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The Impact of Marine Resource-Free Diets on Quality Attributes of Atlantic Salmon

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Abstract: The influence of feeding Atlantic salmon for 90 days on diets that excluded fishmeal (FM) and fish oil (FO) was examined for influence on various quality traits. In addition, the effect of adding krill meal (KM; 0%, 2.5%, and 5%), as a putative feed palatant was also examined. Total replacement of FM/FO had a limited effect on production characteristics, affecting percentage yields of headed and gutted control fish and their standard length ($p < 0.05$). Variances between dietary groups were observed for pigmentation, and plant protein-based KM-free-fed fish returned deeper hues across their belly, NQC (Norwegian Quality Cut), and back portions ($p < 0.03$). No differences were measured for relative fin condition. $\delta^{13}C$ and $\delta^{15}N$ concentrations were lower and higher, respectively ($p < 0.05$) for fish fed the FM/FO-based diet. $\delta^{13}C$:$\delta^{15}N$ likewise differed between treatments with FM/FO-fed salmon expressing higher ratios. Fillet mechanical characteristics varied with fish fed on animal protein-based diets, without KM expressing higher springiness and resilience ($p < 0.05$). Fish fed plant-based diets were generally preferred by younger taste testers. The results from this trial illustrate that FM/FO can be completely removed from salmon diets without problematic effects on quality and palatability attributes.

Keywords: organoleptic; $\delta^{13}C$:$\delta^{15}N$ ratios; relative fin index; algal oil; pigmentation

Key Contribution: Complete elimination of FM/FO from the diet of Atlantic salmon post-smolts and their replacements with alternative animal or plant proteins, combined with algal oil, had no effect on growth. The fillet color was of a deeper hue in fish fed the all-plant protein diet. Both the use of fillet stable isotope concentration ($\delta^{13}C$ and $\delta^{15}N$) and their ratios did not permit distinction between fish fed FM/FO-based diets and those fed FM/FO-free, KM-containing feeds. Complete replacement of FM/FO from Atlantic salmon feeds had little effect on fillet texture or organoleptic profiles.

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1. Introduction

The use of fishmeal (FM) and fish oil (FO) in salmon feeds has declined markedly over the last two decades and this trend is predicted to continue [1]. The reduction in FM/FO has been facilitated, to a certain extent, by an improved understanding of salmon nutrition, genetic selection, advances in feed technologies, and innovation in feed delivery methods [2–5]. Other attributes driving the reduction in the use of FM/FO have been the rising price of both commodities in the international marketplace [6] and the emergence of a more refined and opinionated consumer. Prices for FM/FO have risen as competition for both resources has increased while availability has generally weakened. Reduced catches of forage fish, which provide the raw material for the production of FM/FO, have emerged due to over-fishing, natural and climate-induced effects (e.g., El Niño–Southern Oscillation, the Madden–Julian Oscillation), and the increased consumption of forage fish resources by society [7–10]. Another concern that has forced the aquafeed sector to reduce its reliance on FM/FO is public awareness of the negative direct and indirect effects of forage fisheries on marine and coastal ecosystems [11]. Consumers are now more mindful of issues surrounding sustainability, including losses to biodiversity such as the consequences of intense fishing operations on populations of marine mammals, seabirds, and reptiles [12–14]. Put plainly, nowadays consumers insist that food production systems are safe, ethically, and environmentally reactive and follow the principles of the circular economy with zero discard, combined with more rigorous criteria for animal health and welfare.

In response to the above and other issues, aquafeed manufacturers and researchers have continued to examine the prospect of further reducing or eliminating FM/FO from Atlantic salmon feeds. While this has met with varying degrees of success, the most satisfactory responses have been gained using animal protein-based diets and those comprising blends of plant protein concentrates, and their combinations cf. [15–23]. Indeed, some longer-term studies support the concept that, with judicious selection and blending of various alternative proteins and oils, it may prove possible to completely replace FM/FO in salmon feeds [24–30]. Due to the potential savings inherent in employing FM/FO alternatives, even given poorer Feed Conversion Ratios (FCRs) and reduced growth rates, profit margins can increase [31–33] while simultaneously addressing some consumer concerns. In fact, previous studies reveal that shoppers will pay higher prices for salmon produced under sustainable conditions, provided account is taken of fish welfare, and that food safety is guaranteed [34–36]. For example, consumers are prepared to pay a 25.3% premium for organic salmon in the UK [37], 20% in Denmark [38], 15% in Norway [39], 11–20% in Croatia [40], and an 11% premium in France [41].

Comparatively few studies have evaluated the quality attributes of Atlantic salmon fed on completely marine resource-free feeds. Most reports have evaluated fish fed diets that substitute either FM or FO rather than excluding both ingredients simultaneously [24,42–47]. Since refined consumers will not accept sub-standard foodstuffs, there is a need to determine whether concurrent FM/FO replacement negatively influences the overall quality of farmed Atlantic salmon. Accordingly, we examined the impact of feeding post-smolt Atlantic salmon with various diets over a 90-day period and assessed the impact of each on the mechanical and organoleptic/palatability characteristics of resultant fillets. Fillet color characteristics were examined as a quality indicator and fin and eye condition indices were assessed to determine whether the marine resource-free diets influenced overall animal welfare. In addition, we explored the value of uncovering carbon and nitrogen isotope ratios as a means of confirming the marine resource-free nature of feeds.

