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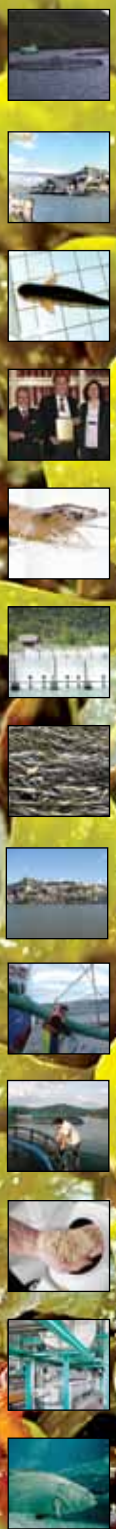

Seaweeds as Ingredients in Aquatic Feeds

A new and revolutionary type of feather meal for fish feed

New dimension in the production of hygienized feed meal

Chromium

- the forgotten mineral that plays an essential role in the utilization

THE INTERNATIONAL MAGAZINE FOR THE AQUACULTURE FEED INDUSTRY

Seaweeds as Ingredients in Aquatic Feeds

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Powdered seaweed or seaweed flour is already used as an ingredient in terrestrial and aquatic feeds. The seaweed is usually a single species and publications over the years have demonstrated a range of benefits - for instance improved resistance to viral and bacterial pathogens.

There are many species of seaweed and they contribute a wide range of 'micronutrient' benefits as well as a reasonable quantity of protein with a good amino acid balance and oil with high n-3 content.

The polysaccharides in seaweeds contribute to animal health in several ways, helping for example to promote gut health. It is also theoretically possible to replace all of the added premix nutrients with seaweed, thereby making a feed that is entirely 'chemical free'. The seaweed powder described is a carefully balanced mix from several species of seaweed, this product being made by Ocean Harvest Technology Ltd with the name 'Oceanfeed®'.

Table: Table 1: Proximate Analysis

	%
Moisture Content	10%
Crude Protein	12%
Crude Fat/Lipid	4%
Crude Fibre	5%
Ash	18%

After several years of research it is now possible to achieve a 'chemical free' feed by adding sustainably harvested seaweeds. This concept has been recently tested on Atlantic Salmon with some very interesting results not only in terms of the effect of the seaweed enhanced feed but also what can be achieved through careful husbandry.

Trial protocol

The trial was run at the Trial Feed Unit owned and operated by Marine Harvest (Scotland) at Loch Eilort, 40 miles north of Fort William on the Scottish west coast. The feeds, both reference and test, were manufactured by EWOS (UK) Ltd at their processing unit in Westfield, Scotland. Thanks are extended to both companies for the very professional, thorough and helpful way they contributed to this trial.

The fish were mixed-sex Atlantic Salmon smolts with a starting weight of 145gms. The two diets each had three replicates, each replicate being 625 fish (making allowance for post transfer mortalities) in 5.25m2 cages, six cages in total with a target starting number of 3600 fish in total. Each cage was fitted with Sterner feeders controlled by an Aquasmart AQ300 adaptive feed control system. Fish were fed to satiation,

Table 2: Amino acid profile of the protein from the seaweed mix

Amino Acid	Requirement	Oceanfeed®
Met(+Cys)	1.7(3.3)	1.4-2.6
Lys	4.8	3.5-4.4
Trp	0.6	0-0.3
Thr	2.0	4.5-6.8
Ile	2.0	2.3-3.6
His	1.6	1.2-2.1
Val	5.3	4.0-5.1
Leu	3.6	4.6-6.7
Arg	4.4	4.3-8.7
Phe(+Tyr)	2.7(5.3)	5.0-7.1

light regime followed natural photoperiod and water temperature was ambient.

The cages were inspected daily for signs of fouling and damage and, when required, were lifted periodically for power washing. Temperature and salinity were recorded at five and 10 metres depth on a daily basis (note the cages were five metres deep), and mortalities were recorded on a daily basis.

Fish were sampled and assessed against standard farming KPI's (Key Performance Indicators). Evaluation included growth rate, feed conversion ratio (FCR), condition factor, yield, pigmentation, fat analysis, lice counts, pigmentation, lipid content and profile, flesh flavour and flesh texture.

