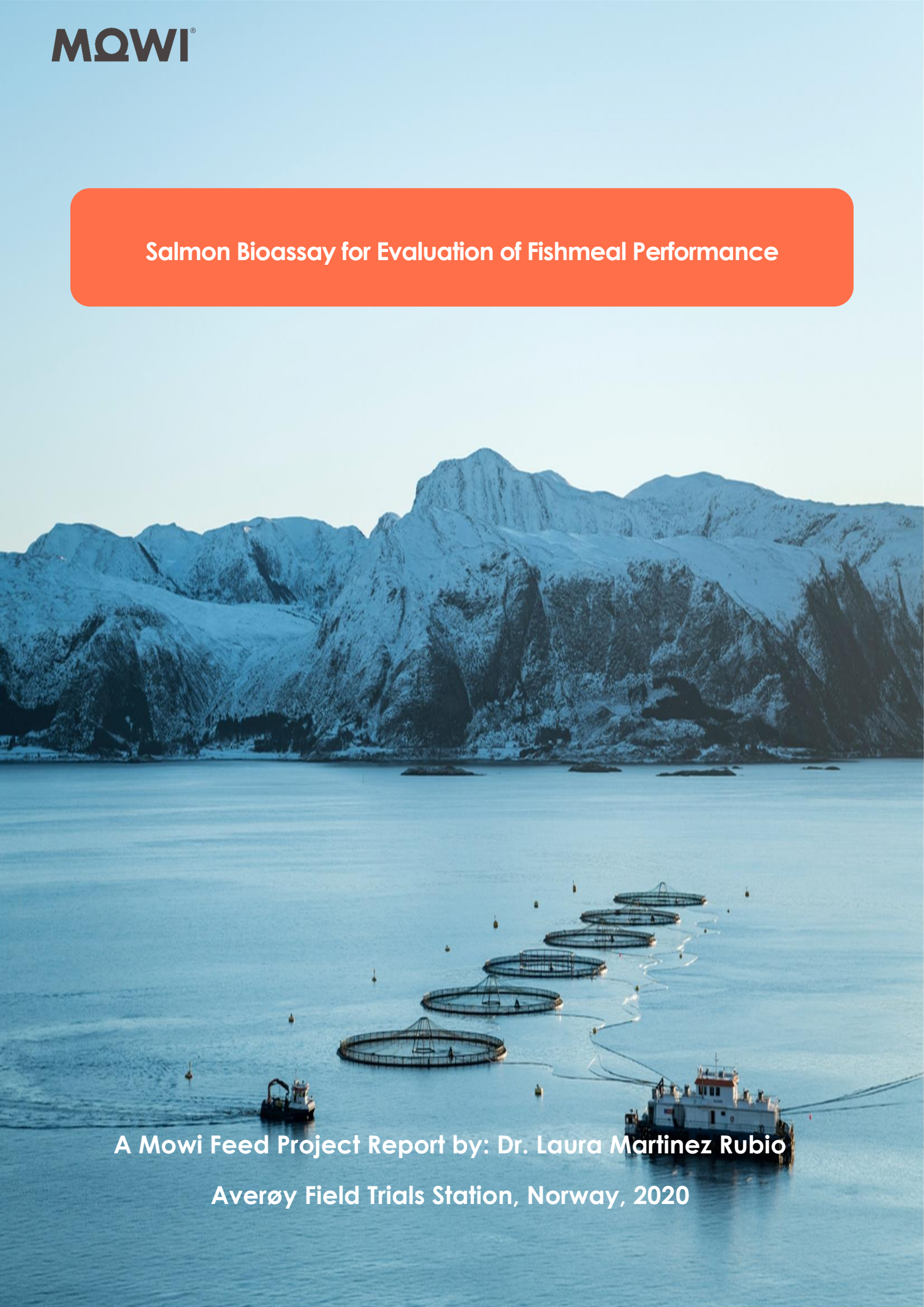
Salmon Bioassay for Evaluation of Fishmeal Performance