2. Materials and Methods

2.1. Raw Material

The fish employed in the present study were derived from a 90-day growth trial which was undertaken to assess the response of Atlantic salmon to dietary krill meal (KM) inclusion. The results of these experiments, which evaluated seven diets, have been presented previously [6], and this study is a further examination of morphological, functional, and consumer acceptance attributes of the fish that were not originally addressed. Four fish were randomly taken from four replicated tanks for each of the seven diets, which comprised a control FM/FO-based feed (C1), plant-based feeds incorporating 0% (P1), 2.5% (P2), and 5% (P3) KM, and animal-based formulas that incorporated 0% (A1), 2.5% (A2), and 5% (A3) KM (n = 16 fish per dietary treatment). The main protein source in the animal-based feeds was poultry meal while that of the plant-based feed was soy protein concentrate. Both animal and plant-based feeds incorporated *Schizochytrium* sp. and canola oils while the control feed contained FO only. Since each tank accommodated 45 individuals (180 fish per treatment), the sample size represented ~9% of the original trial's total population. Fish used for image acquisition (n = 8 per dietary treatment) were sacrificed using an overdose of benzocaine (100 mg L^{-1}) and photographed from the left side using a Canon EOS T7i +

18–55 mm lens. Photographs were taken under fluorescent light and each image incorporated a 30 cm divided ruler. Acquired images were rasterized in Photoshop 2022 (Adobe Systems Inc., San Jose, CA USA) and employed for determining the presence of cataracts and degree of fin erosions. Salmon used for filleting, textural analyses, and assessment of $\delta^{13}C$: $\delta^{15}N$ ratios were fasted for 48 h to avoid fecal contamination, immobilized in ice, and bled by severance of the gill arches (n = 8 per dietary treatment). Since there is the potential for significant processor variation in fillet yields, filleting was carried out by one individual to minimize this possibility.

2.2. Assessment of Surface Area, Eyes and Fins

Body surface area (BSA) was approximated as a function of mass using the formula (13.9 $W^{0.61}$), as determined by [48].

$$\mathrm{BSA} = 13.9 \times W^{0.61}$$

All fish images were evaluated for the presence of cataracts (n = 8 per diet) using a 0–4 scale as described in [49]. The dorsal fin was employed to evaluate fin erosion (n = 8 per diet) and was selected since this appendage is one of the most sensitive nociceptive zones [50]. Fin erosion was quantified using a modification of the relative fin index (RFI) as described in [51]:

$$\mathrm{RFI} = \frac{(\text{fin length} \times 100)}{\text{standard length}}$$

Standard length (SL) was used in preference to total length to avoid measurement inaccuracies due to caudal fin fraying. Dorsal fin height was measured from the point of insertion of the anterior-most fin ray to its tip. The degree of erosion (fraying, splitting, abrasion, and other forms of deterioration; Figure 1) was assessed using a 5-point ordinal scale adapted from MacLean et al. [51]. A 0 score indicated 0–10% fin erosion representing good condition; 1, 10–20% loss demonstrating moderate condition; 2, 20–30% erosion characterizing moderate damage; 3, 30–60% being indicative of severe damage; and 4, >60% erosion, signifying extensive damage (Figure 1). The overall external condition of each fish was examined visually.

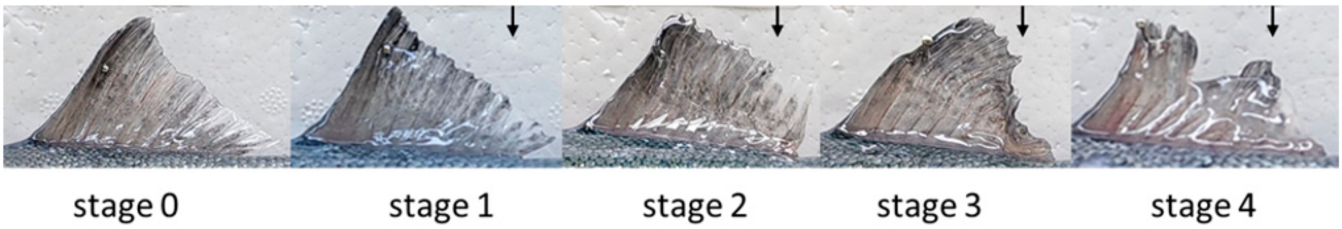

Figure 1. The effect of experimental diets on fin erosion was assessed using a categorical method based on an ordinal scale of 0–4 as suggested by Person Le-Ruyet et al. [52]. Arrows indicate positions of posterior-most intact rays.

