BONUS OPTIMUS: WP6

Deliverable Report (D6.2-D6.4)

Lead Scientist preparing the Deliverable: Ivar Lund (DTU AQUA)

Other Scientists participating: None

Objective: Undersized blue mussels grown for mitigation purpose (WP 1-5) may have a potential as use in feeds for aquaculture as mussels contain high protein levels with an optimal amino acid composition and a good fatty acid composition.

Description WP 6- from the DOW:

Deliverables including month of delivery	**Month**
D6.1 Development of new cost-efficient technique for processing mussel meal.	14 –expected month 28
D6.2. Production of two different meal types (with or without byssus) for fish feed	16- expected month 24
D6.3. Growth of juvenile rainbow trout on processed mussel products	32
D6.4. Digestibility of rainbow trout on processed mussel products	32

BONUS OPTIMUS has received funding from BONUS (Art 185), funded jointly by the EU and the Innovation Fund Denmark, the German Ministry for Education and Science (BMBF), the Swedish Agency for Marine and Water Management and the National Centre for Research and Development, Poland

Deliverable D6.2.

Production of two different meal types (with or without byssus) for fish feed (Month 16).

Blue mussel byssus threads as a protein source in fish feeds

Blue mussels attach to the sediment or when grown on lines by byssus threads, which consists mainly of protein fibres and amino acids are the main molecular building blocks of the byssus. The byssal thread comprises a corrugated proximal section connected to the soft tissues of the mollusc, and a smooth distal part that is anchored to the substrate. Byssal threads comprise a significant part of the mussel production at harvest and an objective was to examine if byssus threads could be used as a protein source along with the mussel meat protein to increase sustainable use and to minimize processing costs by separation.

Results:

In autumn 2017 DTU Aqua, Section for Aquaculture obtained byssus threads from the Limfjord, DK and separated from the mussel prior to examination.
Byssal proteins are cross-linked by complexation with metals such as Zn2+, Cu2+ and Fe3+ which improves the integrity and structural strength of the byssus (Harrington and Waite, 2008; Lucas et al., 2002). Different processing methods were thus examined to convert byssus threads to a homogenous sludge and to break down protein bonding for further processing.

Autoclavation:
Byssus material was autoclaved and cooked in a steam pressure chamber at 110 C for various time periods up to 6 hrs.

Acid hydrolysis:
Hydrolysis of byssus material was carried out by treatment with acid. Acid hydrolysis with constant-boiling HCI at 110 "C for more 6 hrs was tested. Aditionally a 6 M HCl containing 50% acetic acid was tried in order to shorten the hydrolysis time

Freeze drying- lyophilisation:
After either autoclavation or acid hydrolysis the material was freeze dried for 24 hr. i.e a dehydration process used to preserve a perishable material.

Results - discussion

It was very difficult to break down the byssus threads by any of the above methods most likely due to the helix structure of the protein indicated.

Figure 1: Photos of byssus threads after various processing, picture right after autoclaving and homogenization

Table 1 indicate the overall proximate composition. Dry matter content was app. 14.2 %. N x 6.25 content was relatively high 13 % (w.w.) (87 % dm), while oil content was very low. The lipid content was comparable to one of a few other studies on byssus threads, i.e. Cook (1970) who reported a lipid content of 8% dry weight, which mainly comprises phospholipids (65%). Glycine was the main amino acid in the fibers with 18% of the total residues analyzed, followed by alanine 9–10%, glutamic acids/glutamine 8–9%; aspartic acid/asparginine 8–9%; arginine 7.5%, lysine 7%, and proline 6–7%.

