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Microalgae suspension as a source of n-3 long-chain PUFA in feed for Atlantic Salmon (*Salmo salar* L) – Technical constraints and nutritional quality

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ABSTRACT

The aim of this research was to study possible inclusion levels of AlgaPrime™ DHA LS microalgae (*Schizochytrium* sp.) plant oil suspension in feed mixes prior to extrusion and in the post drying vacuum coating step and evaluate microalgae digestibility in Atlantic salmon (*Salmo salar* L). In the technical evaluation, physical quality, expansion, and microstructure parameters were studied for AlgaPrime™ DHA LS inclusion in fishmeal and plant-based diets prior to extrusion. A target lipid level of 361 g/kg DM was achieved for all feeds. High physical quality and water stability were measured and mixture design models with R^2 in the range of 0.950 to 0.999 ($P <$ 0.001) were established for specific mechanical energy (SME), apparent dough viscosity in the extruder die (V_{die}), expansion, and microstructure parameters. Addition of AlgaPrime™ DHA LS resulted in a feed mix lipid level in the range of 27 to 204 g/kg and was the main contributor for the observed variation in this study. Commercially, the most interesting feed was the centroid point with a feed mix lipid content of 92 g/kg. This equals an AlgaPrime™ DHA LS content of 48 g/kg in the final feed. Alternatively, the AlgaPrime™ DHA LS can be added in the vacuum coating process. In this study the pellet pores size distribution allowed high amount of microalgae suspension to be absorbed (50% of total oil) with no significant impact on oil leakage. Based on the findings in the technical trial, 4 diets were produced for the fish experiment; 1) control feed with a 1:1 fish oil and rapeseed oil mixture, 2) a feed where the oil mixture was partly replaced by 100 g/kg AlgaPrime™ DHA LS prior to extrusion, 3) a feed where 100 g/kg of oil mixture were replaced in the vacuum coating step by 100 g/kg AlgaPrime™ DHA LS and 4) where 100 g/kg oil mixture was replaced both prior to extrusion and in the vacuum coating step by AlgaPrime™ DHA LS. All diets showed high nutrient digestibility in Atlantic salmon, also for the saturated fatty acids. This documents no need for extra cell wall rupture for the microalgae suspension prior to extrusion or vacuum coating. Lower content of tri-saturated triacylglycerols than reported in previous *Schizochytrium* biomasses have limited the negative effects on the digestibility of saturated fatty acids in Atlantic salmon.

1. Introduction

The lipid-rich microalgae biomass from heterotrophic *Schizochytrium* sp. is a novel ingredient developed to be an important source of long chain omega-3 polyunsaturated fatty acids in animal feeds (Hakim, 2012; Madeira et al., 2017). The microalgae biomass has traditionally been included in the feed mix prior to extrusion, substituting up to 100% fish oil in Atlantic salmon (*Salmo salar*) diets (Carter et al., 2003; Kousoulaki et al., 2015; Kousoulaki et al., 2016; Miller et al., 2007). However, recent developments and innovations have resulted in a microalgae suspension that can be added the dried feed pellets in a vacuum coating step which ease applicability in logistics and use by aquafeed manufactures. This can be extracted microalgae oil or microalgae biomass in a co-product with an oil.

The availability of nutrients with optimal digestibility in fish has been a challenge for microalgae producers (Annamalai et al., 2021). Nutrients in the microalgae cells must be available for the fish and therefore processing steps during the production of ingredients or feeds that disrupt the cells are crucial for optimal nutrient digestibility. The thermomechanical treatment in the extrusion process has previously been shown to be adequate for optimal digestibility of *Schizochytrium* sp. biomass in feeds for salmon (Kousoulaki et al., 2015). Furthermore, the

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microalgae biomass has high content of docosahexaenoic acid (DHA) and saturated fatty acids. Kousoulaki et al. (2016) documented lower digestibility of these saturated fatty acids in Atlantic salmon, and it was later assumed that this was caused by a high content of tri-saturated glycerides, particularly tripalmitin (Bogevik et al., 2018). Product development to ensure optimal nutrient availability for the fish is therefore crucial for algae products developed for the use in a vacuum coating operation.

Fish feed extrusion is based on thermomechanical processing of moistened feed ingredients. The process involves starch gelatinization, protein plasticization and texturization to durable and expanded feeds which is dried and added oil in a vacuum coating operation. High physical feed quality is important to avoid crushing and feed loss in the transport chain and pneumatic feeding to the sea cages and is normally controlled by the addition of starch and the adjustment of water, steam, and mechanical energy (Blanche and Sun, 2004; Sørensen et al., 2010; Aarseth et al., 2006; Aas et al., 2011). However, protein and lipid rich ingredients also impact the process and physical feed quality. Soy protein concentrate (SPC) requires higher water content and increased temperature and mechanical shearing to produce durable pellet compared to fishmeal (FM) and wheat gluten (Draganovic et al., 2011; Draganovic et al., 2013; Samuelsen et al., 2013, 2014; Samuelsen et al., 2018; Sørensen et al., 2009). The FM technical quality is dependent on the content of water-soluble protein where, due to its plasticizing effect, pellet hardness and durability can be improved with increased level in FM (Samuelsen and Oterhals, 2016). The new and sustainable tunicate meal has shown higher pellet expansion and larger pores compared to SPC due to lower viscosity in the extruder barrel at same temperature (Samuelsen et al., 2022). Oil-rich microalgae powder has previously been shown to lubricate the extruder screws and give dense pellet with poor physical quality at high inclusion levels above 13% in the feed mix (Samuelsen et al., 2018). However, when SPC was replaced by microalgae powder, a positive lubrication effect with increased physical pellet quality was observed up to 13% microalgae inclusion in the feed mix. This corresponded well to the maximum recommended oil level of 12% considered having little or no effect on feed quality (Rokey, 1994). Therefore, for high energy salmon feeds, due to this limitation, most of the oil must be added in a vacuum coating operation after drying (Strauch, 2005). Microalgae can alternatively be added in this step, when in a liquid form (fine milled microalgae in oil suspension or extracted oil). Liquid viscosity, degree of pellet expansion and the microstructure parameters such as open porosity and pore size distribution will define the microalgae addition level and how well it is adsorbed and retained in the feed (Dethlefsen, 2017; Draganovic et al., 2013; Samuelsen et al., 2022). The latter is important to prevent oil leakage from feed pellets, which will give challenges in the transport chain and feeding due to blocking of silos and pipes and in addition change the nutritional composition of the feed.

The objectives of this study were to (1) quantify the effect of microalgae suspension inclusion levels in fishmeal (FM) and soy protein concentrate (SPC) based diets on the extrusion process, physical pellet quality, expansion parameters and microstructure; (2) assess the maximum microalgae suspension level possible to use in the vacuum coating process; (3) based on the results from (1) and (2), produce experimental feeds with defined levels of microalgae suspension added in the feed mix or in the coating step and (4) evaluate the effects of algae suspension inclusion in the feed mix or in the coating step on dietary lipid digestibility in salmon.

2. Material and methods

2.1. Microalgae suspension

The microalgae suspension (AlgaPrime™ DHA LS) was provided by Corbion (Amsterdam, The Netherlands). The AlgaPrime™ DHA LS (AlgaPrime) is a dried and fine milled microalgae (*Schizochytrium* sp.) biomass suspended in rapeseed oil. The particle size distribution of the tested product is given by the supplier to be Dv(90) <200 μm and Dv(50) <50 μm. The chemical composition of AlgaPrime is presented in Table 1. AlgaPrime is a high-lipid oil suspension (863 g/kg crude lipid). The AlgaPrime lipid structures are mainly triacylglycerols (TAG; 854 g/kg), thereof a high content of isomers with unsaturated fatty acids with 5–10 double bounds (483 g/kg). The content of tri-saturated TAGs (i.e., tripalmitin) is barely detected compared to what is previously analyzed in *Schizochytrium* biomasses (Bogevik et al., 2018). The content of palmitic acids is however high in this product (120 g/kg), and thus esterified in TAG isomers with unsaturated fatty acids (Table 1).

2.2. Diet formulation for the technical extrusion trial

For the technical extrusion trial, 7 feed mixes were prepared by replacing AlgaPrime with either FM and/or SPC (Table 2). The level of AlgaPrime, FM and SPC was predetermined by the chosen range in a three-component mixture design (Table 3).