2.3. Texture and Color Determination

Sample preparation: Frozen fillets were thawed in the refrigerator (<4 °C) for 24 h. Samples were then brought to room temperature (20 °C) over a two-hour period prior to texture analysis. This ensured that all samples were analyzed at the same temperature and were verified by a thermometer. Raw salmon fillets (3 fillets per tank, 28 tanks) from each treatment group underwent texture profile analysis (TPA) using a TA.XT Plus Texture Analyzer outfitted with a spherical TA-18 Probe (Texture Technologies Corp., Hamilton, MA, USA). Measurements were taken two times along the lateral line (anterior and posterior) for each fillet and subsequently averaged. The TPA method employed to determine hardness, springiness, resilience, and cohesiveness was adapted from [53]. Fillet color was visually assessed using a DSM *Salmo*Fan™ Lineal. Inter-observer differences in color determination were avoided by using one individual only.

2.4. Sensory Evaluation

A taste trial was undertaken using fillets derived from each of the experimental and control dietary groups. Thirty-four untrained active consumers of Atlantic salmon were sent blind samples to prepare by baking at 80 °C for 15 min. Each was then asked to establish whether there were differences in the following four main characteristics: odor, flavor, texture, and visual appearance. Within each characteristic, a subset of descriptors was applied, encompassing, odor: sweet odor, off-odor; flavor: sweet flavor, off-flavor, fresh oily flavor; texture: dryness, fiberness, juiciness, softness, chewing residue; and appearance: dry, protein stains, discoloration. After sample evaluation, each panelist was requested to select their favored fillet(s). When several people gathered for the test, each received the identical part of the different fillets to inspect. Ethnicity, gender identity, and age grouping of participants are summarized in Table 1.

Table 1. Ethnicity, gender, and age-group breakdown of thirty-four untrained taste testers employed during the organoleptic evaluation of the various salmon fillets.

		Female	Male
Ethnicity	Caucasian	4	15
	African/Afro-American	2	2
	(Eur)Asian	7	1
	Latino/Hispanic		3
Age group	18–24	2	1
	25–34	2	6
	35–44	3	6
	45–54	2	2
	55–64	2	4
	65+	2	2

2.5. Authenticity of Fillets

Finally, the muscle (taken dorsal to the lateral line, between the second dorsal and caudal fins) of three fish per treatment were also sampled for carbon and nitrogen isotope ratios, as well as strontium analysis. Samples were collected and sent to the Marine Biological Laboratory, Woods Hole, MA, Stable Isotope Laboratory, where they were dried, pulverized, and analyzed for $\delta^{15}N$ and $\delta^{13}C$ using a Europa 20-20 continuous-flow isotope ratio mass spectrometer interfaced with a Europa ANCA-SL elemental analyzer. The analytical precision based on replicate analyses of isotopically homogeneous international standards is +/− 0.1‰ for both $\delta^{15}N$ and $\delta^{13}C$ measurements, and about 1% relative to the % N and % C measurements.

2.6. Statistical Analyses

All statistical analyses were performed using JASP software (JASP Team, 2019, Version 0.11.1) at the $\alpha = 0.05$ level of significance. Differences between treatment means were examined by one-way ANOVA and significant differences were isolated using Tukey's studentized range (honestly significant difference) test. Any potential tank effect or associated handling/treatment stress was assumed to be identical for each dietary group.

3. Results

There were no differences in weight, length, or *K* between dietary groups and this extended to gutted weights, headed-gutted weights, fillet weights and yields, and body surface area (Table 2). Percentage yield did however vary between groups, ranging from 79.88 to 83.00% with lower ($p < 0.001$) values observed for fish fed experimental diets void of KM and salmon fed the P2 formulation (Table 2). Variances ($p < 0.03$) between dietary groups were apparent for pigmentation in each of the three tested fillet regions and Student's paired *t*-tests revealed that, overall, belly color was lighter than either the NQC or back portions ($p < 0.001$).

Table 2. The impact of dietary treatments (±SD) on morphometric and fillet characteristics of post-smolt Atlantic salmon. Data in a row displaying different superscripts were significantly different ($p < 0.05$). K = condition factor; HG wt = headed and gutted weight. For comprehensive diet formulations see [6].