Table 1: Proximate overall composition (% DM) of byssus threads from blue mussels

Blue mussel byssus threads	% DM
N x 6.25 (Protein)	87.8
Oil	1.1
Ash	14.2

For various potential feedstuff the conversion into protein is total Kjeldahl Nitrogen (analytical method) = N x 6.25, as average nitrogen (N) content in protein is usual 16%. However, this is confounded by the fact, that not all nitrogen is found in protein, but also in other compounds such as amides, free amino acids, peptides, nucleic acids, nitrogenous lipids, ammonium salts, nucleotides, nitrates, creatine, choline and secondary compounds, where it is referred to as non-protein nitrogen (NPN). In the case of byssus threads most N is arranged in protein helix fibre structure non useful for animal utilization.
A better way to analyze actual protein content is analysis of each single amino acid, the sum of amino acids reflect the actual protein content and could be used in comparison with the calculated protein content of the Kjeldahl N analysis.

So despite the relatively high level of calculated protein along with the difficulties in processing byssus threads and an unsuitable amino acid composition byssus threads were considered as having no interest for future use in fish feeds and therefore was not processed further into meals for fish feed studies.

Feed recipes and feed produced

Feed recipes and feed produced

Mussels produced for mitigation are of varying size, with varying shell thickness, thus more - fragile than mussels for human consumption, and mussels may easily be crushed during harvest and transport, which can reduce storage processing time before mussel will undergo qualitative deterioration. Mitigation mussels are often biofouled with other growing organisms a.o. sponges and barnacles, that may possibly influence on the nutritional composition and quality of the final product, which could limit the use as a fish feed ingredient. As fish opposite to poultry cannot utilize the calcium carbonate rich shell fraction, cheap methods are required to obtain the nutrients from the mussels.

Mitigation mussels obtained from the Limfjord were shipped to DTU Food. The entire unprocessed biomass was then crushed in a food grinder, juiced and centrifuged and divided into two parts and subsequent spray dried at two temperatures, respectively 75°C and 90°C. A conventional mussel meal obtained by the Triple Nine, Esbjerg, DK (fish meal factory) was used as a reference. The meal from these mussels were obtained by classical separation / processing of large consumption mussels by cooking and pressure to remove shells allowing the use of only mussel meat tissue, which were dried and grinded into a meal.

Before diet formulation the two experimental meals and the conventional reference meal were analysed for proximate composition and amino acid composition (Table 2). All 3 meals were sent to SPAROS, Portugal (research based fish feed producer) for production of 3 extruded feeds, pelletized as 3 mm. Each of the experimental diets (spray dried at 75 °C (JM75) or 90 °C (JM90) were formulated with either 24%- or 47-49 % inclusion of JM75mussel meal or JM90 mussel meal, which was partly substituted with the conventional mussel meal reference (CONV). The performance of the conventional mussel meal was tested in a reference diet without inclusion of the experimental meals.

The formulation of the diets were balanced with the other ingredients, so that diets were similar in crude protein and lipid content (Table 2).

Table 2. Proximate composition and amino acid content of tested mussel meals (g /100 g mussel meal)

	CONV	JM75	JM90
Protein	63.8	45.3	43.6
Fat	14.8	8.4	7.7
DM	93.9	93.0	92.6
Ash	6.9	26.9	29.9
Phosphorous	1.01	0.98	0.92

Amino acid	CONV	JM75	JM90
Essential			
Met	1.40	0.84	0.75
Val	2.79	2.00	1.80
Ileu	2.63	1.68	1.52
Leu	4.06	2.54	2.30
Phe	2.33	1.49	1.32
His	1.00	0.77	0.69
Arg	3.41	1.59	1.50
Thr	2.84	1.90	1.69
Ala	3.02	2.43	2.23
Lys	4.31	2.18	2.07
Cys	0.00	0.00	0.00
Non Essential			
HyPro*	0.17	0.06	0.06
Asn	0.00	0.00	0.00
Tau	0.72	1.76	1.50
Ser	2.59	1.61	1.45
Gln	0.00	0.00	0.00
Gly	3.32	2.69	2.60
Asp	6.48	3.96	3.61
Glu	7.84	4.14	3.77
Pro	2.45	1.39	1.28
Csn	0.09	0.09	0.1
Tyr	2.26	1.44	1.32
Trp	0.00	0.00	0.00
Sum	53.7	34.6	31.5
Analysed protein content (g/100 g algae meal)	63.8	45.3	43.6
Sum amino acids (% of protein)	84.2	76.4	72.2