Samples of whole guts were taken for microbiological evaluation and samples of lower intestine were taken (being immediately fixed in buffered formalin) for histological examination.

Scottish Quality Cuts (SQC's) of flesh were taken for lipid analysis, lipid profile, protein analysis and pigment analysis. Thanks are extended to the Institute of Aquaculture, University of Stirling, for its assistance in analysing samples.

Table 3:

Fatty Acid	% of Total Lipid
14.0	1.1-4.99
14.1(n-5)	0.29-0.93
16.0	8.21-33.41
16.1(n-7)	1.02-2.46
16.4(n-3)	2.25-14.63
18.0	0.53-5.64
18.1(n-9)	0.67-5.96
18.1(n-7)	3.04-12.79
18.2(n-6)	0.63-5.47
18.3(n-3)	3.1-11.14
18.4(n-3)	1.62-20.44
20.5(n-3)	0.44-3.25
22.5(n-3)	0.4-1.84

Analysis of seaweed

The following shows typical analytical profiles for the mixture of seaweeds (Oceanfeed®) used to make the salmon feed in the trials. First looking at Proximate Analysis (see Table1):

Now look at the amino acid profile of the protein from the seaweed mix. (see Table 2)

Although the total crude lipid from the Oceanfeed® is low at four percent, the fatty acid profile of this lipid is very interesting, as can be seen in Table 3.

The vitamin profile of the seaweed mix is equally interesting, compared to a typical fish feed (see Table 4).

Finally, the mineral profile of Oceanfeed®,

Table 4:

Vitamin	Oceanfeed®	Fish Feed	Units
Vitamin A Retinol	1000	3000	IU/kg
Vitamin B1	1-5	30	mg/kg
Vitamin B2	5-10	45	mg/kg
Vitamin B6	0.1-0.5	45	mg/kg
Vitamin B12	100-6300	0.08	mg/kg
Vitamin C	50-1650	188	mg/kg
Vitamin D3	1000-2000	1000	IU/kg
Vitamin E	260-450	188	mg/kg
Vitamin K	10	30	mg/kg

Table 5:

Mineral	Oceanfeed®	Fish Feed	Units
Manganese	60-347	9.0	mg/kg
Iron	622-1372	27.0	mg/kg
Zinc	12-37	54.0	mg/kg
Copper	4-10	18.0	mg/kg
Iodine	160-780	0.9	mg/kg
Selenium	0.3-1.0	0. 15	mg/kg
Calcium	2,100-7,100	2,000	mg/kg
Molybdate	2-10	0.08	mg/kg
Vitamin K	10	30	mg/kg

again compared to a typical fish feed, is shown in Table 5.

Formulation of feed for the trials

The reference feed was an Organic Salmon feed and the test feed was a very simple feed containing fishmeal, fish oil, Oceanfeed® and wheat. Note that no premixes, pigments or any other 'chemical' additives were used in the test feed. The organic feed had included premixes with typical levels of vitamins, minerals and pigment.

Growth rates

The Phase I diet was fed until October 2009 from June 2009. As expected the proximate gap between the two formulations produced a higher level of growth in the reference population. Diets were switched in October 2009 through to June 2010, with test diet having 15 percent Oceanfeed®. This diet had same protein as the reference diet, but oil content of the Oceanfeed® diet was still lower. Despite this growth rate of the Oceanfeed® fish accelerated with a cumulative difference showing Oceanfeed® diet having 14 percent higher growth rate than the Organic Diet. The differences in proximate analysis are an expected issue when squeezing short runs of product into a busy commercial mill, but despite this performance of the Oceanfeed® diet is significantly better.

Feed Conversion Ratio (FCR)

The FCR for the organic diet was 0.979kg feed per kg whole fish. For the Oceanfeed® diet FCR was 0.957kg feed per kg whole fish, 0.025 percent better than the reference diet. The yield of fish were different, where fish are weighed, gutted and weighed again. Gutted weight as a percentage of whole fish weight is percent yield. For the organic diet this was 88.44 percent. For the Oceanfeed® diet this was 89.83 percent. If FCR is calculated looking at the weight of gutted fish per kg of feed this difference in yield shows a further gap between the two diets. This FCR proc. number was 1.19kg feed per kg gutted fish for the organic diet and 1.16kg feed per kg gutted fish for the OceanfeedTM Diet, a difference of 2.6 percent.