A Mowi Feed Project Report by: Dr. Laura Martinez Rubio

Averøy Field Trials Station, Norway, 2020

Introduction

The quality of fishmeal depends on multiple factors including: fish species; ocean conditions where the fish was caught; seasonal effects; and more importantly, preservation methods that influence the processing, yield and nutritional value of these products. (Einarsson et al. 2019)

Maintaining the freshness of fishmeal is the main challenge for producers as enzymatic degradation of the protein in the raw material before processing starts right after the animal's death and continues during the spoilage of the fish. Biogenic amines (i.e. anisidine), ammonia bases, trimethylamine (TVNs), dimethylamine, and other components produced at that stage, can be indicative of the extent of the spoilage thus they are measured and used as purchasing criteria when buying fish meals.

Increasingly, the need to make salmon feeds more sustainable has led to substantial reductions in the inclusion of fishmeal in most salmon feeds. However, a complete elimination of fishmeal from the feed is not yet achievable without some inconsistency in the levels of performance, robustness and quality of the salmon raised. Thus, the fishmeal that remains in commercial salmon feeds plays an important role to maximise the growth of the salmon and its quality has to be periodically reviewed in our research programme.

Whilst laboratory evaluation of key fishmeal characteristics is suitable for quality control and commercial management, a bioassay of fishmeal represents an all-encompassing tool for comparison of fishmeal properties. With the fishmeal content of feed being much reduced, small changes in fish performance and / or quality may be masked by the underlying variability that occurs in bioassay. On that basis, a bioassay on fishmeal should be carried out using high levels of the test material. The objective for this project was assess whether, having accommodated all the common quality control variables and differences in face value nutrient content, there are factors that could affect performance and health of salmon at grower stages when using feeds with a very high (by today's grower feed standards) fishmeal content.

Materials and Methods

Fishmeals

For this trial we selected four different fishmeals that were representative the fishmeals available for salmon feed production in Europe. Table 1 shows the manufacturer-supplied assumptions for proximate composition at the time of purchase. Only one fishmeal originated in the USA, the rest were all from Scandinavian suppliers using raw material as indicated in Table 1.

Table 1. Data provided by the suppliers of the fish meals used in the salmon bioassay

Name	Main feature	Protein	Fat	Ash	Moisture
MS	Mix of different species	71.8	9.1	14.3	7
BW	Blue whiting 100%	73.3	8.83	13.91	3.96
TO	Trimmings only	67.2	10	15.4	8.6
USA	USA origin	64.3	9.2	19.7	8.3

Prior to formulation of the test feeds, the formulation matrix assumptions for each fishmeal were updated using the results of analysis (Nofima BioLab) as indicated in Table 2. As shown in Table 2, there were small but, important discrepancies between the measured values and those provided by the suppliers. Notable in terms of the protein content would be the determination of protein by the Dumas method by the supplier and reanalysis by Kjeldahl as required by EU regulations for feed manufacture.

Table 2. Macronutrient composition of the fishmeals established by at NOFIMA Biolab.

Name	Protein	Fat	Ash	Moisture
MS	69.7	11.1	13.7	6.5
BW	72	10.9	14.7	6.3
TO	67.6	11.2	15.3	7.8
USA	63	10.3	20	8.5

Feeds

Four feeds with equal specifications for digestible protein (DP) and digestible energy (DE) were formulated including approximately 30% of each fish meal with the balancing totals of DP and DE, amino acid, fatty acid and micronutrients provided by broadly similar inclusion of the other raw materials. Table 3 shows the formulations of the four feeds. The feeds were manufactured on a pilot scale at the Nofima Technology Centre in Bergen, Norway.