The analysed protein and lipid content in CONV mussel meal were much higher than in the experimental meals, while ash content was much higher in the experimental meals, indicating content of inorganic substances, most likely shell fractions as particles less than 200 µm were part of the juice filtration fraction

As indicated in Table 2, the sum of analysed amino acids given as percentage of analysed protein content was higher for CONV than for JM 75 and JM 90. There was an additional effect of temperature, as spray drying at 90° C apparently decreased both the content of most individual amino acids and the overall protein content as compared with mussel meal dried at 70° C.

Results indicate, that some nitrogenous compounds but not proteins were present in the two experimental meals, which likely originated from the processing of the whole biomass, that

include byssus threads, sponges, barnacles etc. and shell remainings, that all contain some nitrogenous compounds. The sum of individual amino acids often may not equal the analysed protein content, which may have several causes apart from the above mentioned, firstly the conversion factor (N x 6.25) to obtain protein content may variate between ingredients of various origin, secondly some free amino acids may not be detected in the amino acid analysis.

Table 3. Inclusion level and proximate analysis of the experimental mussel meal diets

Diet Ingredients (%)	CONV	JM75	JM75	JM90	JM90
Mussel meal conventional	70.0	53.0	36.0	54.0	36.0
Juiced mussel, Spray dried 75 C	0.0	24.0	47.0	0.0	0.0
Juiced mussel, Spray dried 90 C	0.0	0.0	0.0	24.0	49.0
Wheat starch	21.4	13.9	7.3	12.9	5.1
Fish oil	7.6	8.1	8.7	8.1	8.9
Vitamin & mineral premix PV01	1.0	1.0	1.0	1.0	1.0
Yttrium oxide	0.01	0.01	0.01	0.01	0.01
Analysed composition (% W.W.)					
Crude protein	45.8	45.3	45.0	45.7	45.1
Crude lipid	17.9	17.3	17.1	17.6	17.2
NFE + fibre (substracted)	23.2	19.4	14.6	18.4	13.1
Dry matter (DM)	93.4	93.2	93.5	93.4	92.7
Ash	6.5	11.2	15.8	11.7	17.3
Energy (MJ/kg) (calculated*)	16.0	15.1	14.2	15.1	14.0

- Based on gross energy levels: Protein:17.1 MJ/Kg; Lipid:23.6; Carbohydrate: 17.0

Dietary levels of analysed crude protein and lipid content were similar between diets.

The supplementation of various inclusions of wheat starch to compensate for the difference in protein content between the 2 mussel meals lead to some difference in NFE content and consequently energy content. The very high ash content in JM75 and JM 90 meals (Table 2) lead to significant differences in ash content of the diets, which also additional affected gross energy, that varied from 14 MJ/kg – to 16.0 MJ/kg.

Exp. meals and formulated diets had significantly higher ash content than the CONV meal and diet. Microscopic examinations (Leica Mz125) identified very small crushed shell particles, that were not filtered during juicing. These shell components would add an indigestible component in the fish diet and may affect the utilization of the various nutrients and gut passage time. Thus a high ash content in diets for fish are normally not recommended, as ash will take up space for important nutrients and lower the possible energy content.

References:

Cook, M. (1970) Composition of mussel and barnacle deposits at the attachment interface. In R.S. Manly Adhesion in Biological Systems. New York Acawaite, demic Press, pp. 139-150

Harrington MJ, Waite JH. Holdfast heroics: comparing the molecular and mechanical properties of *Mytilus californianus* byssal threads. J Exp Biol. 2007;210:4307–4318

Lucas,J.M., Vaccaro E., Wait, J.H. A molecular, morphometric and mechanical comparison of the structural elements of byssus from *Mytilus edulis* and *Mytilus galloprovincialis*ournal of Experimental Biology 2002 205: 1807-1817.