2.3. Diet formulation for the fish experiment

Based on the results from the technical extrusion trial, 4 diets for a fish experiment were prepared. Feed mix wheat flour and the added fish oil/rapeseed oil mix (1:1) during vacuum coating were partly substituted with AlgaPrime, either at 100 g/kg inclusion in the feed mix (Algablend), 100 g/kg in the coating step (Algacoat) or both 100 g/kg in the feed mix and 100 g/kg in the coating step (Algamix) (Table 4). The remaining ingredients in the diets for both the technical trial and fish experiment are commonly used by the Norwegian salmon feed industry (Aas et al., 2022).

2.4. Pilot extrusion process

All experimental diets were produced at Nofima's innovation facility, the Aquafeed Technology Centre (Bergen, Norway). The diets, calibrated to a production rate of 150 kg/h, were conditioned in an atmospheric double differential preconditioner (Wenger Manufacturing Inc., Sabetha, KS, USA) prior to extrusion on a TX-52 co-rotating, fully intermeshing twin-screw extruder (Wenger). For the technical trial, standardized conditions were used giving a preconditioner outlet temperature of 94 ± 2.2 °C and a moisture content of 187 ± 4.2 g/kg and 255 ± 3.4 g/kg (wet basis) in the preconditioner and extruder, respectively. For the fish experiment the preconditioner and extruder settings were adjusted to produce pellets with the same expansion and oil adsorption capacity. The extruder screws were adapted to produce high energy salmon feed (Samuelsen and Oterhals, 2016) and the outlet restricted by two circular 6.1 mm dies for the technical trial and nine circular 3.2 mm dies for the fish experiment. The wet extrudates were cut at the die surface at constant knife speed for the technical trial and to an equal length for the fish experiment. Sampling was conducted after the achievement of steady state conditions in the preconditioner and extruder. For the technical trial, duplicate runs were performed for four formulations (Table 3) separated by a 5-min interval between the first and second sampling. Specific mechanical energy (SME) was calculated based on the measured extruder torque (kW) and wet flow rate (kg/h) as described in Samuelsen et al., 2018. Apparent viscosity of the feed dough in the extruder die (V_{die}) was calculated by use of the die diameter (6.1 mm) and length (6.3 mm) and the measured pressure and the volume flow rate as described by Samuelsen et al., 2018. All extrudates were dried in horizontal conveyor belt dryer with 2 drying chambers (Mitchell Dryers, CAD Works Engineering LTD, Carlisle, UK) at constant temperature of 95 and 90 °C for the technical trial (chamber 1 and 2 respectively) and 10 °C higher in each of the chambers for the fish trial. The dried pellets were added oil in a Pegasus® vacuum coater (PG-10 VC Lab, Dinnissen BV, Sevenum, Netherlands) to a lipid content of 361 ± 2 g/kg DM for the technical trial and 324 ± 5 g/kg DM for the fish

Table 1
Chemical composition of AlgaPrime™ DHA LS (g/kg DM).

Proximate		Fatty acids		Lipid classes		TAG isomers	
DM	999	16:0	120	TAG	854	SUM tri-saturated	13
In DM		18:0	11	FFA	8	SUM unsaturated 1–4 double bonds	142
Crude protein	61	18:1	141	Cholesterol	<1	SUM unsaturated 5–10 double bonds	483
Crude lipid	863	18:2n-6	41	PC	<1	SUM unsaturated >10 double bonds	96
Total ash	38	18:3n-3	22	PE	<1		
Carbohydrate[1]	28	20:5n-3 (EPA)	2	PI	<1		
		22:6n-3 (DHA)	332	PS	<1		

FFA = free fatty acids; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PI = phosphatidylinositol; PS = phosphatidylserine; TAG = triacylglycerol; SUM saturated TAG isomers includes 1.65 g/kg TG(48:0) = tripalmitin. TAG isomers with unsaturated fatty acids may also contain 1–2 saturated fatty acids.
[1] Calculated.

Table 2
Formulation and proximate chemical composition of the experimental feed mixes fed to the extruder system for the technical trial (g/kg as is)[1].

	EX1	EX2	EX3	EX4	EX5	EX6	EX7
AlgaPrime™ DHA LS[2]	191	0	0	96	96	0	64
Fishmeal[3]	0	206	0	103	0	103	69
Soy protein concentrate[4]	0	0	199	0	99	99	66
Wheat gluten[5]	245	245	245	245	245	245	245
Whole wheat flour[6]	78	78	78	78	78	78	78
Corn gluten[7]	74	74	74	74	74	74	74
Horsebeans[8]	148	148	148	148	148	148	148
Soy protein concentrate[9]	199	199	199	199	199	199	199
Mineral mixture[10]	7	7	7	7	7	7	7
Vitamin mixture[10]	8	8	8	8	8	8	8
Monosodium phosphate[10]	36	36	36	36	36	36	36
Water[11]	15	0	7	7	11	4	7
Chemical composition[12]							
Crude protein	464	606	586	535	525	596	552
Crude lipid	204	45	27	124	115	36	92
Total ash	78	107	84	93	81	96	90
Carbohydrate	257	251	306	254	281	278	271

[1] Proximate chemical composition calculated for diets at a lipid level of 361 ± 2 g/kg DM: Crude Protein, 388 ± 11 g/kg DM; Starch 82 ± 6 g/kg DM; Total ash, 63 ± 5 g/kg.
[2] Provided by Corbion (Amsterdam, The Netherlands).
[3] Purchased from Pelagia (Egersund, Norway).
[4] Purchased from Agro Korn AS (Videbæk, Denmark).
[5] Purchased from Tereos Syral (Aals, Belgium).
[6] Bakery quality (falling number > 200 s), purchased from Norgesmøllene AS (Vaksdal, Norway).
[7] Purchased from Roquette (Lestrem, France).
[8] Purchased from Soufflet (Nogent-sur-Seine, France).
[9] Purchased from Agro Korn AS (Videbæk, Denmark). Constant based level in all feed mixes.
[10] Purchased from Vilomix (Hønefoss, Norway).
[11] All diets were adjusted to 80 g/kg water content.
[12] In g/kg DM. Calculated based on raw material analyses.

experiment.

The dried centroid feed (EX7; Tables 2 and 3) was used in the coating trial with AlgaPrime. Fish oil and AlgaPrime were heated to 60 °C and fish oil was replaced by increasing level of AlgaPrime prior to coating in the Pegasus® vacuum coater (PG-10 VC Lab). For each level, a 500 g pellet sample was coated with 194 g oil mixture, giving a total lipid content of approximate 361 g/kg DM in the final feeds. The following fish oil to AlgaPrime ratios were used: 1.00:0.00, 0.94:0.06, 0.90:0.10, 0.86:0.14, 0.80:0.20 and 0.50:0.50. The fish oil and AlgaPrime were homogeneously mixed prior to coating.

2.5. Fish experiment

The four feeds (Table 4) were given to triplicate groups of Atlantic salmon postsmolt (Benchmark Genetics strain, start weight of 294 ± 3 g) in tanks (2 m^3) at the Nofima Research Station for Sustainable Aquaculture (Sunndalsøra, Norway) in a period of 32 days. Each tank contained 45 postsmolts with a stocking density of 6.5 kg/m^2 at the start of the trial. The fish were reared at a temperature of 12 ± 2 °C, 32 ‰ salinity and oxygen saturation at 93 ± 3%. At termination the fecal contents of all fish in each tank were stripped from the end of the hindgut according to Austreng (1978). Yttrium (III) oxide was added in the feed mix to enable the calculation of the apparent digestibility coefficient (ADC) of nutrients and fatty acids from the formula:

$$\mathrm{ADC} = 100 - \left(100\,x\,\frac{\mathrm{Yd} \times \mathrm{Nf}}{\mathrm{Nd\ x\ Yf}}\right)$$

where d is diet, f is feces, Y is yttrium content and N is nutrient content.