Diet	C1	P1	P2	P3	A1	A2	A3
Weight (g)	603.13 ± 76.95	568.75 ± 47.49	610.63 ± 58.76	606.88 ± 67.03	613.75 ± 36.52	626.88 ± 61.76	605.63 ± 57.54
Length (cm)	36.81 ± 1.93	35.63 ± 0.79	36.81 ± 1.31	36.19 ± 1.10	36.88 ± 0.64	36.69 ± 1.25	36.56 ± 1.05
K	1.21 ± 0.05	1.26 ± 0.09	1.22 ± 0.08	1.28 ± 0.10	1.22 ± 0.03	1.27 ± 0.06	1.24 ± 0.04
Surface area (cm^2)	689.16 ± 53.90	665.57 ± 34.21	694.88 ± 41.16	692.08 ± 46.55	697.48 ± 25.12	706.08 ± 42.67	691.45 ± 39.78
Gutted wt	550.00 ± 73.29	503.13 ± 42.25	547.50 ± 57.63	553.75 ± 60.58	552.50 ± 37.51	564.38 ± 56.28	545.63 ± 51.30
HG wt (g)	501.88 ± 66.44	455.50 ± 40.44	487.50 ± 54.31	500.00 ± 55.16	496.88 ± 33.48	509.38 ± 50.25	493.13 ± 48.10
HG Yield (%)	83.00 ± 1.51 ^{a}	81.13 ± 1.13 ^{b}	79.88 ± 1.64 ^{b}	82.50 ± 0.76 ^{a}	80.88 ± 0.84 ^{b}	81.25 ± 1.28 ^{a}	81.38 ± 0.74 ^{a}
Fillet wt (g)	302.50 ± 43.01	273.75 ± 27.22	293.75 ± 42.74	311.25 ± 46.73	305 ± 40.00	316.25 ± 39.62	303.75 ± 36.23
Fillet yield (%)	50.19 ± 4.13	48.19 ± 3.52	48.02 ± 4.30	51.26 ± 4.96	49.58 ± 4.53	50.42 ± 3.39	50.08 ± 2.22
Color back	20.88 ± 1.13 ^{a}	23.88 ± 0.84 ^{b}	22.75 ± 1.17 ^{a,b}	23.00 ± 1.07 ^{b}	22.38 ± 1.19 ^{a,b}	22.63 ± 1.41 ^{a,b}	23.00 ± 1.77 ^{b}
Color belly	20.38 ± 0.74 ^{a}	22.88 ± 0.84 ^{b}	21.75 ± 1.17 ^{a,b}	22.00 ± 1.07 ^{a,b}	21.38 ± 1.19 ^{a,b}	21.63 ± 1.41 ^{a,b}	22.00 ± 1.51 ^{a,b}
Color NQC	20.88 ± 1.13 ^{a}	23.88 ± 0.84 ^{b}	22.63 ± 1.19 ^{a,b}	23.00 ± 1.07 ^{b}	22.38 ± 1.19 ^{a,b}	22.50 ± 1.51 ^{a,b}	23.00 ± 1.77 ^{b}

Diet had a significant impact on SL, with fish fed the P1 and A3 diets being shorter than those fed the FM/FO-based diet (Table 3). The relative widths (RFW) and heights (RFH) of the dorsal fins did not differ between groups, varying from 42.44 to 45.69 and from 29.81 to 33.88, respectively, with the relative fin index falling (RFI) in the range of 13.4 to 13.8 (Table 3). The number of frays (#F) and fray widths (FW) of the dorsal fin frays also did not differ between treatments (Table 3). No cataracts were discerned in any of the fish, irrespective of dietary treatment.

Table 3. The impact of dietary treatments (±SD) on standard length and dorsal fin characteristics of post-smolt Atlantic salmon. SL = standard length; RFW = relative fin width; RFH = relative fin height; RFI = relative fin index; FW = fray width; #F = number of frays. For comprehensive diet formulations see [6]. Data in a row displaying different superscripts were significantly different ($p < 0.05$).

Diet	C1	P1	P2	P3	A1	A2	A3
SL	33.57 ± 17.9 ^{a}	31.18 ± 10.0 ^{b}	31.46 ± 11.7 ^{a,b}	32.09 ± 12.6 ^{a,b}	33.08 ± 11.3 ^{a,b}	31.78 ± 17.0 ^{a,b}	31.46 ± 11.73 ^{b}
RFW	45.18 ± 3.49	42.44 ± 2.83	42.75 ± 1.67	43.69 ± 3.74	45.69 ± 3.14	43.69 ± 3.70	42.44 ± 2.47
RFH	33.88 ± 2.12	29.81 ± 3.77	30.81 ± 1.83	30.94 ± 4.01	33.63 ± 3.14	30.56 ± 4.25	30.63 ± 3.71
RFI	13.48 ± 1.08	13.60 ± 0.68	13.59 ± 0.50	13.64 ± 1.35	13.80 ± 0.63	13.73 ± 0.49	13.40 ± 0.71
FW	3.50 ± 2.69	1.63 ± 2.15	2.00 ± 1.69	1.81 ± 2.07	0.75 ± 1.49	2.81 ± 4.00	2.31 ± 2.74
#F	2.75 ± 2.25	0.88 ± 0.99	2.00 ± 2.39	1.00 ± 1.69	1.25 ± 3.15	2.00 ± 2.56	1.00 ± 1.31

Mean fillet stable isotope differences ($p < 0.05$, Table 4, Figure 2) were apparent between salmon fed on the control, FM/FO-based feeds, and all other samples, with $\delta^{13}C$ and $\delta^{15}N$ concentrations being lower and higher, respectively. $\Delta^{13}C$ levels also varied with diet when considering the experimental feeds, with fish fed the diets containing 5% KM and the 2.5% animal protein formulation returning elevated concentrations when compared to P1, P2, and A1 feeds. There were no differences in readings, however, for measured fillet $\delta^{15}N$ levels between experimental diets. Nevertheless, the significant variation observed in fillet

$\delta^{13}C$ had no overall impact on fillet $\delta^{13}C$: $\delta^{15}N$ ratios from experimental feeds, which, as illustrated by Figure 2, were all lower ($p < 0.05$) than the control fillets.