Deliverable D6.3-6.4.

Growth; digestibility and nutrient mass balance in juvenile rainbow trout fed on diets from mitigation mussel meals

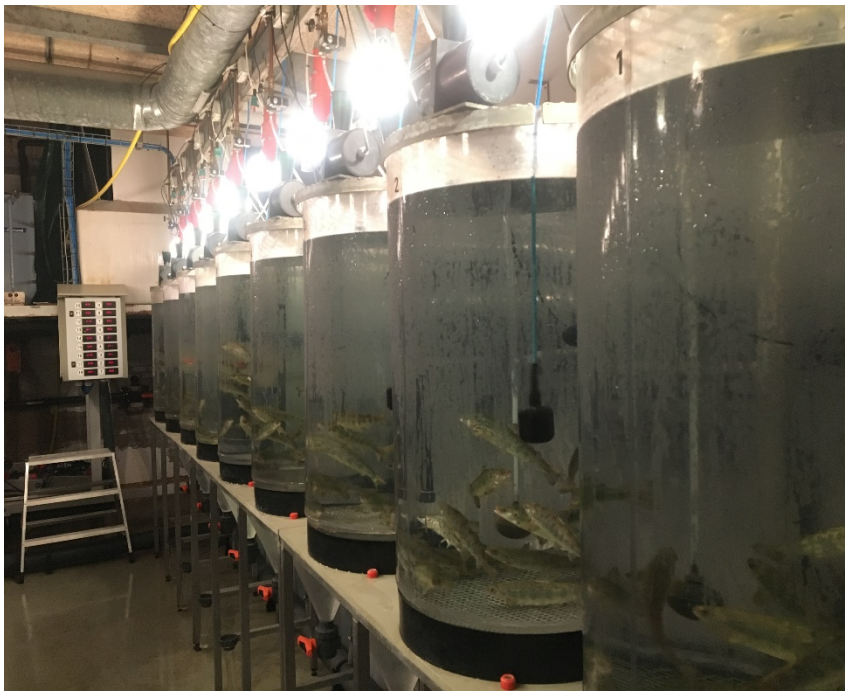
Diets produced in D6.2 were used in experiments with juvenile rainbow trout (Oncorhynchus mykiss). The experiments were performed at research facilities at DTU Aqua, Hirtshals and examined in vivo nutrient digestibility, growth performance, SGR, FCR and N, P mass balance calculations.

Materials & Methods

The growth and digestibility experiment lasted 12 feeding days and was designed as a fully random, single factorial experiment with three replicate tanks for each diet (i.e., n=3, 15 tanks in total) (Fig.1). Fish with an initial mean weight of 172±13.5 g were sorted from a larger batch of fish and randomly distributed among 15, 189 L, cylindrical-conical, flow-through, thermoplastic tanks at a stocking density of 12 fish $tank^{-1}$. The tank setup ensured that all faecal particles were collected in separated sedimentation columns submerged in ice-water as previously described (Dalsgaard and Pedersen, 2011). The tanks were supplied with 10° C tap water at a flow rate of app. 40 L h^{-1}. A 15 h light: 9 h dark diurnal photoperiod was maintained throughout the trial, and oxygen saturation levels were kept between 70 and 100 % during the experiment.

The fish were acclimatized to the experimental conditions and to the diets for 8 days prior to commencement of the experiment. They were individually weighed at the start of the experiment (day 0), and subsequently fed 1.2 % of the estimated biomass d^{-1} (calculated based on an expected FCR) for 9 days. The daily ration was divided into two equal portions, which were fed at 10:00 and 14:00 h, respectively. Feed waste was registered and counted throughout the trial to derive the exact feed intake. All faeces were collected daily prior to feeding at 10:00 h, and samples from each three consecutive days were pooled (i.e. yielding three faecal sampling periods) and stored at -20 °C until chemical analysis was carried out. Faeces from the second and third sampling periods were analysed for protein, lipid, dry matter (DM) and ash. The fish were individually weighed at the end of the digestibility trial (day 10) and returned to the tanks. The experiment was subsequently continued for additionally 3 days. Here they were fed a fixed daily ration for days corresponding to app. 1.2 % of the biomass measured at the end of the digestibility trial.. After the first days of this period, influent water was turned off for 24 h (and air diffusion turned on) and the waste produced was measured as the delta increase derived from water samples collected just prior to feeding at 10:00 and 24 hours later, respectively.