2.6. Chemical analysis and oil viscosity

Chemical analyses were carried out by the accredited laboratory, Nofima BioLab. Dry matter (DM) was measured gravimetrically after drying at 103 ± 1 °C (ISO 6496). Crude protein (N x 6.25) was analyzed by the Kjeldahl method (ISO 5983-2). Ash was determined by combustion of organic matter at 550 °C and gravimetric measurement of the residue (ISO 5984). Total starch and degree of starch gelatinization were measured utilizing a modification of the glucoamylase methodology described by Chiang and Johnson (1977) and Samuelsen and Oterhals (2016). Total lipid in AlgaPrime, diets and fecal samples was quantified according to Bligh and Dyer (1959). Yttrium was determined by inductively couple plasma atomic emission spectroscopy (ISO 11885–1996). Fatty acid methyl esters (FAME) were prepared according to AOCS method Ce1b-89 by transesterification of Bligh & Dyer extracted oils from feed and feces with methanolic NaOH followed by methylation with boron trifluoride in methanol. The FAME solution was extracted and diluted with isooctane to approximately 50 μg/ml. The gas chromatography (GC) analyses were conducted in a Trace GC gas chromatograph (Thermo Fisher Scientific) with flame ionization detector (GC–FID). The FAMEs were identified by comparing the elution pattern and relative retention time with the reference FAME mixture (GLC-793, Nu-Chek Prep Inc., Elysian, MN, USA). Fatty acid composition was calculated by using 23:0 fatty acid methyl ester as an internal standard and reported on a sample basis as g/100 g fatty acid methyl esters. Lipid classes and triacylglycerol (TAG) isomers were quantified by the VTT Technical Research Centre of Finland Ltd. (Esbo, Finland) as described in Bogevik et al. (2018). All chemical measurements were based on averages of duplicate analyses. Viscosity profile for the fish oil, AlgaPrime suspension, and a rapeseed oil were measured on a rotational rheometer (RheolabQC, Anton Paar GmbH, Graz, Austria) using a concentric cylinder geometry (CC39) at 100 s^{-1} and a temperature ramp of 20, 30, 40 and 50 °C.

2.7. Pellet analysis

Hardness (peak breaking force) was measured on coated laying pellets by use of a texture analyzer (TA.XTplusC, Stable Micro Systems Ltd., Surrey, UK) as described in (Samuelsen and Oterhals, 2016). Pellets

Table 3
Pseudo and actual units for the mixture design variables, AlgaPrime™ DHA LS, fishmeal (FM) and soy protein concentrate (SPC) for the technical trial.

Feed mix	Eno	Pseudo units			AlgaPrime (g/kg DM)	FM (g/kg DM)	SPC (g/kg DM)
EX1–1	2a	1	0	0	206	0	0
EX1–2	2b	1	0	0	206	0	0
EX2–1	5a	0	1	0	0	206	0
EX2–2	5b	0	1	0	0	206	0
EX3	3	0	0	1	0	0	206
EX4–1	4a	0.5	0.5	0	103	103	0
EX4–2	4b	0.5	0.5	0	103	103	0
EX5–1	7a	0.5	0	0.5	103	0	103
EX5–2	7b	0.5	0	0.5	103	0	103
EX6	6	0	0.5	0.5	0	103	103
EX7	1	0.33	0.33	0.33	69	69	69

DM, dry matter; ENo, experiment number order; EX, experimental feed mix.

were treated individually and reported values based on the average of 20 analyses. Durability was measured in a Doris tester (AKVAsmart, Bryne, Norway) on a 350 g coated pellet sample as reported in Aas et al. (2011). Doris durability was based on the average of duplicate measurements and defined as percent of unbroken pellet larger than 8.0 mm. Bulk density was measured by loosely pouring the coated pellets from a funnel into a 1000 ml measuring cylinder. Oil leakage was measured on a 20 g coated pellet sample incubated at 40 °C for 24 h in a heating cabinet as described in Samuelsen et al. (2022). Pellet water stability index (WSI) was determined by placing a 20 g coated pellet sample in steel-mesh buckets inside a 1000 ml glass beaker filled with distilled water (500 ml). The beakers were incubated in a thermostat-controlled water bath (23 °C) and shaken (160/min) for 120 min, and the remaining amount of DM was determined (Samuelsen et al., 2022). The three latter analyses were based on the average of triplicates. Micro-CT scanning was preformed using a Skyscan 1275 X-ray microtomograph (Bruker micro-CT, Kontich, Belgium) on dried uncoated pellet for microstructure determination. The pixel size was set to 12 μm, voltage to 30 kV and current to 212 μA. The scans were reconstructed using NRecon (v. 1.7.3.1, Bruker micro-CT, Kontich, Belgium). The porosity calculations were performed using CTan (v. 1.17.7.2, Bruker micro-CT, Kontich, Belgium). The threshold was set using the Automatic Otsu function. Percent open porosity, mean cell size (MCS) and cell size-volumetric and cell wall thickness-volumetric distribution were calculated as described in Ahmad et al. (2019).

Table 4
Feed formulation and chemical composition of experimental feeds for the fish experiment.

Feed mix (g/kg as is)	Control	Algablend	Algacoat	Algamix
AlgaPrime™ DHA LS		100		100
Fishmeal	200	200	200	200
Soy protein concentrate	140	140	140	140
Wheat gluten	140	140	140	140
Whole wheat flour	122	102	102	82
Corn gluten	50	50	50	50
Horsebeans	55	55	55	55
Fish oil	10	–	10	–
Rapeseed oil	10	–	10	–
Mineral mix	5	5	5	5
Vitamin mix	5	5	5	5
Choline chloride	2	2	2	2
Carophyll Pink	0.5	0.5	0.5	0.5
Mono sodium phosphate	20	20	20	20
Yttrium (III) oxide	0.1	0.1	0.1	0.1
Oil to coating (g/kg as is)				
AlgaPrime™ DHA LS			100	100
Fish oil	120	90	80	50
Rapeseed oil	120	90	80	50
Feed composition (g/kg DM)				
Crude protein	433	438	431	431
Crude lipid	330	319	326	322
16:0	29	33	34	38
18:1[1]	111	89	91	73
20:1[2]	21	15	15	9
22:1[3]	28	20	21	12
18:2n-6	38	32	32	28
18:3n-3	14	12	12	10
20:5n-3 (EPA)	11	8	8	5
22:6n-3 (DHA)	13	43	46	75
Sum SFA	46	46	48	48
Sum MUFA	168	131	133	99
Sum n-6 PUFA	39	33	34	29
Sum n-3 PUFA	45	69	73	96
Sum PUFA	86	103	107	125

[1] (18:1(n-9) + (n-7) + (n-5)).
[2] (20:1(n-9) + (n-7)).
[3] (22:1(n-11) + (n-9) + (n-7)).

2.8. Experimental design for the technical trial

The experiment was based on a 3-component simplex-centroid mixture design by varying the content of AlgaPrime, FM, and SPC in the feed mixes (Table 2) and fit to a Scheffe special cubic polynomial model (Cornell, 2002). This represents a triangle with a total of 7 trials including three vertex points (pure blends), three edge points (binary blends), and a centroid point. Two vertex points and two edge points were replicated (Table 3). A range of 0–206 g/kg DM was varied for the three ingredients in the feed mixes. This corresponds to feed mix lipid levels of 27–204 g/kg DM and AlgaPrime levels of 0–164 g/kg DM in the final feeds. The rest of the feed mixes were held constant and with a base level of 199 g/kg SPC in all feed mixes (Table 2). This gave a SPC level normally used in feeds to Atlantic Salmon for the AlgaPrime and FM blends but higher than normally used for the pure SPC blend (398 g/kg; Aas et al., 2022). The experiments were run in random order (Table 3). The extruder, pellet and microstructure responses are given in Table 5.

2.9. Statistical analyses

Design-Expert v10 (Stat-Ease, Inc. Minneapolis, USA) was used for mixture design modelling based on pseudo scaled components for the technical trial. All basic statistics were carried out using STATISTICA v13.5 (StatSoft, Inc. Tulsa, USA). Dietary effects on fish performance and digestibility, and changes in fatty acids composition in the processing steps for the AlgaPrime pure blend were performed by one-way analysis of variance (ANOVA). Analyses by ANOVA were followed by Tukey's post hoc test to rank significant differences among the treatments. Differences were considered significant when $P < 0.05$.

Table 5
Extruder, pellet, and microstructure responses for the technical trial.