Table 3. Formulations of the for diets

Raw materials (%)	USA	TO	BW	MS
Fishmeal mix species	0	0	0	30
Fishmeal trimmings	0	30	0	0
Fishmeal USA	31.93	0	0	0
Fishmeal blue whitting	0	0	30	0
Soya protein conc.	5.34	9.63	7.20	8.49
Wheat gluten	18	15	15	15
Wheat, whole	9.41	10.20	12.74	11.38
Beans dehuled	5	5	5	5
Fish oil	10.38	12.87	12.78	12.68
Vegetable oils	17.17	14.52	14.33	14.47
Vitamins & carotenoids	0.652	0.590	0.575	0.575
Minerals	0.905	1.116	1.124	1.113
Synthetic amino acids	0.811	0.551	0.432	0.454
Yeast derivatives	0.4	0.4	0.4	0.4
Other	0	0.12	0.41	0.44

Fish and husbandry

The feeding trial was conducted at Mowi's Averøy Field Trials Station in Norway between May and October 2019. Atlantic salmon (mean weight 900g) were randomly distributed amongst 16 marine net pens (5x5x5m; 125 m3) at an abundance of 100 fish per pen. The fish were given a period of 7 days acclimation to environmental conditions whilst fed a commercial feed before the study started. Upon commencement of the 146-day feeding period, the fish were fed one of 4 feeds (Table 3) with 4 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 ratio were calculated. Fish health and welfare were monitored daily following Mowi's standard procedures. Water temperature spanned a range between 8.5 °C (May) and 16 °C (August) and a natural photoperiod was observed.

Fish sampling

At the end of the 146-day feeding period, all fish were individually weighed and counted.

The apparent digestibility of key nutrients in the feed including protein, amino acids and energy was determined by reference to an indigestible marker and with faeces collected from euthanised fish by stripping.

Further post-mortem assessments was carried out on 5 salmon / pen (20 fish / feed type) for a histopathological appraisal. From those fish, collection of different sections of the intestine (pyloric caeca, mid intestine, distal intestine) and the liver was performed.

Results and Discussion

Nutrient composition of the different feeds

Analyses of key nutrients in the feeds showed very little differences between the 4 feeds used in this trial (Table 4) with a satisfactory correlation between formulated and actual outcomes. The content of docosahexaenoic acid (DHA) of the USA feed was slightly lower than that of the other feeds (3.4% vs 4.0-4.3% g/100g fat) resulting in feeds with an EPA+DHA content of around 6.4% of the fat in the USA feed (7.1 to 7.4% for the other 3 feeds). Our expectation is however, that all four feeds still provided sufficient EPA+DHA to meet the minimum requirements for essential fatty acids and that any differences in fish performance were still driven by fishmeal quality rather than by dietary EPA+DHA content

