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Do Yeast Hydrolysate and Supplementary Marine Phospholipids Work as Aids to Total Fishmeal Replacement in Feeds for Atlantic Salmon, *Salmo salar*, in the Seawater Production Phase?

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Introduction

The ability to formulate salmon feeds without reliance on individual feed materials is of both tactical and strategic importance for the salmon farming industry. Whilst it is possible to successfully farm salmon using feeds devoid of fishmeal, the success of this approach is not guaranteed and there is a need to more fully understand the reasons why this is in order to achieve maximum protein source flexibility. Inconsistency in the performance of plant-based fishmeal alternatives has been attributed to, amongst other causes, failure to fully consider all the variables in digestible nutrient contribution to the feed formulation and / or failure to deactivate or remove the anti-nutritional factors in lower quality feed materials. One area that also merits further investigation relates to the proportion and nature of peptides and free amino acids associated with protein sources and the role they may play when attempting to replace fishmeal with non-animal proteins. Additionally, fishmeal is a feed material that supplies an abundance of phospholipids which, under ideal conditions, are considered not be essential nutrients for large salmon. However, given the diversity of roles attributed to phospholipids in both metabolism and potentially in digestion and nutrient transport, the value of pre-formed phospholipids in feeds devoid of fishmeal should be reappraised.

If the industry is to lessen its reliance on fishmeal and its intrinsic content of small peptides, it is necessary to establish if fishmeal equalling performance can be achieved using non-fishmeal protein hydrolysates. On that basis, the functional protein source used in this study was a high protein, purified yeast extract obtained from primary culture of a proprietary *Saccharomyces cerevisiae* strain. The yeast extract contains over 60% crude protein, with a high proportion of glutamic acid, nucleic acids and low molecular weight peptides. Marine phospholipids (MPLs) supplied by the fishmeal and oil trade are initially the most likely candidates for the supply of replacements for the phospholipids previously provided by traditional fishmeals. Whilst not necessarily breaking the industry's reliance on marine ingredients, the material used in this study represents a suitable tool to examine the principle of phospholipid supplementation.

Objectives

The objective of the present study was to establish whether the addition of yeast hydrolysate alone or in conjunction with a marine phospholipid extract can improve the performance of fishmeal-free feeds when fed to Atlantic Salmon in a semi-commercial environment. In this study, the salmon were given feeds containing either 30, 10 or 0% fishmeal where the feeds made without fishmeal were further modified to contain yeast hydrolysate at levels expected to mimic the peptide fingerprint of feeds made with 10, 20 or 30% fishmeal. Additionally, the feed with yeast hydrolysate mimicking the 20% fishmeal fingerprint was further supplemented with a proprietary marine phospholipid-rich supplement at a level of 2% of the total feed with the intention of providing phospholipids at a level equivalent to that found in feeds containing approximately 20% fishmeal (7 feeds in total). The feeds were fed to large salmon in seawater net pens on a semi-commercial scale where fish performance (growth and feed conversion ratio), fish health and welfare and routine quality traits e.g. yields and pigmentation were the key performance indicators (KPIs).

Materials and Methods

Feeds

The feeds (9mm diameter x length) were formulated by Mowi Feed and produced at the Nofima Feed Technology Centre in Bergen (Norway). Seven experimental feeds were formulated to secure equal outcomes for digestible protein (DP) and digestible energy (DE) and to be matched with regards to the profile of digestible amino acids, essential fatty acids and key micronutrients.

Feed FM_{30} was formulated with a high level of fishmeal (30%) whilst feeds FM_{10} and FM_0 were formulated to deliver the same nutritional outcomes except with plant ingredients and necessary additives used to reduce the fishmeal content to 10 and 0% respectively. Feeds YE_L, YE_M and YE_H were derivatives of FM_0 with yeast hydrolysate being added to the feed at low, medium and high levels to recreate the peptide fingerprint of feeds containing 10, 20 or 30% fishmeal respectively. Feed YE_M MPL was a further derivative of YE_M with a proprietary marine phospholipid (MPL) blend being included to supply a level of marine phospholipids equivalent to that typically found in feeds containing 20% fishmeal. Yeast extract and additives displaced balanced proportions of the vegetable products whilst the MPL source displaced a mixture of fish and rapeseed oils. Analysis of feed was carried out by Nofima BioLab, in Bergen, Norway.