	Extruder		Pellet					Microstructure	
Feed mix	SME (Wh/kg)	V_{die} (Pa s)	Hardness (N)	Doris Durability (%)	Bulk density (g/l)	Oil leakage (g/kg)	WSI (%)	Open porosity (%)	MCS (μm)
EX1–1	19.5	70	120.7	98.9	697	36.8	98.0	12.5	214.8
EX1–2	19.2	49	117.4	98.7	702	38.6	96.5	11.0	227.8
EX2–1	55.9	353	167.7	95.3	440	76.7	93.3	59.9	247.6
EX2–2	56.6	307	148.6	97.2	431	83.7	94.5	58.7	259.5
EX3	48.5	410	167.8	95.0	416	86.5	92.7	61.7	260.0
EX4–1	26.6	169	158.9	97.4	671	32.8	97.6	20.2	194.1
EX4–2	26.1	162	136.8	98.7	659	33.0	98.4	19.2	194.3
EX5–1	32.1	248	170.8	96.1	695	38.1	97.5	13.1	219.1
EX5–2	32.3	231	156.8	98.0	694	36.6	98.2	12.7	213.8
EX6	52.3	321	195.8	94.9	535	60.0	97.8	50.2	373.4
EX7	34.6	307	135.9	97.9	635	40.4	98.0	22.0	208.9

MCS, mean cell size; SME, Specific mechanical energy; V_{die}, Apparent viscosity in the extruder die; WSI, water stability index.

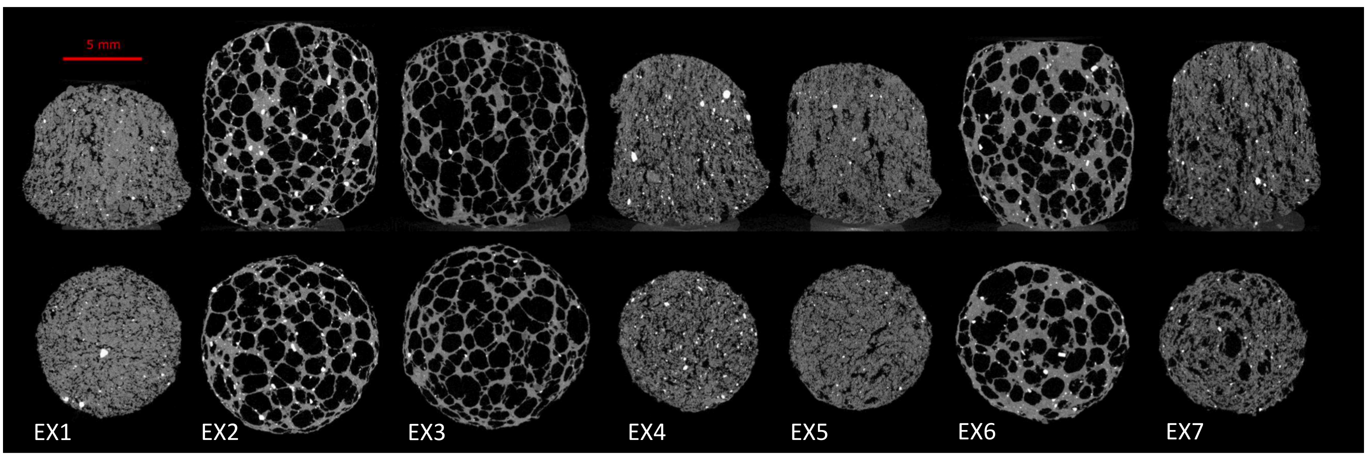

Fig. 1. X-ray microtomography images in longitudinal (top) and radial (bottom) views for the pellet samples. Black corresponds to air, grey to solid structure, and white to minerals and fish bone fragments. Detailed composition data are given in Table 2.

Table 6
Mixture models.

Variables	Pseudo component model	P - value	Lack of fit (P)	R^2
SME[2]	19.4 A + 56.3 B + 48.6C - 46.4 AB - 7.8 AC -1.6 BC	< 0.0001	0.099	0.999
V_{die}[1]	58.9 A + 316.2 B + 409.5C	< 0.0001	0.119	0.950
Bulk density[3]	699.5 A + 435.5 B + 416.0C + 390.0 AB +546.3 AC + 435.7 BC - 930.0 ABC	< 0.0001	na	0.999
Oil leakage[2]	37.5 A + 80.0 B + 86.1C - 100.2 AB - 94.4 AC - 85.7 BC	< 0.0001	0.168	0.990
Open porosity[2]	11.8 A + 59.3 B + 61.7C - 63.4 AB - 95.3 AC - 41.4 BC	< 0.0001	0.969	0.999
MCS[3]	221.3 A + 253.5 B + 260.0C - 173.0 AB - 96.8 AC + 466.5 BC - 1563.5 ABC	0.0003	na	0.993

A = AlgaPrime™ DHA LS, B = fishmeal, C = soy protein concentrate.
na = not applicable, because the degrees of freedom are used for modelling.
[1] Linear model.
[2] Quadratic.
[3] Special cubic model.

3. Results

3.1. Technical extrusion trial

The experimental design resulted in different surface morphology of the dried pellets (Fig. 1). All pellet samples showed high degree of starch gelatinization, i.e., 96.0 ± 1.2% of a total starch content of 82.0 ± 6.0 g/kg. A low variation and high values of Doris durability (97.1 ± 1.5) and WSI (96.6 ± 2.1) were found for all blends (Table 5). Hardness, ranging from 117.4 to 195.8 N, was reduced with increased AlgaPrime addition. Modelling was focused on extruder, expansion, and microstructure responses. Highly significant mixture models ranging from $R^2 = 0.950$ to 0.999 were found for all the responses (Table 6) and no outliers were detected. The linear (V_{die}) and quadratic (SME, oil leakage, and open porosity) models showed insignificant lack of fit. For bulk density and MCS, lack of fit could not be estimated because the number of runs (degrees of freedom) were used for modelling of the special cubic models. The response SME had the highest values on the vertex of the pure FM blend and lowest on the vertex of the pure AlgaPrime blend (Fig. 2A). Viscosity had the highest value on the vertex of the pure SPC blend and lowest on the vertex of the pure AlgaPrime blend (Fig. 2B). Bulk density had the lowest value with zero AlgaPrime addition and with denser pellet adding AlgaPrime in the feed mix (Fig. 2C). The opposite trend was found for oil leakage, open porosity and MCS