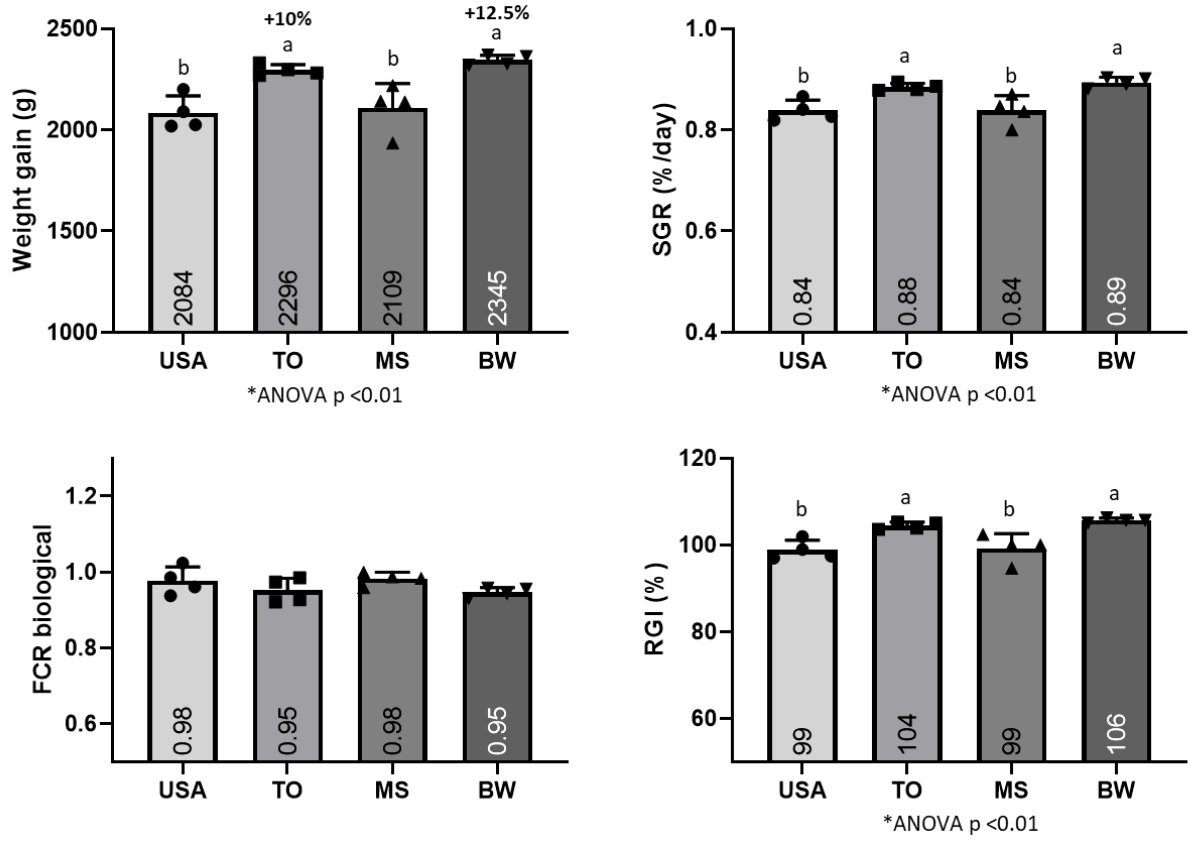
Table 4. Nutrient composition of the feeds.

	USA	TO	BW	MS
Protein (%)	42.1	43.1	42.3	41.9
Fat (%)	31.5	31.7	31.1	31
Moisture (%)	6.4	6.2	7	6.8
Ash (%)	7.5	6.1	5.6	5.7
Asta (mg/kg)	61	61	62	61
Phosphorous (%)	1.4	1.2	1.1	1.1
Choline (mg/kg)	3096	3141	3006	2909
Fatty acids				
EPA (g/100g of Fat)	3	3.1	3.1	3.1
DHA (g/100g of Fat)	3.4	4.3	4.3	4
EPA+DHA (g/100g of Fat)	6.4	7.4	7.4	7.1
Saturated (g/100g of Fat)	14.3	14.7	13.5	14
Monounsat (g/100g of Fat)	48.1	48.1	46	48
PUFA (n-6) (g/100g of Fat)	12.7	11.5	11	11.1
PUFA (n-3) (g/100g of Fat)	17.4	18	17.4	17.3
n-6/n-3	0.74	0.64	0.63	0.64
Amino acids				
Lysine (g/100g)	2.4	2.4	2.4	2.5
Methionine (g/100g)	0.9	0.93	0.89	0.92
Threonine (g/100g)	1.5	1.5	1.5	1.5
Histidine (g/100g)	0.82	0.85	0.78	0.85
Valine (g/100g)	1.7	1.8	1.7	1.8
Isoleucine (g/100g)	1.6	1.7	1.6	1.7
Leucine (g/100g)	2.7	2.8	2.8	2.9
Phenylalanine (g/100g)	1.7	1.7	1.7	1.7
Arginine (g/100g)	2	2.1	2	2.2
Aspartic acid (g/100g)	2.7	2.9	2.9	3
Glutamic acid (g/100g)	8.7	8.2	8.3	8.4
Hydroxyproline (g/100g)	0.46	0.29	0.31	0.3
Serine (g/100g)	1.7	1.8	1.7	1.8
Glycine (g/100g)	2.2	2.1	2	2
Alanine (g/100g)	1.7	1.7	1.7	1.7
Proline (g/100g)	3.2	2.9	2.8	2.9
Tyrosine (g/100g)	0.96	0.99	1.1	1.1
Cysteine (g/100g)	0.66	0.6	0.59	0.61
Starch (%)	9.47	9.9	10.9	11
Fibre (%)	0.76	0.83	0.8	0.9