Stock average weight difference

The second phase of the feeding trials adjusted the diet for several reasons, one being the difficulty in making a feed robust enough to sustain physical stresses of handling in a traditional feed plant, where the first feed tended to break up in handling, resulting in lower growth rates and high stock average weight difference. When the feed was changed

Table: 6 Proximate Analysis, carried out at the University of Stirling

	Oceanfeed® Phase 1	Oceanfeed® Phase 2	Organic Phase 1	Organic Phase 2
Moisture Content %	11.24	11.2	6.59	6.6
Protein Content %	42.14	43.3	43.09	43.2
Oil Content %	22.61	2.8	24.78	25.0
Ash Content %	17.56	15.9	10.92	10.9
Fibre Content %	1.03	2.0	3.15	3.6
Carbohydrate Content %	6.94	9.0	13.09	13.1
Total n-6 PUFA % of oil	3.23	3.43	4.28	4.18
Total n-3 PUFA % of oil	30.15	29.87	25.95	24.00
Total PUFA % of oil	37.93	37.71	32.08	30.1

Note: that two production runs of the feeds were made. The first batch of test diet had 25% of Oceanfeed® and the second batch 15%. The consequence of running a small trial batch of product on a large plant led to the first batch having lower protein and lower oil than the reference diet. Also during handling and storage the first batch tended to break up into small pieces. For these reasons the second batch was formulated with 15% Oceanfeed® which made a stronger product. The reformulation also brought back protein and oil to similar levels when compared to the reference product.

to a much more physically robust feed it was interesting to monitor improved growth rates (14 percent better than reference) and a significant drop in average weight difference, as show in the following graph.

The OceanfeedTM average fish weight at this time was 4.065kg, with fish growing 14 percent faster than reference and FCR 2.6 percent lower (or better).

Flavour and texture

A focus group was selected from a group of individuals experienced in organoleptic evaluation of salmon. A score chart was constructed so that the fish could be marked on a points basis from 1 (excellent) to 5 (poor). The evaluation criteria were appearance, texture, colour, smell and taste for both raw and cooked fillets of salmon. The OceanfeedTM fish scored 2.1 and the organic-feed fish scored 3.0, with all panellists noting a significant improvement in the OceanfeedTM fish on taste and texture.

Mortality

Of the populations of 1800 fish on each diet OceanfeedTM scored 19 mortalities and the organic diet scored 53 mortalities. There was a consistent pattern throughout the trial with no single factor being responsible for the mortalities. This is 1.09 percent mortalities for OceanfeedTM and 2.9 percent for the organic feed. Anecdotal observation suggest that the OceanfeedTM fish were healthier and more active, for instancing reviving much more quickly from anaesthetic when being sampled for length and weight measurement.

"Comment about flesh texture and flavour after cooking was that the Oceanfeed® salmon had a lighter more delicate flavour with no oily overtone, the flavour being similar to wild caught salmon."

Intestinal morphology

Samples of intestine were taken at mid-point and were dropped into buffered formalin immediately post mortem. The samples were sectioned and stained at the University of Stirling. The following pictures show a healthy cross-section of the intestinal lumen, with the

Table 7: Weight gain ratios

	Seaweed Diet	Reference Diet	Difference
Starting Weight (g)	162	158	-
Weight at 4 weeks (g)	348	372	6.9%
Weight at 14 weeks (g)	809	964	19.2%
Weight at 22 weeks (g)	1,108	1,301	17.4%
Weight at 24 weeks (g)	1,380	1,640	18.8%
Weight at 40 weeks (g)	3,111	3,279	5.4%

intestine well filled with a complex folded lining. There was no observable difference between the intestinal morphology of both populations. As a comparison a slide is shown from a salmon fed a diet high in soybean meal, showing the lumen poorly filled with very few folds on the intestinal wall.