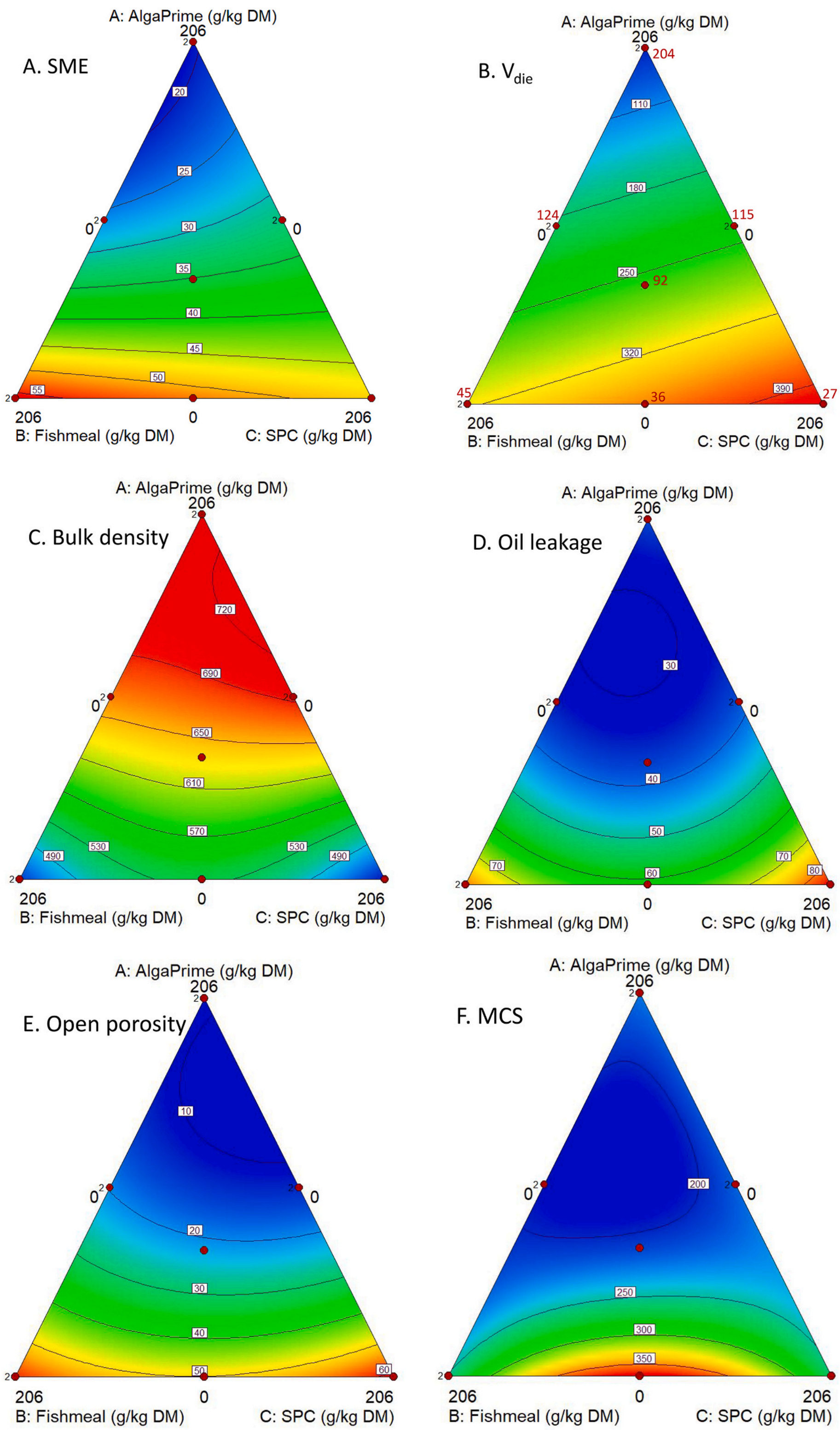

Fig. 2. Contour plots for the extruder, expansion, and microstructure responses. Actual inclusion levels (g/kg DM) for the three components are given for the pure (vertex) and binary (edge) points. The contours are flagged with the actual unit of the respective response. Solid red circles represent the design points. A) Specific mechanical energy (SME; Wh/kg), B) Apparent dough viscosity in the extruder die (V_{die}; Pa s). Red numbers at the design points represent the respective feed mix lipid levels in g/kg DM., C) Bulk density (g/l), D) Oil leakage (g/kg), E) Open porosity (%), F) Mean cell size (MCS; μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 7
Correlation (*R*) between design variables, oil content in the feed mix and extruder, pellet and microstructure responses.

	AlgaPrime	FM	SPC	Oil in feed mix	SME	V_{die}	Bulk density	Oil leakage	Open porosity	MCS
AlgaPrime	1.00									
FM	-0.61[a]	1.00								
SPC	−0.40	−0.48	1.00							
Oil in feed mix	1.00[b]	−0.54	−0.48	1.00						
SME	−0.95[b]	0.65[a]	0.30	−0.93[b]	1.00					
V_{die}	−0.94[b]	0.38	0.61[a]	−0.96[b]	0.88[b]	1.00				
Bulk density	0.86[b]	−0.60	−0.26	0.85[b]	−0.92[b]	−0.80[b]	1.00			
Oil leakage	−0.78[b]	0.48	0.31	−0.78[b]	0.89[b]	0.76[b]	−0.98[b]	1.00		
Open porosity	−0.88[b]	0.62[a]	0.26	−0.87[b]	0.93[b]	0.80[b]	−0.99[b]	0.96[b]	1.00	
MCS	−0.56	0.24	0.35	−0.57	0.66[a]	0.47	−0.54	0.54	0.62[a]	1.00

[a] Denotes significant correlation coefficients (P < 0.05).
[b] Denotes significant correlation coefficients (P < 0.01).

(Fig. 2D, E, F).

The lipid content in the feed mixes fed to the extrusion system was a function of AlgaPrime addition and were negatively correlated to SME and V_{die} (Table 7). This had an impact on expansion parameters and microstructure where oil leakage, open porosity and MCS, were positively correlated to SME and V_{die}, and bulk density negatively correlated to the two responses (Table 7). Open porosity, bulk density and oil leakage were highly correlated to each other.

Closed porosity was only 1.14% ± 0.47 of the total analyzed pore volume. This shows that most of the pore volume was open, also documented in other studies (Ahmad et al., 2019; Draganovic et al., 2013; Samuelsen et al., 2022). The pellets with zero AlgaPrime addition i.e., pure FM (EX2), pure SPC (EX3) and the FM/SPC blend (EX6) showed the highest variation compared to the other design points. They had an expanded structure, high percentage of open pores, broad cell size distribution and thicker cell walls (Figs. 1 and 3) and were floating feeds at the target lipid level of 361 g/kg DM. The low lipid content for EX2, EX3 and EX6 (Fig. 3B) resulted in flow instabilities (extruder surging) giving challenges during sampling of the dried pellet.

For the pure AlgaPrime blend (maximum AlgaPrime addition; EX1) the feed mix, wet extrudate and dried pellets were analyzed for fatty acid composition (Table 8). There were observed significant differences for some fatty acids between the samples, but no trend towards reduction of omega-3 fatty acids in the processing steps compared to the feed mix.

3.2. Technical coating trial

The dried centroid feed (EX7; Tables 2 and 3) was used in the coating trial and all the AlgaPrime levels listed in 2.4., were possible to add. AlgaPrime addition above 50% replacement was not possible due to blocking of the pellet pores during vacuum coating and a target total lipid level of 361 g/kg DM was not possible to achieve. Results from the oil leakage test of the coated pellet are found in Fig. 5. Some variations in oil leakage were observed but no significant trends.

3.3. Fish experiment

After one month feeding with the test diets, the postsmolt salmon ended up with a final body weight of 492 ± 9 g with a growth ratio of 1.61 ± 0.04 per day (Supplementary data file; Table S1). There were no significant differences in start or final weigh, or specific growth ratio, between the different treatments (ANOVA; $P > 0.05$).

The dietary inclusion of AlgaPrime at 100 g/kg (Algablend and Algacoat) and 200 g/kg (Algamix) was reflected in the fatty acid composition increasing the content of 16:0 and DHA and decreasing the content of monounsaturated and n-6 polyunsaturated fatty acids in the feeds by increasing the dietary inclusion of AlgaPrime (Table 4). To maintain similar protein and lipid content in the diets (Table 4) wheat flower meal was reduced with increased inclusion of AlgaPrime. The apparent digestibility coefficient of dietary nutrients showed overall minor differences among the treatments. Feeds with 100 g/kg AlgaPrime included either prior to extrusion or oil coated after extrusion showed no significant differences in apparent nutrient digestibility ($P > 0.05$). High inclusion of AlgaPrime (200 g/kg) affected apparent digestibility, showing significantly lower crude protein apparent digestibility compared to salmon fed the control and Algacoat diets. The lipid and fatty acid apparent digestibility were also affected resulting in significantly higher apparent digestibility of total lipid and the fatty acids 18:1, 18:2, 18:3 and DHA in salmon fed the Algamix as compared to the Algablend diet (Table 9).

4. Discussion

4.1. Technical extrusion trial

The impact of increased level of AlgaPrime on the measured extruder and pellet responses is discussed below.