Fish performance

Overall, a satisfactory rate of growth was achieved with the relative growth index (RGI, Mowi's model for fish performance) indicating growth rates between 99 and 106% of expected levels. Salmon given the feeds containing the blue whiting (BW) and trimmings-only (TO) fishmeals gained more weight and at a significantly higher rate than those fed the American (USA) and mixed species (MS) fishmeals. However, the utilisation of the feeds (FCR), was not significantly influenced by feed type (Figure 1)

Figure 1. Performance results expressed as weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and relative growth index (RGI)



Digestibility and estimated digestible nutrient levels

There were no significant differences (ANOVA) in the apparent protein and fat digestibility of the four test feeds (Table 5). However, the apparent protein digestibility of the USA feed was numerically the lowest in this study.

Table 5. Apparent Digestibility coefficients (ADC) (%) of the different diets based on the faecal collection.

ADC (%)	USA	TO	BW	MS
ADC Protein	86.56	88.27	88.51	89.26
ADC Fat	95.72	96.13	94.78	95.82
ADC LYS	91.09	92.25	91.13	90.86
ADC MET	88.84	91.19	89.78	90.52
ADC THR	87.91	87.89	87.26	86.30
ADC HIS	90.02	90.37	89.50	89.34
ADC ARG	90.19	94.03	91.95	93.38
ADC VAL	91.14	91.38	89.99	90.58
ADC ISO	92.50	92.22	90.67	91.54
ADC LEU	92.73	92.60	91.61	92.04
ADC PHE	91.58	91.72	90.47	90.86
ADC ASP	82.10	79.41	78.99	75.37
ADC GLU	94.76	95.01	93.86	94.54
ADC HYD	50.48	92.06	92.08	91.40
ADC SER	88.19	89.19	87.69	88.11
ADC GLY	77.18	87.01	87.31	85.80
ADC ALA	84.80	89.62	88.33	88.46
ADC PRO	91.34	94.06	92.91	93.55
ADC TYR	90.02	89.83	89.74	90.37
ADC CYS	70.83	75.14	74.57	70.28
Sum ADC AAs	1546	1615	1598	1593

In order to establish if fish growth was correlated with digestibility and / or the net content of digestible protein and amino acids, the ADC outcomes (Table 5) were applied to the dietary amounts of crude protein and amino acids (Table 4) to establish the actual DP and DAA content of the test feeds. (Table 6).

When ranked in terms of crude protein (nitrogen) digestibility, the feed containing the American (USA) fishmeal was the least digestible but, in terms of the sum of digestible amino acids (g/kg feed), this feed was ranked only 3rd. A high protein digestibility (1st place) coupled to a relatively poor apparent digestibility of the individual amino acids (3rd place) still resulted in an overall first place in terms of contribution to the g digestible AAs in the feed made with mixed species fishmeal (MS). Ultimately, the feeds containing blue whiting (BW) and the trimmings-only fishmeal (TO) accumulated the lowest (4th place) and 2nd highest (2nd place) ranks with regards content of digestible essential amino acids. Regression analysis of the data for digestible protein and amino acid content and fish performance outcomes indicated that neither the apparent digestibility coefficients of protein and essential amino acids (% of the protein or amino acid) or the absolute amounts of DP and DAAs in the feed (g/kg feed) correlated with the growth performance observed. (Table 7).This leads to the assumption that feed performance was not linked to the supply of digestible protein and / or amino acids. A similar process was applied to the DE content of the feeds with the result that the feed

containing the American fishmeal was ranked 4th in terms of ultimate DE content (MJ/kg feed) and fish growth (weight gain) whilst the fastest growth was observed in the fish fed the feed with the 3rd ranked feed in terms of DE content (BW). Thus, it appears that fish growth was not correlated with the dietary energy of the feeds.