Table 4. The impact of various dietary treatments on mean (±SD) stable isotope concentrations of $\delta^{13}C$ and $\delta^{15}N$ and $\delta^{13}C$: $\delta^{13}N$ ratios in fillets of post-smolt Atlantic salmon. For comprehensive diet formulations see [6]. Data in a row displaying different superscripts were significantly different ($p < 0.05$).

Diet	C1	P1	P2	P3	A1	A2	A3
$\delta^{13}C$	-19.3 ± 0.3^{a}	-20.0 ± 0.2^{b}	-20.6 ± 0.4^{b}	-21.1 ± 0.4^{c}	-20.6 ± 0.3^{b}	-20.9 ± 0.4^{c}	-20.9 ± 0.3^{c}
$\delta^{15}N$	10.8 ± 0.4^{a}	6.7 ± 0.2^{b}	6.4 ± 0.3^{b}	6.2 ± 0.3^{b}	6.2 ± 0.1^{b}	6.3 ± 0.2^{b}	6.6 ± 0.2^{b}
$\delta^{13}C$: $\delta^{15}N$	-1.8 ± 0.1^{a}	-3.0 ± 0.1^{b}	-3.2 ± 0.2^{b}	-3.4 ± 0.2^{b}	-3.3 ± 0.1^{b}	-3.3 ± 0.1^{b}	-3.2 ± 0.1^{b}

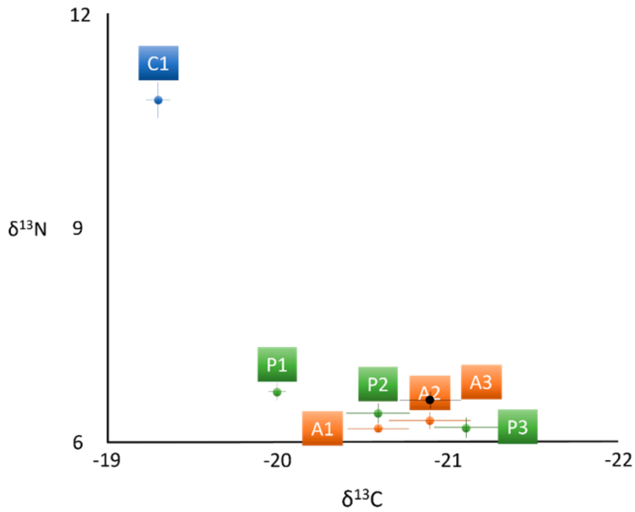

Figure 2. Isotope values for $\delta^{15}N$ and $\delta^{13}C$, for Atlantic salmon post-smolts fed one of seven diets. Values are means + 95% confidence intervals. For A, P, and C diet formulations see [6].

Tables 5 and 6 summarize measurements for hardness, cohesiveness, springiness, and resilience of the anterior and posterior halves of the fillets derived from the different dietary treatments, respectively. There were no differences discerned in any measured parameter when comparing the anterior fillets. However, evaluation of springiness and resilience for the posterior half of the fillet revealed a higher value for springiness ($p < 0.005$) in A1-fed salmon fillets when compared against all others, which returned comparable results (Table 6). Additionally, A1 fillets expressed higher resilience readings than those observed in other experimental diets ($p < 0.007$). The fillets from the FM/FO-fed fish (C1), however, did not differ in resilience to any of the experimental feeds (Table 6).

The average age of the untrained taste testers was 43.1 ± 16.3 years. Declared preferences for specific fillets were in the following order: P3 > P2 > no preference > A2 = A3 ($p > 0.05$; Figure 3). Testers of Asian, African, and Latino descent expressed preference only for fish fed plant-based, KM-containing feeds (P2 and P3), whereas Caucasians were apparently less discerning, selecting fillets derived from each dietary group except for the control (C1). Interestingly, taster testers who selected no preference were generally older (66.4 ± 12.1 years) than those who selected the P3 fillets (42.2 ± 4.6 years).

Table 5. Mean (±SE) measurements for various texture variables taken from the anterior section of fillets of post-smolt Atlantic salmon fed on different diets over a 90-day period. For comprehensive diet formulations see [6].