"Of great importance in today's climate is the ability to introduce to the public a step forward in the use of sustainable ingredients that help create, with the right husbandry, a healthier happier and tastier product"

Sea Lice

All through the trial regular samples were taken to evaluate sea lice (lepeophtheirus salmonis) infestation. There had been an expectation that sea lice counts would be reduced on the OceanfeedTM fish, because of the previously known effect of the diet on stimulating the immune system and stimulating formation of a more viscous layer of mucous on the outside of the fish. Recruitment of juveniles seemed to be broadly similar in both populations, but there were significant differences throughout the trial in the count of adults, especially gravid females. Work is ongoing using positive challenge with a version of OceanfeedTM specifically designed to combat lice infestation to follow up from these observations. The following chart shows a typical analysis of sea-lice, with green being the OceanfeedTM results and blue the organic or reference feed results. Note that all the fish were treated for lice several times during the trial when population levels in any of the cages approached statutory limits that require treatment. Further work in isolated systems that allow positive challenge work is ongoing.

Table 8: Analysis of Feed (expressed as mg/kg)

	Oceanfeed®	Organic Feed
Astaxanthin (Ax)	1.31	1.51
Beta Carotene	5.20	5.63
Lutein	0.98	0.12
Unidentified Esters	18.00	8.58
Total Carotenoids	25.49	15.84

Table 9: Analysis of fish flesh: (Salmofan as number; Pigment results expressed as mg/kg)

	Oceanfeed®	Organic Feed
Salmofan Colour	22.81	22.88
Astaxanthin (Ax)	1.17	1.23
Canthaxanthin (Cx)	0.04	0.04
Lutein	0.05	<LOQ
Total Carotenoids	1.26	1.28

Pigmentation

The organic diet contained a small quantity of natural pigment. Organic-farmed salmon do not require the deep flesh colour of 'standard' fish, a target being a Salmofan colour of approximately 23 for the organic fish. The OceanfeedTM diet contained only natural pigments contributed by the seaweed mix. Colour development for both populations was broadly similar with no statistical difference throughout the trial. Analysis of the pigments at Stirling University showed some interesting results, as shown below:

Pigment uptake was very similar in both populations. The higher level of total carotenoids in the OceanfeedTM diet may help to partly explain increased growth rates and reduced FCR's, these substances having an essential effect in, for instance, lipid metabolism.

Husbandry

The Marine Harvest Feed Test Unit at Ardnish has very low water flow rates, which would not be expected to produce a fish with firm flesh. This was not the case in this trial. The feeding system assured that all the fish would be fed to satiation. Stocking densities ranged throughout the trial from 2kg/m3 up to 20kg/m3. No grading of the fish took part at any time during the trial. Both populations of fish were in apparent good health but the Oceanfeed® fish had brighter scales, appeared to be much more active, had an excellent shape and had thicker mucous over the scales. The following picture shows the Oceanfeed® salmon.

Final harvest

The fish were harvested on August 8, 2010 and processed at the Marine Harvest factory in Fort William. They were further filleted and either vacuum packed fresh or smoked then vacuum packed by Highland Smoked Salmon in Fort William. Thanks are extended to both these companies for the highly professional and helpful way they helped with this project. The sides of salmon were distributed to a wide range of retailers, food service companies and chefs for evaluation of quality. The results that came back were almost all the same. Comment was made about light flesh colour. This was a 'natural' or 'organic' colour and obviously end users still seem to prefer the darker red colour, which can easily be achieved by using one of the accepted natural colouring sources available.

Comment about flesh texture and flavour after cooking was that the Oceanfeed® salmon had a lighter more delicate flavour with no oily overtone, the flavour being similar to wild caught salmon. It was also noted that flesh texture was firm, but not too firm. Almost 100 percent of the professionals who tasted these salmon asked when and where they could buy them from.

Conclusion

The results of this trial were encouraging in many ways. The Oceanfeed® diet seemed, by comparison to a very high quality organic salmon diet, to result in improvements in growth rate, FCR, Mmortalities, fish flesh flavour and texture and sealice infestation. This is despite lower oil and energy content throughout the trial. The use of dried seaweed as an ingredient in aquacultural feeds is well established, but this work takes the use of seaweeds to a higher level, where different species of seaweed are carefully formulated to make a complex mix with targeted performance improvements that will give the farmer better yields of healthy products that taste significantly better. Of great importance in today's climate is the ability to introduce to the public a step forward in the use of sustainable ingredients that help create, with the right husbandry, a healthier happier and tastier product.

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