Table 6. Calculated outcomes for digestible energy (DE) digestible protein (DP) and all essential and non-essential amino acids. Ranking based on those values is included on the right for a more comprehensive understanding.

	USA	TO	BW	MS	USA	TO	BW	MS
DE (MJ/kg as fed)	21.91	22.47	22.08	22.20	4	1	3	2
DP (g/kg)	364.17	380.14	374.36	373.75	4	1	2	3
DP/DE ratio (g/MJ)	16.62	16.92	16.96	16.84	4	2	1	3
DLYS (g/kg)	21.86	22.14	21.87	22.71	4	2	3	1
DMET (g/kg)	8.00	8.48	7.99	8.33	3	1	4	2
DTHR (g/kg)	13.19	13.18	13.09	12.95	1	2	3	4
DHIS (g/kg)	7.38	7.68	6.98	7.59	3	1	4	2
DARG (g/kg)	18.04	19.75	18.39	20.54	4	2	3	1
DVAL (g/kg)	15.49	16.45	15.30	16.30	3	1	4	2
DISO (g/kg)	14.80	15.68	14.51	15.56	3	1	4	2
DLEU (g/kg)	25.04	25.93	25.65	26.69	4	2	3	1
DPHE (g/kg)	15.57	15.59	15.38	15.45	2	1	4	3
Total DEAAs	139.36	144.88	139.16	146.13	3	2	4	1
DASP (g/kg)	22.17	23.03	22.91	22.61	4	1	2	3
DGLU (g/kg)	82.44	77.91	77.90	79.41	1	3	4	2
DHYD (g/kg)	2.32	2.67	2.85	2.74	4	3	1	2
DSER (g/kg)	14.99	16.05	14.91	15.86	3	1	4	2
DGLY (g/kg)	16.98	18.27	17.46	17.16	4	1	2	3
DALA (g/kg)	14.42	15.23	15.02	15.04	4	1	3	2
DPRO (g/kg)	29.23	27.28	26.02	27.13	1	2	4	3
DTYR (g/kg)	8.64	8.89	9.87	9.94	4	3	2	1
DCYS (g/kg)	4.67	4.51	4.40	4.29	1	2	3	4
Total DNEAAs	195.87	193.85	191.33	194.18	1	3	4	2
Total DAAs	335.23	338.73	330.49	340.31	3	2	4	1

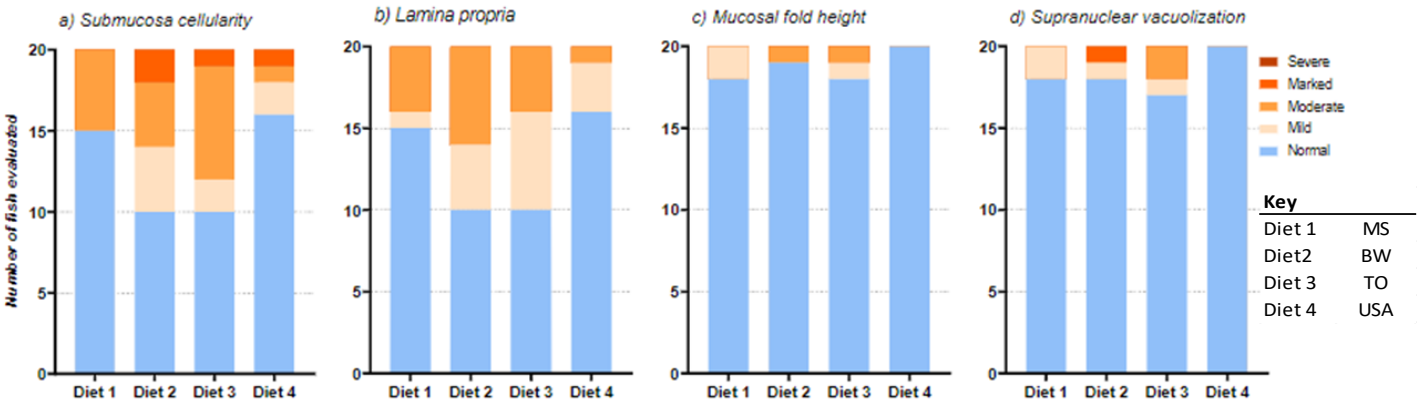
Table 7. Regression analysis showing the correlations between weight gain and digestible energy (DE) digestible protein (DP), digestible amino acids: essential (DEAA) non-essential (DNEAA) and total (DAA).