Table 1: Formulation and content of key nutrients of the seven experimental feeds (results on as-fed basis)

	FM_{30}	FM_{10}	FM_0	YE_L	YE_M	YE_H	YE_M MPL
Ingredients (%)							
Fishmeal	30	10	0	0	0	0	0
Fish oils	15.71	16.2	17.49	17.4	17.38	17.35	14.58
Yeast hydrolysate	0	0	0	1.85	3.7	5.5	3.7
Marine phospholipids	0	0	0	0	0	0	2
Soy protein concentrate	4.01	12.34	24.45	21.92	20.4	18.92	20.4
Corn gluten	5	5	5	5	5	5	5
Wheat gluten	10	18.5	18.5	18.5	18.5	18.5	18.5
Guar meal	4	4	3.51	4	4	4	4
Vegetable oils	13.18	13.29	13.86	13.87	13.87	13.87	14.62
Wheat, whole	7.39	7.08	2.91	2.92	4.69	5.86	4.62
Field beans, dehulled	8	8	7.26	7.56	5.46	4	5.52
Vitamins and carotenoids	0.62	0.75	0.82	0.82	0.82	0.82	0.82
Minerals	1.16	2.09	2.49	2.43	2.37	2.31	2.42
Amino acids	0.55	1.75	2.05	2.05	2.06	2.05	2.06
Other	0.38	0.99	1.67	1.69	1.76	1.82	1.76
Post-production analysis							
Moisture (%)	6.3	5.8	6.3	5.5	4.1	5.4	6.1
Crude protein (%)	41.4	41	41.1	41.7	41.5	42.4	41.6
Crude fat (%)	32.9	31.9	32.2	32.6	32.5	31.4	32.4
Total starch (%)	9.5	10.7	8.2	7.9	8.3	8.5	7.9
Water soluble crude protein (%)	9.2	7.3	6.5	8.1	9.4	10.9	9.3
Free astaxanthin/ mg kg^{-1}	47	41	48	48	49	47	47

FM_0= diet without fish meal (YE) inclusion; YE_L, YE_M and YE_H = 1.85, 3.7 and 5.5% inclusion of yeast extract protein, respectively. YE_MMPL; 3.7% yeast extract protein and marine phospholipids.

Fish and husbandry

The feeding trial was conducted at Mowi's Averøy Field Trials Station in Norway between December and May 2021. Atlantic salmon (mean weight 1.25kg) were randomly distributed amongst 21 marine net pens (5x5x5m; 125 m^3) at an abundance of 120 fish per pen. The fish were given a period of 7 days acclimation to environmental conditions whilst fed a commercial feed (Mowi RI 1200) before the study started. Upon commencement of the 173-day feeding period, the fish were fed one of 7 feeds (Table 1) with 3 pens allocated to each feed. The salmon were fed in excess using a combination of automatic feeders and a waste feed collection system. A daily over-feed of 15% was targeted and the excess feed amount was confirmed gravimetrically on dry weight corrected, recovered waste feed before feed intake and feed conversion factor were calculated. Fish health and welfare were monitored daily following Mowi's standard procedures. Water temperature spanned a range between 3.2 °C (February) and 9 °C (May) and a natural photoperiod was observed.

Fish sampling

At the end of the 173-day feeding period, all fish were individually weighed and counted with further post-mortem assessments being carried out on 15 salmon / pen (45 fish / feed type). Each fish was subjected to the following: measurement of individual weight and fork length; gutting and weighment of the gutted carcass and liver; visual assessment of the internal organs; removal of the Norwegian Quality Cut (NQC) and preparation of fillet portions; visual colour assessment (SalmoFan™ Lineal); and instrumental measurement of flesh colour (L*, a* and b*) using a Minolta CR-200 Chroma Meter (CR-410, C illuminate). Based on these activities, indices such as condition factor (round and gutted weight basis) and viscero- and hepato-somatic index were calculated. Colour outcomes were further derived in terms of chroma and hue. NQC fillet portions were also despatched for measurement of flesh composition at Mowi Lab (Ulvan, Norway). The total fat content, proportions of key fatty acid groups, astaxanthin and total carotenoid pigment content of the flesh was determined by near infra-red spectrometry (NIRS).

Blood plasma

Blood samples were withdrawn from the caudal vein complex of euthanised fish using size 0.50×16 mm lithium heparinised syringes. Samples were then centrifuged at 3000 rpm for 10 min and plasma was removed and stored in cryo tubes at −20°C until analyses were performed. 8 fish per pen (168 in total) were sampled for blood plasma analysis and analysed by Biovivo-Tech, Norway. Concentrations or activities of albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, chloride, cholesterol, CK, creatinine, glucose, phosphate, potassium, sodium, total protein and albumin were determined.

Statistical analysis

All statistical analyses were performed using the free software environment R (R Development Core Team, 2011). All data were statistically evaluated with a one-way ANOVA and Tukey post-hoc test to find differences due to dietary treatments. All data were analysed for normality using a Shapiro Wilk's test, as well as being evaluated graphically by QQ-plots before parametric tests were performed. Body weight was added to models as a covariate where appropriate. Where significant differences were detected by ANOVA, data was subjected to Tukey's honest significance post-hoc test for homogenous subsets. Differences in health and welfare scores for the final sampling point were analysed for statistical significance using ordinal logistic regression run in the R statistical package (version 4.0.4; 2021) within the RStudio interphase (version 1.4.1103; 2021. Differences were regarded as significant when $p \leq 0.05$. All data are presented as means and standard error(SE), unless otherwise stated.