4.1.1. Feed mix lipid content and physical feed quality

Increased AlgaPrime addition increased the feed mix lipid content in the range of 27–204 g/kg DM which is the main contributor of the observed variation in this study. A final and target lipid content of 361 g/kg DM was reached for all feeds in the design and with a significant reduction in oil leakage with increased AlgaPrime or lipid level in the feed mix (Fig. 2D) due to a denser pellet, smaller pores (Fig. 2C and F) and less oil to be coated in the vacuum coating step. Within the boundary of the chosen design near equal effect of AlgaPrime addition on the FM and SPC based diets were found (Fig. 2). All feeds in this trial had acceptable physical quality (Table 5). Despite the expected reduction in SME and denser pellet at increased AlgaPrime/oil addition (Fig. 2A, C; Samuelsen et al., 2018) there has been sufficient viscous heat dissipation for proper starch gelatinization and protein plasticization in all the design points. This was confirmed by the high degree of starch gelatinization and high values for Doris durability and WSI (Table 5). For the two latter responses the highest values were found for pellet with AlgaPrime addition (Table 5). This may be related to a much denser structure (Fig. 1) and in line with other studies (Sørensen, 2012). Pellet hardness showed an opposite trend where pellet without AlgaPrime had highest values (EX2, 3 and 6, Table 5). Thicker cell walls (Fig. 3B) and a larger area of compression during hardness testing may explain this finding. The observed reduced SME and expansion can be counteracted for by reduced moisture and/or flow rate and/or increased screw speed (Akdogan, 1996) but this was not in the scope of this study. Commercially the center point with a feed mix lipid content of 92 g/kg DM (Table 2) gave the most interesting feed in this design. This corresponds to an AlgaPrime content of 48 g/kg DM in the final feed which is within the recommended limits (30–50 g/kg; personal communication with Corbion). An interesting observation is the possibility to make high physical quality feed with the highest AlgaPrime addition. This may indicate that feed mix lipid level above 120 g/kg can be used when lipid

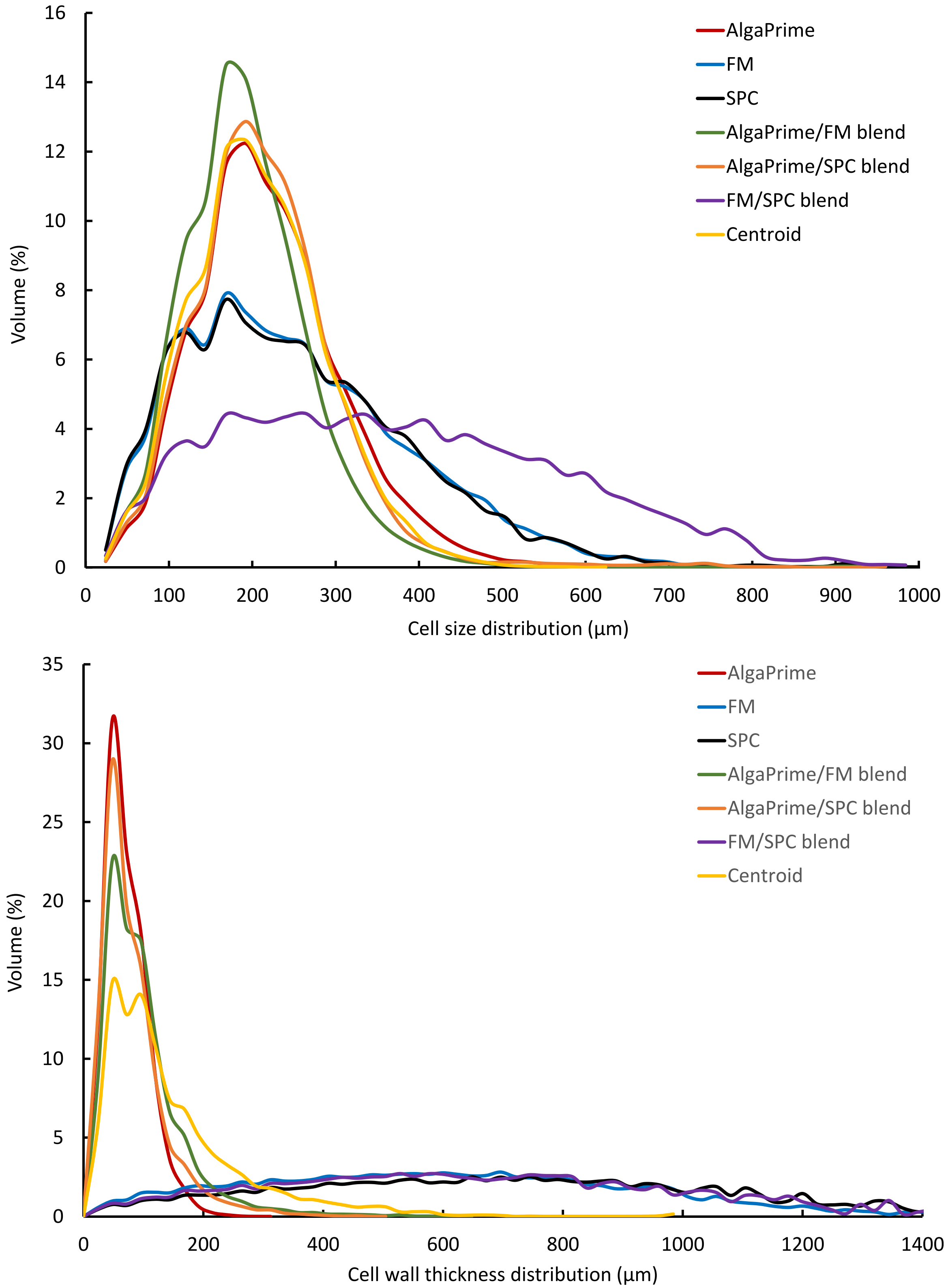

Fig. 3. A) Cell size-volumetric distribution for the pellet samples, B) Cell wall thickness-volumetric distribution for the pellet samples.

is added as a particulate ingredient compared to a pure oil. This finding is supported by Rokey (1994).

The protein fraction of *Schizochytrium* sp. microalgae constitutes of high amounts of water-solubles (~39%; Kousoulaki et al., 2016) having a plasticizing effect in the extrusion process improving starch gelatinization and protein plasticization (Samuelsen and Oterhals, 2016). There is however only 61 g/kg DM protein in the AlgaPrime suspension (Table 1) and the water-soluble content in the AlgaPrime blends will therefore be low (<0.5%). Triglycerides have no plasticizing effect (Kalichevsky et al., 1992). However, the opposite has been found for free fatty acids (Di Gioia and Guilbert, 1999; Pommet et al., 2003). The oil fraction in the AlgaPrime suspension is mostly triglycerides (99%). The

Table 8
Fatty acid composition in the feed mix, extrudate and dried feed for the diet with maximum level of AlgaPrime™ DHA LS (EX1).

FA/total FA	Feed mix	Extrudate	Dry pellet	ANOVA (*P*-values)
16:0	14.99 ± 0.01^{b}	14.84 ± 0.04^{a}	14.92 ± 0.04^{ab}	0.039
18:1	17.23 ± 0.04^{a}	17.27 ± 0.04^{a}	17.57 ± 0.01^{b}	0.004
20:1	0.32 ± 0.01	0.32 ± 0.02	0.35 ± 0.00	0.134
18:2n-6	11.66 ± 0.03^{c}	11.14 ± 0.05^{a}	11.38 ± 0.03^{b}	0.002
18:3n-3	2.72 ± 0.02	2.69 ± 0.04	2.72 ± 0.02	0.495
20:5n-3 (EPA)	0.23 ± 0.00^{a}	0.28 ± 0.00^{b}	0.27 ± 0.00^{b}	0.001
22:6n-3 (DHA)	35.89 ± 0.08	36.38 ± 0.31	36.29 ± 0.03	0.138
Sum SFA	16.91 ± 0.02	16.78 ± 0.07	16.75 ± 0.06	0.111
Sum MUFA	17.81 ± 0.06^{a}	17.78 ± 0.06^{a}	18.21 ± 0.01^{b}	0.006
Sum n-6 PUFA	11.91 ± 0.03^{c}	11.40 ± 0.04^{a}	11.65 ± 0.03^{b}	0.002
Sum n-3 PUFA	39.44 ± 0.06	39.97 ± 0.34	39.87 ± 0.00	0.142
Sum PUFA	51.35 ± 0.03	51.36 ± 0.38	51.53 ± 0.03	0.700
Sum identified FA	86.07 ± 0.10	85.92 ± 0.24	86.48 ± 0.08	0.081
Sum unidentified FA	13.93 ± 0.10	14.08 ± 0.24	13.52 ± 0.08	0.081

Mean ± standard derivation; $n = 2$. Statistics by one-way for dietary effects at $P < 0.05$, followed by Tukey post hoc test. Superscripts showing different letters are significant different.

composition of lipids and water-solubles have therefore limited plasticizing effect and cannot explain the observed acceptable physical feed quality at high AlgaPrime levels in this study.