Diet	Hardness	Cohesiveness	Springiness	Resilience
C1	638.0 ± 34.6	40.9 ± 1.4	99.9 ± 0.1	13.7 ± 0.7
P1	610.5 ± 41.6	37.2 ± 1.2	99.9 ± 0.0	11.5 ± 0.5
P2	608.5 ± 40.4	41.1 ± 1.5	100.2 ± 0.3	13.4 ± 1.3
P3	536.0 ± 25.1	38.9 ± 1.5	100.0 ± 0.0	12.2 ± 0.8
A1	633.4 ± 17.2	38.9 ± 0.8	100.6 ± 0.5	14.4 ± 1.3
A2	552.8 ± 45.4	39.7 ± 1.8	100.1 ± 0.2	13.3 ± 0.8
A3	537.8 ± 45.4	39.2 ± 1.4	101.6 ± 1.3	16.1 ± 2.7

Table 6. Mean (±SE) measurements for various texture variables taken from the posterior section of fillets of post-smolt Atlantic salmon fed on different diets over a 90-day period. For comprehensive diet formulations see [6]. Data in a row displaying different superscripts were significantly different ($p < 0.05$).

Diet	Hardness	Cohesiveness	Springiness	Resilience
C1	764.3 ± 133.5	38.3 ± 1.2	100.2 ± 0.3 ^{b}	13.3 ± 1.0 ^{a,b}
P1	691.5 ± 62.8	35.6 ± 1.6	99.8 ± 0.0 ^{b}	10.9 ± 0.5 ^{b}
P2	681.5 ± 63.6	38.3 ± 1.5	99.9 ± 0.0 ^{b}	12.8 ± 0.9 ^{a,b}
P3	619.8 ± 73.9	36.2 ± 1.2	99.9 ± 0.0 ^{b}	11.9 ± 0.7 ^{b}
A1	711.7 ± 41.1	36.4 ± 1.2	102.4 ± 1.0 ^{a}	17.8 ± 2.6 ^{a}
A2	729.1 ± 63.2	37.0 ± 1.4	99.9 ± 0.0 ^{b}	12.3 ± 0.6 ^{b}
A3	659.3 ± 38.5	36.1 ± 0.9	99.0 ± 0.0 ^{b}	11.7 ± 0.6 ^{b}

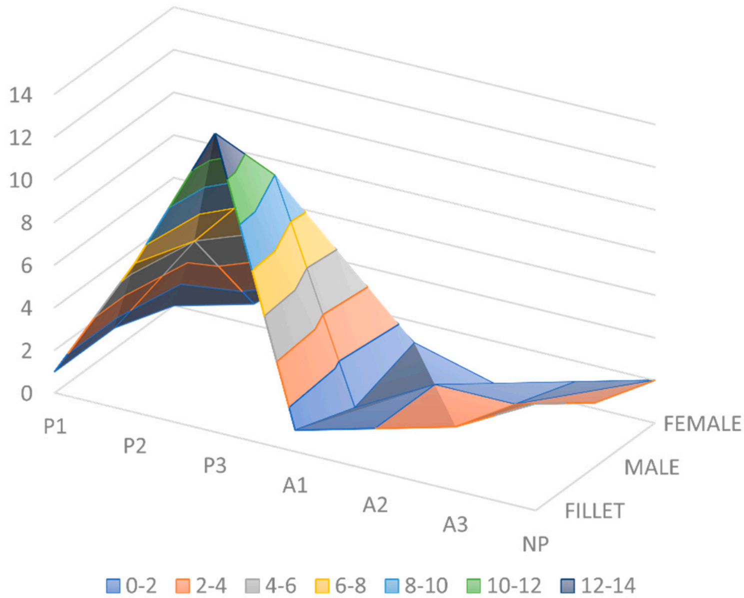

Figure 3. Differences in fillet preference, by gender. Fish fed the P3 diet (for formulations see [6]) were favored by both sexes. NP = no preference.

4. Discussion

Seafood consumers generally make purchasing decisions based on safety and quality. Willingness to pay (WTP) for products by more sophisticated buyers, however, may include consideration of a variety of other factors. These include issues that are predominantly aligned to commercial fisheries (e.g., by-catch and at-sea discards, ghost fishing, toxic fisheries subsidies, carbon emissions from fleets, human rights infringements) as well as those that are more affiliated with aquaculture (e.g., animal welfare, environmental degradation, genetic pollution, carbon emissions from feed manufacturers, human rights infringements in production and supply chain sectors) [34,54–60]. Irrespective of whether a salmon is fished or farmed, however, the first impression a consumer obtains is generally visual. How the salmon presents, therefore, represents a significant element in a purchaser's decision-making process and WTP for the product.

During cultivation, Atlantic salmon are exposed to various stressors that can influence their overall appearance. For example, eyes can become variably opaque due to the presence of cataracts [61]. This condition, which is generally considered to be irreversible, may be caused by nutritional, environmental, chemical, and infectious insults [62]. During the culture of Atlantic salmon, cataracts and the attending poorer vision can lead to abnormal behavior, reduced feed intake, inferior performance, and lowered survival, which also influences profitability. Sissener et al. [63] observed a 6–14% occurrence of cataracts with post-smolts 3 months following transfer to net pens, while Tröße [64], reported mild cataracts and smaller-sized lenses in Atlantic salmon fed on a substantially plant-based diet for 12 months. She suggested that increased risks for cataracts existed due to the low histidine and N-acetylhistidine content of the plant diet. In the present trial, however, there were no signs of eye opacity or hemorrhaging between treatment groups. Thus, over the duration of the trial, the rearing environment and nutrition were acceptable, an observation identical to that of Sissener et al. [63]. Nonetheless, the possibility of cataract development should be thoroughly examined, especially if FM/FO-free feeds are to be deployed over a full production cycle since it has been suggested that dietary levels of histidine should be 14.4 g kg^{-1} feed [65], which is higher than used herein.