Results

Feed analysis

The outcomes for moisture, crude protein and crude fat in the test feeds were in accordance with the formulated values (Table 1). With the exception of the FM_{10} feed, the astaxanthin content of the feeds was typically 47-49 mg/kg though the outcome for FM_{10} was considerably

lower at just 41 mg/kg. For the yeast extract-containing feeds, increasing the content of yeast hydrolysate increased the proportion of small soluble peptides (SSPs) in line with the objectives and the feed with added MPL (YE_MMPL) contained approximately the same amount of SSPs as its companion product YE_M. However, the fishmeal-containing feeds contained less SSPs than was expected (mostly, less than the YE feeds) with SSP outcomes not responding dose-wise to fishmeal level in feed ($FM_{10} \leq FM_0 < FM_{30}$).

All experimental feeds were readily accepted by the fish and the physical properties of the feeds supported correct functioning of the waste feed recollection system.

Table 2: Percentage of small soluble peptides < 500 kDa (SSP) in the different feeds according to Nofima BioLab.

Diet	g/kg water soluble protein	Peptides 500-200 kDa/g kg^{-1}	Peptides 0- 200 kDa/g kg^{-1}	Total SSP
FM_{30}	92	5.15	33.76	38.91
FM_{10}	73	4.38	29.35	33.73
FM_0	65	3.9	29.9	33.8
YE_L	81	7.37	34.1	41.47
YE_M	94	10.43	41.27	51.7
YE_H	109	13.73	49.7	63.43
YE_MMPL	93	10.6	41.85	52.45

FM_0= diet without fish meal (YE) inclusion; YE_L, YE_M and YE_H = 1.85, 3.7 and 5.5% inclusion of yeast extract protein, respectively. YE_MMPL; 3.7% yeast extract protein and marine phospholipids.

Salmon performance, biometrics and quality

Overall, the feeds supported acceptable growth, minimal mortality and acceptable feed conversion ratios (Table 3). No significant differences were found amongst the standard fish growth and feed utilisation performance KPIs.

Table 3: Fish growth, mortality and feed utilisation criteria.

	FM_{30}	FM_{10}	FM_0	YE_L	YE_M	YE_H	YE_MMPL	SEM	ANOVA
Growth parameters									*p* - value
Initial weight, kg	1.25	1.25	1.25	1.25	1.25	1.25	1.25	30.06	0.99
Final weight, kg	2.28	2.48	2.38	2.29	2.31	2.21	2.35	0.15	0.45
Weight gain, kg	1.03	1.23	1.13	1.06	1.06	0.96	1.11	0.15	0.44
SGR, %day^{-1}	0.35	0.4	0.38	0.35	0.36	0.33	0.37	0.09	0.43
Feed conversion ratio, biological	0.99	0.93	0.98	0.99	1	1	0.98	0.04	0.59
Feed conversion ratio, economic	0.99	0.93	0.98	0.99	1.01	1.01	0.99	0.01	0.46
Relative feed intake, % assumed biomass day^{-1}	0.36	0.39	0.39	0.37	0.37	0.35	0.38	0.19	0.35
Mortality, %	0.55	0.00	0.28	0.00	0.55	0.55	0.83	0.86	0.48

FM_0= diet without fish meal (YE) inclusion; YE_L, YE_M and YE_H = 1.85, 3.7 and 5.5% inclusion of yeast extract protein, respectively. YE_MMPL; 3.7% yeast extract protein and marine phospholipids. SGR = specific growth rate; Significant differences p ≤0.05; one-way ANOVA were recorded among the dietary groups. Growth and nutritional indices were calculated as followed: Feed conversion ratio (FCR) = feed intake (g)/fish weight gain (g) where biological FCR is the net amount of feed used to produce one Kg of fish and economic FCR takes into account all the feed used, including the effects of feed losses and mortalities.