4.1.2. Viscosity, pellet expansion and microstructure

The V_{die} was highly negatively correlated to increased AlgaPrime level in the feed mix (Table 7). This is a result of an increased lubrication effect of the lipids and is the main contributor to the observed reduction in pellet expansion, increased bulk density, smaller pores, and reduced oil leakage (Fig. 1, Fig. 2C, D and 3a). This relationship is valid for any type of lipid rich ingredient or oil added in the extrusion process (Rokey, 1994; Samuelsen and Oterhals, 2016) and may be explained by two mechanisms; 1) Extrudate expansion to a porous structure at extruder die exit is dependent on superheated steam nucleation, growth of bubbles entrapped in the feed melt and, due to the high vapor pressure, bubble rupture to atmosphere. The reduction in V_{die} has resulted in weaker cell walls that reduced bubble wall extension before rupture (Moraru and Kokini, 2003). 2) For the fixation of the porous structure, viscosity after rupture must be below the critical level of 10^{7}–10^{8} Pa s to prevent the structure from collapsing. For extrudates with low V_{die} this may not be fulfilled giving increased structure collapse and a denser pellet (Fan et al., 1994; Moraru and Kokini, 2003).

4.1.3. Flow instabilities and lubrication

An important observation in this study is the dramatic change in pellet expansion and microstructure increasing the lipid level from 27 to 45 g/kg DM for the pure and binary blends of FM and SPC (EX2, EX3 and EX6) to 92 g/kg DM for the centroid point (EX7; Fig. 1 and 2). The very low lipid content for EX2, EX3 and EX6 has resulted in flow instabilities (extruder surging), floating feeds at the target lipid level of 361 g/kg DM and increased oil leakage due to larger pores (Fig. 2D, F and 3A) Flow and extrudate instabilities may be a result of oscillation breakup of the compacted feed mix in the extruder screws and/or stick-slip movements due to adhesion failure at the die wall (Denn, 2001; Fenner et al., 1979). The lubricity effect of the added lipids has most probably improved feed melt plasticity and mixing performance in the extruder screws resulting in a more stable process (Ilo et al., 2000; Rokey, 1994) and a denser pellet with narrower pore size distribution and smaller pores (Fig. 2 and 3A). In this study, this positive lubrication effect was achieved by adding AlgaPrime to the feed mix in the mixing step. Alternatively, in large scale production, the suspension or lipids can be added in the preconditioning step prior to extrusion. The AlgaPrime addition level will be a variable depending on the targeted lipid and DHA fatty acid content in the feed mix. High inclusion level of other lipid rich ingredients as FM or insect meal will also reduce the amount of AlgaPrime possible to add. A higher lipid content in the extrudate also demands less oil to be vacuum coated to meet the target final lipid content. A lower degree of expansion can therefore be accepted with a positive effect on oil leakage.

4.1.4. Preservation of omega-3 fatty acids in the process

For the pure AlgaPrime blend (EX1) there were no significant changes in omega-3 fatty acids in the processing steps compared to the

Table 9
Apparent digestibility of nutrients of the experimental feeds in Atlantic salmon.

	Apparent digestibility (%)				ANOVA
	Control	Algablend	Algacoat	Algamix	(*P*-values)
Dry matter	64.9 ± 0.9	63.9 ± 0.9	64.3 ± 1.7	63.6 ± 0.9	0.606
Crude protein	88.9 ± 0.6^{b}	88.4 ± 0.8^{ab}	88.7 ± 0.3^{b}	87.2 ± 0.6^{a}	0.026
Crude lipid	95.8 ± 0.5^{b}	94.4 ± 0.6^{a}	95.2 ± 0.1^{ab}	95.6 ± 0.2^{b}	0.012
16:0	96.8 ± 0.6	95.5 ± 1.0	96.0 ± 0.7	96.1 ± 0.4	0.239
18:1	96.8 ± 0.3^{ab}	95.5 ± 0.7^{a}	96.0 ± 0.3^{ab}	96.1 ± 0.2^{b}	0.026
20:1	98.3 ± 0.4	97.5 ± 0.9	98.1 ± 1.0	98.9 ± 0.1	0.188
22:1	97.8 ± 0.5	96.9 ± 1.0	96.9 ± 1.1	98.0 ± 0.2	0.177
18:2n-6	97.2 ± 0.4^{ab}	96.4 ± 0.8^{a}	96.2 ± 0.4^{ab}	97.6 ± 0.2^{b}	0.038
18:3n-3	98.0 ± 0.2^{ab}	97.0 ± 0.7^{a}	97.6 ± 0.3^{ab}	98.5 ± 0.1^{b}	0.012
20:5n-3 (EPA)	98.6 ± 0.2	97.9 ± 0.8	98.2 ± 0.7	99.1 ± 0.2	0.123
22:6n-3 (DHA)	97.6 ± 0.4^{ab}	97.3 ± 0.9^{a}	98.4 ± 0.4^{ab}	98.8 ± 0.1^{b}	0.035
Sum SFA	97.0 ± 0.6	95.7 ± 0.9	96.0 ± 0.5	96.1 ± 0.4	0.116
Sum MUFA	98.0 ± 0.4^{ab}	97.2 ± 0.7^{a}	97.6 ± 0.5^{ab}	98.6 ± 0.2^{b}	0.048
Sum n-6 PUFA	97.9 ± 0.3^{ab}	97.0 ± 0.8^{a}	97.7 ± 0.4^{ab}	98.5 ± 0.1^{b}	0.026
Sum n-3 PUFA	98.4 ± 0.3^{ab}	97.5 ± 0.8^{a}	98.5 ± 0.2^{ab}	98.9 ± 0.1^{b}	0.033
Sum PUFA	98.2 ± 0.3^{ab}	97.4 ± 0.8^{a}	98.3 ± 0.1^{ab}	98.8 ± 0.1^{b}	0.026

Mean ± standard derivation; $n = 3$ tanks. Statistics of digestibility by one-way ANOVA for dietary effects at $P < 0.05$, followed by Tukey post hoc test. Superscripts showing different letters are significantly different.

feed mix (Table 8). The AlgaPrime suspension is added antioxidants to prevent oxidation (Butylated Hydroxyanisole, Butylated Hydroxytoylene and Ascorbyl Palmitate). Antioxidant addition, low oxygen level and a short residence time during preconditioning and extrusion may explain the observed preservation in these processing steps. No significant oxidation was observed in the pilot belt dryer under the used dryer conditions (95 and 90 °C in chamber 1 and 2, respectively). A higher heat load may increase oxidation in the drying step. The effect of dryer conditions on microalgae lipid oxidation should be studied in more detail. However, this was out of the scope of this study.

4.2. Technical vacuum coating trial

The AlgaPrime™ DHA LS is a dried and fine milled microalgae biomass suspended in rapeseed oil. To enable vacuum coating of such a product the pellet pores must be large enough to adsorb the microalgae fragments (particulate matter). If not, the particles will block the porous structure and target lipid levels will not be achieved. The tested version of the AlgaPrime suspension had a particle size distribution of Dv(90) <200 μm and Dv(50) <50 μm. All feeds in this trial had a pore size distribution and MCS with a potential for high inclusion level of AlgaPrime (Fig. 2F and 3A). The dried centroid feed (EX7; Tables 2 and 3) with a MCS of 209 μm was used in the coating trial and 50% replacement of fish oil with AlgaPrime was achieved in the vacuum coating process before blocking of the pellet pores. This 50% replacement corresponds to an AlgaPrime level of 14% in a final feed with 36% lipids, which is far above the relevant industrial application levels. The pellet pore size distribution and MCS is dependent on choice of ingredients and process conditions and setup, and the volume of the pores should ideally be distributed among many small pores for reduced oil leakage (Dethlefsen, 2017; Draganovic et al., 2013; Samuelsen et al., 2022). In the study of Samuelsen et al. (2022) the MCS were ranging from 113 to 423 μm and different AlgaPrime addition levels would be expected for these feeds before blocking of the pellet pores. High AlgaPrime addition for pellet with low MCS demands a finer particle size distribution for the AlgaPrime suspension.

As the viscosity of the AlgaPrime suspension was higher than the used fish oil and rapeseed oil (Fig. 4) at any temperatures, reduced oil leakage could be expected replacing fish oil with AlgaPrime. The highest AlgaPrime addition level (50%) had the lowest value of oil leakage. However, no significant relationship between Algaprime level and oil leakage was found (Fig. 5). All pellets in this trial leaked out a low amount of oil compared to the findings in Samuelsen et al. (2022).