More obvious than cataracts are external injuries to the skin and fins. High-density holding of Atlantic salmon has been reported to elevate fin erosion [66]. However, in the current study stocking densities were maintained below that which is considered stressful [66,67]. Moreover, there were no signs of skin or opercular damage at trial end, indicating a low level of aggressive interaction within tanks and further evidence that the rearing environment used was appropriate. However, cultured salmonids often suffer from fin erosion—damage to the epidermis, dermis, and fin rays—resulting in fraying, splitting, and changes in histology and fin size [68]. Fin erosion is considered an important welfare issue in fish since caudal, dorsal, and pectoral fins are nociceptive. The causes of fin erosion, which can achieve a prevalence of 60–90%$^{+}$ [63,69–71], appear diverse [68,72–75] but, unlike fin rot, it is not due to bacterial infection. Nor is fin erosion transmissible, but it may, nonetheless, open fish to secondary bacterial infections. In salmonids, damaged fins can result in downgrading by processors on aesthetic grounds and reduced WTP by consumers [76]. In the present study, fin erosion was noted in approximately 45% of the fish examined with no differences between dietary treatments. Moreover, the average fray width of affected fish was 2.12 mm, with the majority only exhibiting modest levels of erosion. A lower prevalence of fin erosion has been observed by others [77,78], although the latter studies used fish maintained in net pens at stocking densities one-quarter to one-tenth that used during the present trial—differences that may provide partial explanations for the detected variations. Nevertheless, further examination of the causes and mitigation of fin damage is warranted.

Flesh color is another important visual quality characteristic, and, in the marketplace, the pink-red color of salmon not only separates them from other species but represents an indicator of freshness and flavor [79]. Most salmon are sold as fillets, and therefore, color is more apparent than eye or fin attributes. The color of salmon also contributes to a consumers' general enjoyment and gastronomic delight [80]; a detail that increases a shopper's WTP for redder fillets [79]. Most studies with Atlantic salmon evaluate production characteristics using much larger fish (4+ kg) than employed here, with the consequence that comparison of morphological relationships and sensory characteristics is problematic. For example, several authors have commented on the relationship between the development of flesh color and fish size, which itself is linked to the duration and quantity of pigmented feed fed, dietary lipid concentration, pigment intensity, type, digestibility, deposition, and retention [81–83]. In the current study, the belly regions of examined fillets were lighter than the back or Norwegian Quality Cut (NQC), which corroborates the report of Young et al. [84] and others who examined larger fish. However, herein, the overall intensity of color attained for fish of the size range evaluated was in accord with previous observations [85]. Interestingly, there was a tendency toward a more vivid hue in salmon fed the alternative protein diets when compared to the control group, which contrasts with the observations with salmon and trout, where increased fillet lightness has been reported [86–88]. Because alternate lipid sources appear to have no influence on pigmentation [30,31,89], the color differences encountered in the current study are difficult to resolve since the darkest hues, as measured by the *Salmo*Fan™, were from fish fed the plant-based diet void of krill meal. The likeliest explanation for the more pronounced coloration of the alternative protein diets, therefore, was the incorporation of *Schizochytrium* sp. oil at 4.5% of the diet. The oil from these heterotrophic microalgae, belonging to the order Thraustochytriales, not only provides a source of docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA) but is also known to contain astaxanthin and other pigments [90].

In the present study, irrespective of the animal's dietary background, instrumental measurements failed to reveal any differences in texture for anterior fillets. However, some subtle differences were observed in springiness and resilience for one of the posterior fillets. Indeed, herein, overall comparisons of anterior and posterior fillets failed to reveal significant anteroposterior differences within treatments. In contrast, under normal conditions of rearing, using FM/FO-based feeds, Casas et al. [91] reported differences in fillet textural measurements taken at anterior and posterior positions of samples from 3^{+} kg animals, with posterior sites registering greater hardness but lower cohesiveness and springiness. These anterior-to-posterior differences in muscle characters were recorded earlier by [92,93] and others. The noted divergence between the present and other studies may simply reflect disparities in the age/size of fish examined, the dietary formulations employed, or even the season and method of slaughter used [94]. Indeed, it is difficult to compare the findings from the present study with those of others since most have examined the effect of supplementary ingredients or only partial replacements of FM with alternative proteins, and often with larger fish. Nevertheless, both [95,96] reported increased firmness in salmon flesh fed supplementary glutamate, while [44], using an FM-free diet and similarly sized post-smolts to those used here, observed no effect of diet on shear force. Augmented muscle hardness, gumminess, and chewiness have also been reported for other species in which diets were supplemented with high levels of plant and animal proteins and distiller's grains [89,97,98] although contrary effects have also been encountered [99,100]. KM has been demonstrated to increase fillet firmness in Atlantic salmon and the impact of poultry and plant-based meals on the mechanical and organoleptic characteristics of fillets has received attention elsewhere [2,44,101].