a) ***b)***

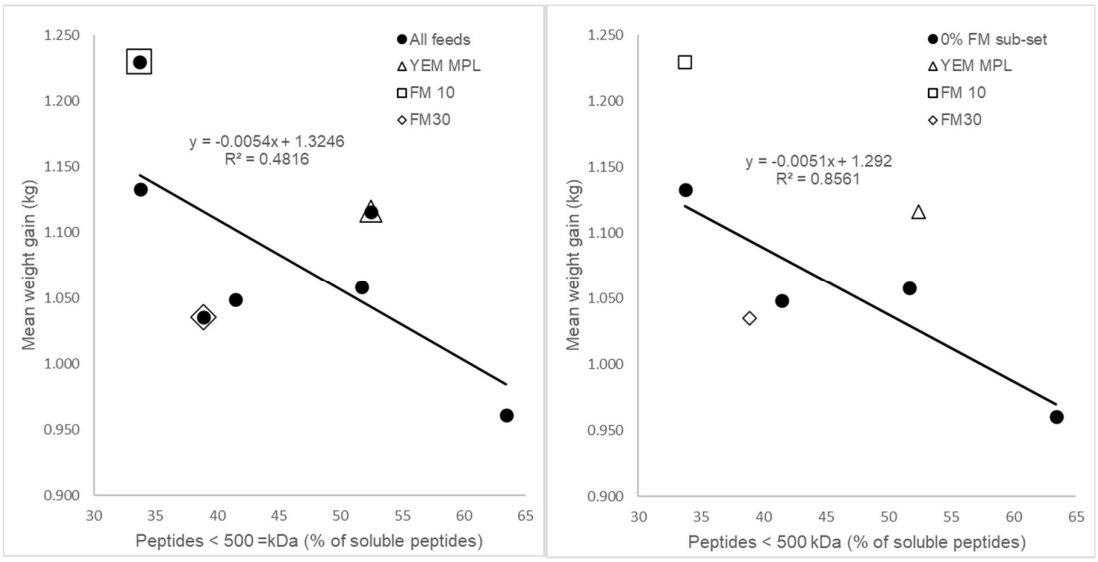


Figure 1: Relationship between % of small soluble peptides (< 500 kDa) in dietary soluble protein and salmon growth (total weight gain, Kg / fish). a) regression analysis shows mean weight gain plotted against peptides < 500kDa from all 7 feed types and b) regression applies only to the subset of 4 feeds without fishmeal or MPL (FM0, YE L, M & H). The marine phospholipid supplemented feed and the 10 & 30% fishmeal feeds are superimposed for reference in Figure 1b.

There was no statistically significant relationship between dietary small soluble peptide content and fish growth as measured by total weight gain between the 7 feeds (Figure 1a, p= 0.08). However, a subset of the 0% fishmeal diets revealed a significant negative association between small soluble peptide inclusion and weight gain (p=0.035). The slope coefficient was -0.008, thus, the total weight gain of fish decreased by 8g for each extra % increase in dietary small soluble peptides in feeds without fishmeal. When comparing the feeds with the intermediate level of yeast protein (YE_M), there was a tendency (non-significant) for salmon fed the MPL supplemented feed to have gained more weight than those without the added MPL.

Although the population average weights of the fish were unaffected by the feeds given (Table 3), the fork length, whole and gutted weights of the individuals selected for biometric analysis (15 fish/pen) were significantly different according to feed type (Table 4). Amongst the salmon fed feeds without fishmeal or MPL, sample weight tended to decrease in response to higher levels of yeast protein extract and the largest fish overall had previously been fed the feed with 10% fishmeal. However, none of the other biometrical parameters including carcass yield, HSI and condition factor showed any statistically significant difference among treatments. (Table 4).

Table 4: Yields and morphometrics of Atlantic salmon fed diets with increasing replacement of fish meal with yeast extract protein n=45/ feed type.

	FM_{30}	FM_{10}	FM_0	YE_L	YE_M	YE_H	YE_MMPL	SEM	ANOVA
Yields and condition									***p* - value**
Whole body weight, g	2271.0^{bc}	2421.9^{a}	2289.0^{b}	2231.0^{bc}	2182.3^{cd}	2086.4^{d}	2213.6^{bc}	693.25	**<0.001**
Weight after gutting, g	1993.6^{b}	2125.0^{a}	2006.3^{b}	1959.3^{b}	1920.1^{bc}	1839.4^{c}	1953.8^{b}	586.11	**<0.001**
Fork length, cm	55.15^{bc}	56.21^{ab}	55.50^{a}	54.77^{bc}	54.71^{bcd}	53.79^{d}	54.48^{cd}	5.18	**<0.001**
Condition factor round, K_{round}	1.35	1.37	1.34	1.36	1.33	1.34	1.37	0.1	0.21
Condition factor gutted, K_{gutted}	1.19	1.2	1.17	1.19	1.17	1.18	1.21	0.09	0.11
Carcass yield, %	87.8	87.7	88.7	88.6	87.9	88.1	88.2	1.61	0.23
Hepato-somatic index, %	0.94	0.99	0.99	0.97	0.99	0.97	0.95	0.15	0.29

FM_0= diet without fish meal (YE) inclusion; YE_L, YE_M and YE_H = 1.85, 3.7 and 5.5% inclusion of Yeast Extract protein, respectively. YE_MMPL; 3.7% Yeast Extract protein and marine phospholipids. Values are means and standard error (SE). Significant differences p ≤0.05; one-way ANOVA were recorded among the dietary groups. Indices were calculated as follows: Condition factor round(K_{round}) = body weight (g)/Length^3(cm)*100. Condition factor gutted (K_{gutted}) = gutted weight (g)/Length^3(cm)*100. Carcass Yield = gutted weight (g)- body weight (g) *100. Hepatic Somatic Index (HSI) = liver weight (g)/body weight (g) *100.

