Resources; Funding acquisition; Methodology; Writing - review & editing. **Veronika Sele:** Methodology; Writing - review & editing. **Liv Søfteland:** Project administration; Writing - review & editing. **Harald Sveier:** Conceptualization; Resources; Writing - review & editing. **Martin Wiech:** Conceptualization; Resources; Project administration; Writing - review & editing. **Antony J. Prabhu Philip:** Conceptualization; Funding acquisition; Project administration; Writing - review & editing. **Marc Berntssen:** Conceptualization; Funding acquisition; Project administration; Roles/Writing - original draft; Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no competing financial interests that could have influenced the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtemb.2023.127319.

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