4.3. Fish experiment

The high-lipid (60–65%) spray dried biomass of heterotrophically produced *Schizochytrium* sp. with a high content of tri-saturated TAG (63%; Bogevik et al. (2018)) has previously shown to have low apparent digestibility of saturated fatty acids compared to fish oil in salmon diets (Kousoulaki et al., 2016). AlgaPrime in the present study was analyzed to have low content of tri-saturated TAGs (Table 1; 1.7% tripalmitin), and no differences in the apparent digestibility of saturated fatty acids were observed in salmon fed increasing inclusion levels of the ingredient (Table 9). Processing steps during ingredient or feed production are seen as essential to disrupt microalgae cells for increased availability of nutrients (Annamalai et al., 2021), whereas the thermomechanical treatment in the extrusion process has been essential for high lipid digestibility of the microalgae biomass from *Schizochytrium* in salmon feeds (Kousoulaki et al., 2015). Inclusion of AlgaPrime either prior to extrusion or in the vacuum coating step showed no differences in apparent fatty acid digestibility between the dietary groups in this study. This documents that there is no need for extra cell wall rupture based on thermomechanical treatment in the extrusion process for the AlgaPrime product. However, a significant difference in digestibility was observed with higher apparent protein digestibility in Algacoat and control compared to Algamix. This may be related to lower digestibility of the protein fraction in AlgaPrime. The results are however not consistent with increased inclusion, as Algablend and Algamix showed similar protein digestibility. Furthermore, a higher lipid and fatty acid apparent digestibility in Algamix compared to Algablend is found. The apparent digestibility of dietary protein (87.2–88.9) and lipid (94.4–95.8) were overall high, with minor differences between dietary groups, and no differences in fish growth were observed. Feed intake and gastrointestinal passage rate, which were not measured in the present study, are known to affect nutrient digestibility (Bogevik et al., 2021), and might be the reason for some of the differences observed in the present trial. To verify the differences in digestibility and feed efficiency requires a prolonged trial to multiply the initial body weight of the fish. Nevertheless, the main results of this study document that nutrient

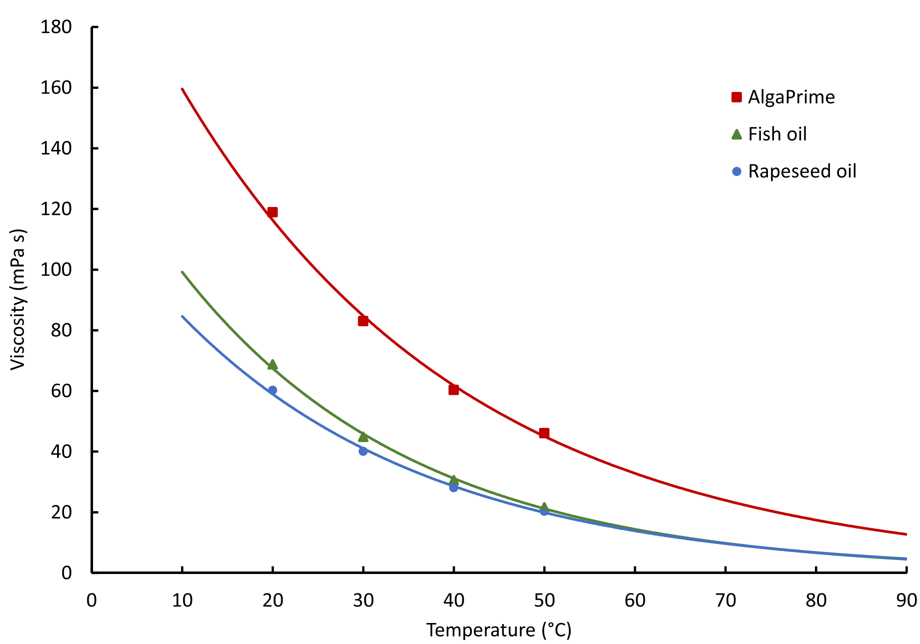

Fig. 4. Viscosity profile at 100 1/s for AlgaPrime™ DHA LS compared to fish oil and rapeseed oil.

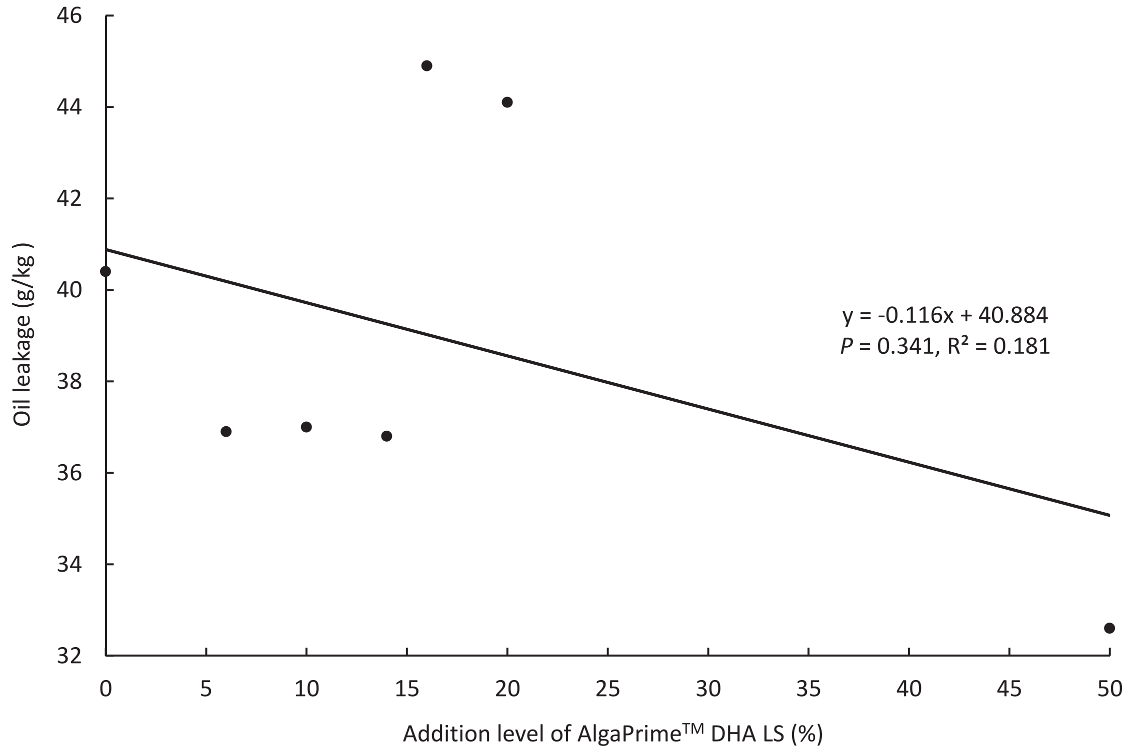

Fig. 5. Oil leakage as a function of addition level of AlgaPrime™ DHA LS in fish oil prior to coating.

digestibility is not affected by AlgaPrime inclusion prior to extrusion or in the coating step.

5. Conclusion

AlgaPrime can be added in FM or SPC based diets prior to extrusion with low impact on hardness, Doris durability and WSI at the studied levels. AlgaPrime addition increased the feed mix lipid content which was the main contributor for the observed variation in the measured viscosity, expansion, and microstructure responses. Low feed mix lipid levels resulted in flow instabilities, expanded pellet with larger pores, floating feeds at the target lipid level and increased oil leakage. AlgaPrime/lipid addition improved feed melt plasticity and mixing performance resulting in a more stable process with reduced pellet expansion. The chosen experimental extruder and dryer settings gave no significant loss of omega-3 fatty acids in the processing steps. The pellet pores for the centroid feed were large enough to adsorb a high amount of AlgaPrime suspension in the vacuum coating step with no significant change in oil leakage. The AlgaPrime processing method enables use of the product with no extra cell wall rupture or thermomechanical treatment in the extrusion process. This make it possible to coat the product at high inclusion levels in feeds to Atlantic salmon without having negative effects of apparent nutrient digestibility.

CRediT authorship contribution statement

Tor Andreas Samuelsen: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Katerina Kousoulaki:** Conceptualization, Funding acquisition, Methodology, Project administration, Writing – review & editing. **André Sture Bogevik:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.740459.

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