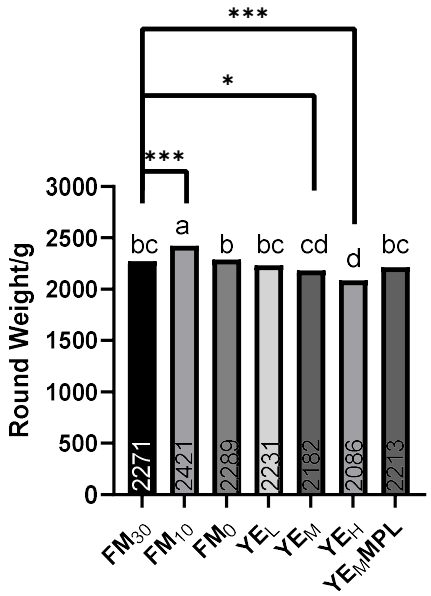


Figure 2: Round weight (g) of Atlantic salmon sampled from population (n=15/pen) Mean values are displayed within bars and displayed letters represent different groups based on Tukey post-hoc analyses, $F_{(6,308)}$ = 17.17, p <0.001.

Flesh composition (NQC)

Table 5: Flesh fat, carotenoid content and colour in NQC portions.

	FM_{30}	FM_{10}	FM_0	YE_L	YE_M	YE_H	YE_MMPL	SEM	ANOVA
									p value
Fat and fatty acids									
Total fat, g/100g	11.82	11.3	12.22	11.87	11.94	11.4	12.46	0.72	0.81
Sum saturated fatty acids, % total fat	16.43	16.93	16.53	17.09	16.79	16.92	16.65	0.34	0.47
Sum n-3 fatty acids, % total fat	18.25^{a}	17.75^{ab}	17.29^{b}	17.59^{ab}	17.38^{b}	17.30^{b}	17.31^{b}	0.59	**0.02**
Sum n-6 fatty acids, % total fat	14.64	14.92	15.02	15.34	15.14	15.51	15.65	0.67	0.19
EPA+DHA, % total fat	8.32^{a}	7.69^{ab}	7.54^{ab}	7.96^{ab}	7.95^{ab}	7.72^{ab}	7.41^{b}	0.52	**0.048**
Carotenoids									
Astaxanthin, mg/kg	3.94	4.14	4.22	4.29	4.19	4.2	4.33	0.22	0.34
Total carotenoid pigments, mg/kg	4.32	4.55	4.64	4.71	4.6	4.61	4.75	0.22	0.34
Pigmentation and colour									
SalmoFan	24.84	25.04	25.93	25.46	25.79	25.46	26.18	0.68	0.15
Minolta l*	25.39	25.99	25.93	25.46	25.79	25.46	26.17	0.58	0.84
Minolta a*	33.24	33.93	33.76	33.44	33.48	33.08	33.93	0.59	0.11
Minolta b*	49.81	50	50.21	49.59	50.4	50.37	50.51	0.64	0.45
Chroma	59.92	60.45	60.54	59.83	60.53	60.3	60.87	0.50	0.78
Hue	56.31	55.86	56.11	56.01	56.42	56.72	56.12	0.24	0.34

FM_0= diet without fish meal (YE) inclusion; YE_L, YE_M and YE_H = 1.85, 3.7 and 5.5% inclusion of yeast extract protein, respectively. YE_LMPL; 3.7% yeast extract protein and marine phospholipids. Values are means and standard error (SE). Significant differences p ≤0.05; one-way ANOVA were recorded among the dietary groups. The reflection of the surface provided by the Minolta reader is integrated according to the CIE-XYZ tristimulus curves and transformed to the uniform L*, a*, b* colour space. The L*, a*, b* colour space is a three dimensional colour space, where L* represents the lightness of the colour (100 being diffuse white), a* the mix of red and green and b* the mix of yellow and blue. Minolta indices are calculated as followed: Chroma = sqrt(a^{*2}+b^{*2}) Hue = arctan(b*/a*) where h ∗ = 0° for reddish hue and h ∗ = 90° for yellowish hue.

Statistically significant differences were only observed for sum of n-3 fatty acids and EPA and DHA as % of total fat from all of the flesh quality parameters measured by NIRS and for colour outcomes according to the SalmoFan and instrumental colour measurement by Chromameter (Table 5). Summarising the majority of the observed differences, it can be noted that treatments containing up to 3.7% of yeast extract and supplemented with marine phospholipid (YE_MMPL) resulted in higher total fat, omega 6 fatty acids, total astaxanthin/pigment and the highest SalmoFan and flesh chroma measurements, compared to the other treatments. The EPA (20:5n-3) and DHA (22:6n-3) content in fish fillets were significantly affected by feeds, with the highest levels recorded in fish fed FM_{30}. The mean sum of n-3 fatty acids was also significantly higher in the FM_{30} group (x= 18.25), YE_M. Fatty acid data are shown in terms of % fat in order to eliminate the overriding total fat and fish size effects.