	Weight Gain	DE	DP	DP/DE	DEAA	DNEAA	DAA
USA	2084	21.91	364.17	16.62	139.36	195.87	335.23
TO	2296	22.47	380.14	16.92	144.88	193.85	338.73
MS	2109	22.2	373.75	16.84	146.13	194.18	340.31
BW	2345	22.08	374.36	16.96	139.16	191.33	330.49
R^2 value linear regression vs Weight gain		0.218	0.496	0.721	0.023	0.725	0.722
p-value		n.s	n.s	n.s	n.s	n.s	n.s

Gut health

The morphology of the pyloric caeca and mid intestine was predominantly normal with no differences observed relating to feed type. However, mild to marked inflammatory changes were observed in the distal intestine of some individuals from each of the feed groups. The inflammation was largely characterised by an infiltration of the submucosal layer (Figure 2a) and the lamina propria (Figure 2b) by inflammatory and immune cells. Reduction in mucosal fold height and loss of supranuclear vacuolisation by enterocytes constituted part of the morphological changes in relatively fewer individuals (Figures 2c, and 2d, respectively).
25% of the salmon fed feeds MS and USA, exhibited moderate (Feed MS) or mild to marked (Feed USA) inflammatory changes.

Figure 2. Number of distal intestine tissue sections that were scored to determine the health status of this tissue.



A histological survey of the salmon prior to the start of feeding indicated inflammation in approximately 25% of the individuals indicating that a degree of inflammation was already evident in the population. The results of the current trial are not considered to be extra ordinary under normal farming conditions.

Conclusions

All four of the test feeds were formulated to yield similar outcomes for key nutrients including digestible protein, energy, amino acids and fatty acids and with broadly comparable approaches to the feed material composition. Despite this, the bioassay technique revealed that attention to formulation outcomes for nutrients alone is insufficient to eliminate the impacts of more subtle quality differences between fishmeal types.

The outcomes also indicate that whilst the lower protein content of the American fishmeal (USA) was accommodated in the formulation resulting in levels of DP, DE and DAA in the feed that was not significantly different from that of the other feeds, this fishmeal was associated with inferior performance to that of the leading two NE Atlantic fishmeals i.e. TO and BW

(though, no poorer than that of the poorer performing European fishmeal, MS). Additionally, the data indicated that, despite its lower content of crude protein and higher fat level, the trimmings-only (TO) fishmeal was capable of supporting growth on a par with that of the single-species, blue whiting fishmeal (BW).

The survey of the gut tissues indicated that a return to fishmeal contents in the order of 30% did not eliminate subtle but, noticeable signs of gut inflammation. Indeed, the feed materials chosen to accompany the fishmeal in the test feeds would all be considered to be low risk in terms of anti-nutrient content raising a question regards what really constitutes a "normal" appearance for farm raised salmon.

All three of the fishmeals from the NE Atlantic (TO, MS and BW) broadly fell into the classification "NSM" according to the fishmeal marketing criteria and should have supported similar performance in the fish. A return to applying what would now be considered very high fishmeal levels represents a tool for fishmeal evaluation though, the slow turnaround time for this form of bioassay limits its commercial relevance at least, if making judgments on fishmeal on a lot-by-lot basis.

References

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