Quality and sensorial analyses of Atlantic salmon have generally employed the so-called NQC as being a typical sample [46,101]. However, as pointed out previously [102–104], the distribution of fat in salmon is not homogenous and this can affect organoleptic qualities and distribution of omega-3 fatty acids along the fillet. Accordingly, Nøstbakken and colleagues [105] suggest that the whole fillet rather than just the NQC be used to obtain an impartial compositional analysis, and here we extended this concept to organoleptic evaluation. Additionally, because a small, trained taste panel could never represent the varied perceptions of a naïve target market [106,107], we elected to use regular consumers of salmon (naïve assessors) for the sensory evaluation. Indeed, many studies have determined a null effect of training over consumer assessments for various foods [108–110]. Quality elements generally considered relevant to the judgment of seafood include taste, texture, and appearance, and, for salmonids, [80] indicates that taste and texture are especially significant in revealing consumer preferences for salmonid flesh. It is the triumvirate of taste, odor, and color, however, that has true physiologic importance since these attributes stimulate appetite and gratification. An individual's perceived quality determinants do, however, differ with age, gender, income, educational attainment, racial origin, culinary heritage, and other factors [111–114]. The latter may explain differences in preference reported here since the naïve testers were of varied age, gender, ethnicity, and educational attainment, while also being of differing culinary heritage (Caucasian, Afro-American, Hispanic, Asian) and geographic origin.

The methods of cooking employed, including baking, as used in the present study, do not appear to interfere with salmon fillet lipid quality or omega-3 fatty acid dynamics [115–117] and, by way of confirmation, Barrows et al. [6] reported that a 75 g serving of fish from the present study, irrespective of dietary treatment, was sufficient to exceed daily intake recommendations for EPA+DHA. The use of algal oil as a replacement for FO was suitable given the study findings. The association of fish size to body fat levels and the relationship between fillet fattiness and sensory quality in Atlantic salmon is well documented [118–121], but size (this study) and body fat did not vary between treatments [6], implying that neither issue impacted sensorial evaluations. Interestingly, Atlantic salmon fed with non-FM/-containing feed, but with 5% KM (P3), proved preferable to all other treatments, with 42% of the respondents having a positive view. Of the remainder, 21% of the panel expressed a liking of P2-fed fish, 15% had no preference, while fillets derived from A2 and A3 feeds polled 6% and 9%, respectively. Noteworthy was that the 'no preference' group was of the greatest mean age whereas the P3 group was younger. This is perhaps not surprising given that a higher prevalence of taste disorder has been described in older people from various geographic regions [122–125], and also reflects the age skew of the taste testers.

Contemporary consumers demand safer and higher-quality food and, when paying premium prices, insist on increased transparency relating to product identity. For salmon, whole, filleted, and cutlet products are generally easy to recognize. Less straightforward, however, is the determination of provenance and production processes for specific seafoods, such as marine resource-free or organic variations. Indeed, according to the FAO, fish products are among the food products most susceptible to fraud [126]. For this reason, a variety of methods have been examined to authenticate, for example, wild and farmed salmon, adulteration (particularly of processed foodstuffs), organic products, and to determine pigment and element levels in salmon muscle [126–133]. In this regard, stable isotope analyses have proven especially useful [131], and we have previously demonstrated its utility in verifying the marine resource-free status of farmed largemouth bass [133]. In the current trial, however, evaluation of muscle $\delta^{13}C$: $\delta^{15}N$ ratios at the trial end did not provide the degree of discrimination previously encountered, for example, with largemouth bass. While there was a clear separation between FM/FO and the alternative plant and animal protein diets, the method was unable to separate fish fed diets supplemented with KM. The reason for this remains obscure but further studies are required to substantiate the suitability of the method as a technique for verifying the fidelity of marine resource-free farmed salmon.

5. Conclusions

Total replacement of FM/FO from Atlantic salmon post-smolts had limited effect on growth, influencing only % yields of headed and gutted fish and their standard length. Fillet color was impacted, however, with plant protein-based diets generally providing better coloration. Both $\delta^{13}C$ and $\delta^{15}N$ and their ratios were also affected but this result did not allow discrimination of marine resource-free (KM) animals from those devoid of KM. There was limited impact of treatment on texture or organoleptic profiles, although springiness and resilience were both affected in the posterior section of the fillets. Notable, however, was that fish reared using plant-based diets were generally preferred by younger taste testers. This study demonstrates categorically that FM/FO can be completely removed from salmon diets without untoward effects on quality attributes. Future studies must be undertaken in order to optimize dietary formulations and fish performance further.

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