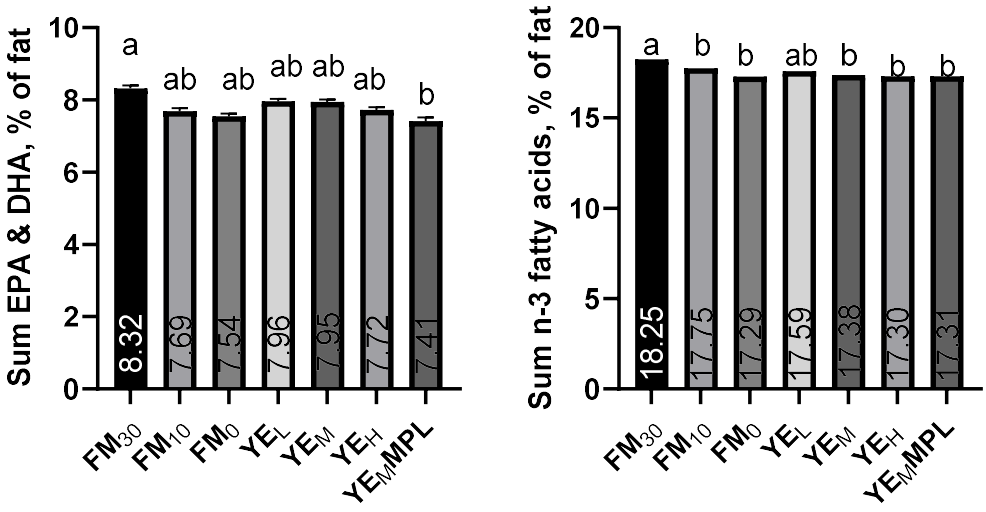


Figure 3: Flesh composition (Norwegian quality cut, NQC) according to near infra-red spectroscopy (NIRS) featuring sum of EPA + DHA, g/100g fat in flesh. P-values and means are provided in Table 5.

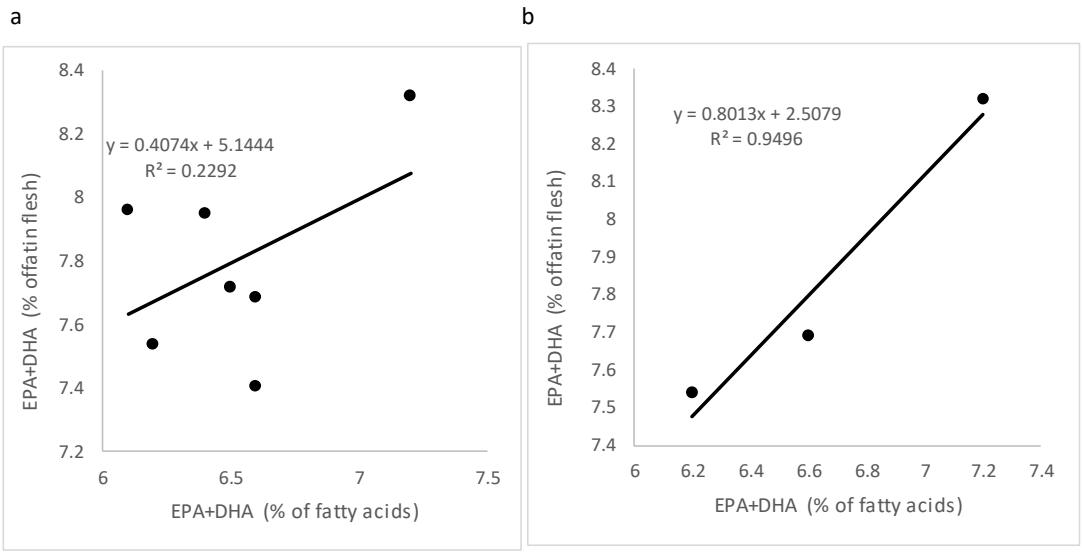


Figure 4: Relationship between EPA and DHA in fish flesh (as % of total fat) and EPA and DHA values in feed (% of fatty acids) a) data are plotted for all 7 feed types b) regression applies only to outcomes for salmon fed the fishmeal-containing feeds.

Figure 4b shows that the EPA+DHA content of the sub-set of fishmeal-containing feeds had a strong influence on the EPA and DHA content of the flesh fat amongst the fish consuming those feeds. However, when the data for fish fed the yeast product were added to the same regression there was no relationship between analysed EPA and DHA in the feed and the amounts found in fish flesh (Figure 4a).

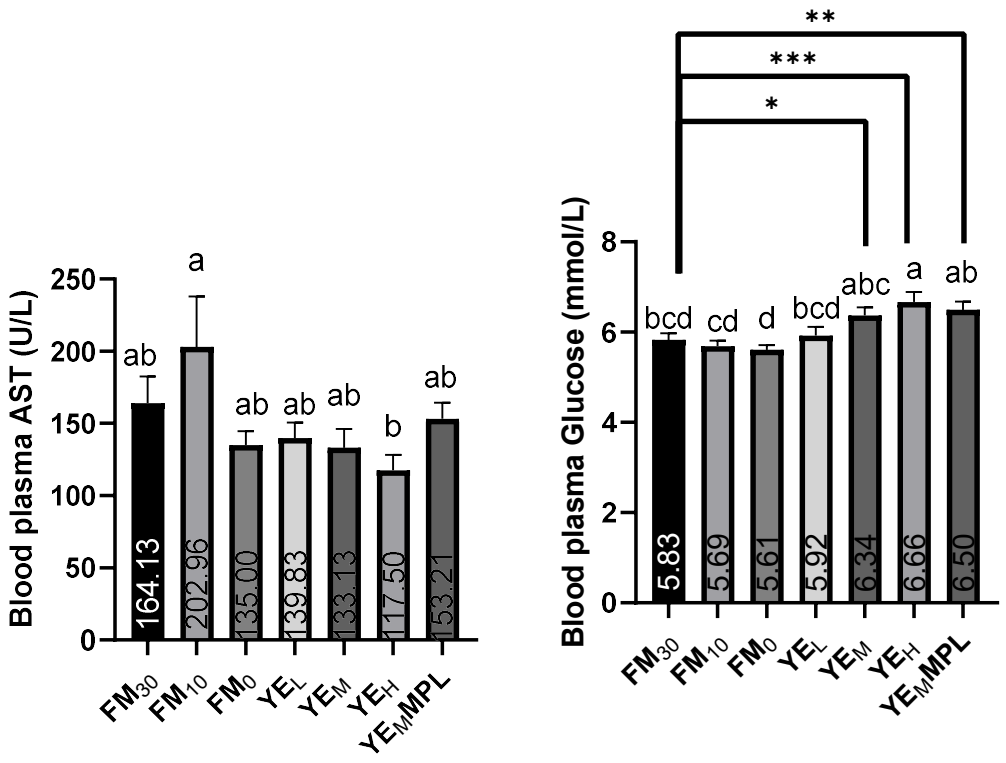
Blood plasma analysis

Salmon fed feeds with the highest hydrolysate inclusion had a significantly increased in blood plasma glucose concentration (Table 6). Thyroxine (T4), uric acid (UA) and aspartate aminotransferase (AST) were also statistically different between treatment groups with the highest UA and T4 found in fish fed the YE_M feed. AST appeared to decrease with increasing dietary hydrolysate $F(_{6,158})=2.45$, $p=0.025$ and a significant difference was also found in plasma glucose between treatments; FM_0 had the lowest mmol/L glucose (x=5.60 mmol/L+/-0.103) and YE_H the highest (x=6.67 +/-0.221 mmol/L) $F(_{6,161})=6.29$, $p=5.83^{e-6}$. A clear relationship was not observed with the increasing addition of yeast hydrolysate and the various blood parameters analysed.

Table 6: Plasma clinical chemistry of Atlantic salmon (n=168) fed feeds with increasing replacement of fish meal with yeast extract protein.

	FM_{30}	FM_{10}	FM_0	YE_L	YE_M	YE_H	YE_MMPL	SEM	ANOVA
Plasma Parameters									*p*- value
AST, U/L	164.13^{ab}	202.96^{a}	135.00^{ab}	139.83^{ab}	133.13^{ab}	117.50^{ab}	153.20^{b}	135.06	**0.016**
BA, umol/L	1.16	6.54	1.45	0.08	6.58	4.25	1.25	13.3	0.387
CK, U/L	3083.67	2573.19	2393.38	2860.53	2715.46	1226.13	1757.58	3152.83	0.121
UA, umol/L	4.21^{ab}	4.88^{ab}	7.54^{ab}	5.96^{ab}	8.75^{a}	4.88^{ab}	1.71^{b}	11.26	**0.04**
GLU, mmol/L	5.83^{bcd}	5.69^{cd}	5.61^{d}	5.92^{bcd}	6.37^{abc}	6.67^{a}	6.50^{ab}	2.06	**<0.001**
CA, mmol/L	3.36	3.43	3.41	3.45	3.45	3.49	3.45	0.18	0.68
PHOS, mmol/L	1.42	1.19	1.46	1.49	1.34	1.42	1.39	0.41	0.158
TP, g/L	39.70	40.21	40.13	40.96	40.13	37.71	39.96	4.95	0.146
ALB, g/L	27.21	27.25	27.25	27.25	27.42	25.08	27.63	4.23	0.146
Na+, mmol/L	168.17	165.25	166.29	166.88	167.7	167.21	168.22	5.31	0.291
Chol, mmol/L	6.51	6.97	6.58	6.76	6.6	6.13	6.83	1.32	**0.**098
T4, nmol/L	30.63^{ab}	25.50^{b}	25.83^{b}	27.78^{b}	34.29^{a}	30.04^{ab}	30.25^{ab}	12.76	**0.019**

FM_0= diet without fish meal (YE) inclusion; YE_L, YE_M and YE_H = 1.85, 3.7 and 5.5% inclusion of yeast extract protein, respectively. YE_MMPL; 3.7% yeast extract protein and marine phospholipids. AST = aspartate aminotransferase; BA = bile Acid, CK= creatine kinase, UA= uric acid, GLU = glucose, CA= calcium, PHOS= phosphorous, TP= total protein, ALB= albumin, NA^+ = sodium, Chol = cholesterol, T4= thyroxine. Values are means and standard error (SE). Significant differences $p \leq 0.05$; one-way ANOVA was performed between the dietary groups.



Figures 5a) mean aspartate aminotransferase and glucose levels taken from plasma samples of 168 Atlantic Salmon fed with control, low fishmeal and experimental diets. b) plasma Glucose concentrations.

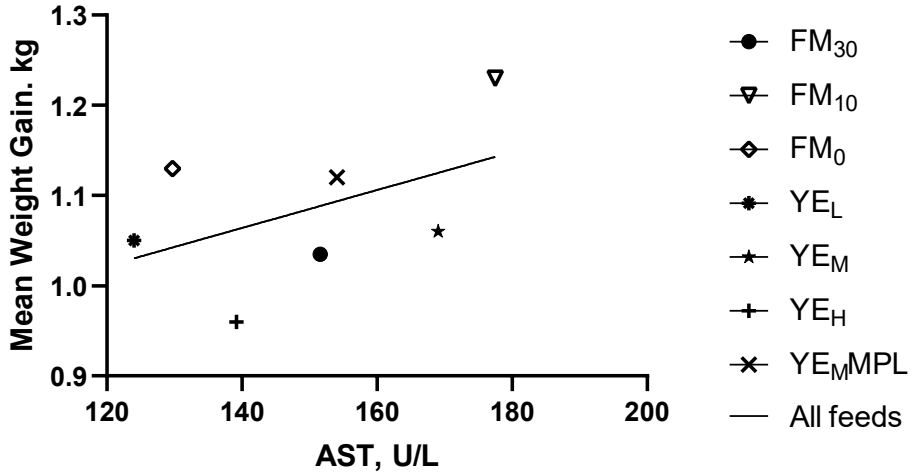


Figure 6: Mean AST plotted against mean weight gain shows a significant positive correlation between increasing AST and performance as measured by total weight gain ($F_{(6,14)}$ = 0.397,p= 0.048, r^2=0.23).

Discussions and Conclusions

The results from the present study represent a good example of the challenges of carrying out this kind of trial on a semi-commercial scale. The initial findings indicated no significant feed-related impacts on fish performance including growth rate, mortality and feed conversion ratio when feeding either 0, 10 or 30% fishmeal with or without the yeast hydrolysate product. Furthermore, supplementation of the feed with marine phospholipids also did not result in significantly enhanced growth or performance of the fish. These findings were contrary to those found in available literature, with Oliva-Teles & Gonçalves (2001) establishing that protein from *S. cerevisiae* yeast can successfully replace up to 50% of fishmeal protein without negative effects on fish growth performance. When isolating the 0% fishmeal diets into a smaller group, it can be observed that increasing the content of yeast hydrolysate resulted in numerically (but not significantly) poorer growth, culminating in the worst performance from the diet containing the most yeast product. This was further supported by the size of the fish sampled for flesh quality and morphometric testing being significantly smaller in response to increasing yeast hydrolysate level in the feed.

Peptide analysis revealed that the non-fishmeal feeds with elevated small soluble peptide inclusion (linked elsewhere to improved palatability) did not promote improvements in weight gain or average feed intake. Thus, it can be said that inclusion of this hydrolysate product did not compensate for a reduction of fishmeal in the test feeds. At this stage, it is not possible to confirm that, in principle, supplementation with peptides might not be beneficial but, for what ever reasons, the current hydrolysed yeast product is not a suitable vehicle for those peptides.

Significant differences were observed in the sum of the omega-3 fatty acids, EPA and DHA (% of total fat), with data indicating a strong relationship between feed and fish levels amongst salmon fed the fishmeal-containing feeds. This would be in line with expectations. However, salmon fed feeds with additional yeast did not display this relationship indicating little or no impact in this regard and this was an unexpected outcome. Of note, the levels of total fat, total astaxanthin/pigment as well as the highest SalmoFan scores and flesh chroma (colour intensity) and redness (Minolta a*) were found amongst salmon fed the YE_MMPL feed. These data indicate advantageous impacts for pigmentation and so, the use of marine phospholipids as a tool for flesh quality improvement merits further exploration.

Blood plasma analysis yielded some convoluted results with no clear relationship between an increase in feed yeast hydrolysate content and the significant differences recorded in thyroxine or uric acid levels. There were only significant differences in the aspartate aminotransferase values observed between the salmon fed feed with 10% fishmeal and those without fishmeal but with the highest amount of yeast hydrolysate. There was a weak but significant relationship between plasma AST levels and weight gain. This is contrary to expectations since, as a commonly applied liver function test, elevated AST is assumed to be indicators of liver damage in fish as well as other livestock (Zou *et al.,* 2016).

Overall the findings from this study indicate that the addition of the yeast hydrolysate alone or in conjunction with a marine phospholipid extract did not improve the performance of fishmeal-free feeds when fed to Atlantic salmon in a semi-commercial environment. Whilst this proprietary yeast hydrolysate is deemed to have limited potential for inclusion within Atlantic salmon feed, there remains some merit in further exploration of the value of marine products including MPL supplements as vehicles to improve salmon pigmentation.

References

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