Fig. 1. Exp. facility for test of mussel meal diets on rainbow trout



Chemical analysis

Samples of the formulated diets were homogenized using a Krups Speedy Pro homogenizer and analyzed for dry matter and ash (NMKL, 1991), crude protein (ISO, 2005; crude protein = Kjeldahl N x 6.25), crude lipid (Bligh & Dyer, 1959) and total phosphorus (ISO, 1998). The amino acid composition of the meals were analyzed by hydrolyzing the amino acids in order to cleave the peptide bonds in the proteins to release the free amino for analysis using HPLC.

Faecal samples from sampling period 2 and 3 in experiment 1 were thawed, homogenized using an Ultra Turrax, and analyzed for DM, ash, protein, lipid and TP as described for the diets.

Water samples were analyzed for total nitrogen (ISO, 1986, 1997), total ammonia nitrogen (TAN; DS, 1975), and TP (ISO, 2004).

Carcass analyses of initial and final fish samples were carried out by removing the digestive system of the fish to avoid contamination from any undigested feed. The pooled carcasses were autoclaved for 1 h (120 °C), homogenized using a Braun hand processor, and analysed for protein, lipid, DM, ash and TP as described for the diets.

Calculations

Nitrogen-free extract (NFE) was calculated as DM less the sum of crude protein, crude lipid, and ash. The apparent digestibility coefficients (ADCs, %) of dietary nutrients and minerals, as obtained from the direct and total collection method of measuring, were calculated as (Jobling, 1994):

$ADC_i = 100*(C_i-F_i)/C_i$,

where *i* corresponds to a dietary macronutrient or mineral (i.e., protein, lipid, NFE, ash, TP , C is the consumed amount of *i*, and F is the faecal loss of *i*.

Complete N and P mass-balances were set up based on the total duration of the experiment (13 days), and following the approach by Cho et al. (1994) modified to measure dissolved waste directly:

X consumed = X retained + SWX + DWX,

where X refers to N or TP, SWX refers to solid waste N or TP, and DWN refers to dissolved waste N or TP . Retained N or TP was calculated based on whole body chemical composition analyses of fish sampled at the start and at the end of the experiment as (Jobling, 2001):

$X\ retained = (X\ in\ biomass_{end} - X\ in\ biomass_{start})/X\ consumed$

The solid waste output of N or TP was calculated as:

$SWX = (1 - ADC_X)*X\ consumed.$

The dissolved output of N or TP (including suspended solids) was measured directly in the water and for inclusion in the mass-balances calculated as:

$DWX = (DWX_{t24} - DWX_{t0})*L/X\ consumed,$

where DWX_{t0} and DWX_{t24} refer to N or TP concentrations in water samples obtained just prior to feeding and 24 hours later, respectively, in a tank with closed valves, and L = volume of the tank in litre. Excretion of TAN was derived similarly to DWX.

The feed conversion ratio (FCR, g g^{-1}) was calculated based on the biomass weight gain and the registered feed intake (feed administered – feed waste) as (Guillaume, 2001):

FCR = feed intake (g)/weight gain (g)

The specific growth rate (SGR, % d^{-1}) was calculated based on the overall biomass gain in the tanks as well as on the gain of individuals (Hopkins, 1992):

$SGR = 100*(\ln W_t - \ln W_0)/\Delta t$

where W_t refers to average weight at day t, W_0 refers to the average weight at day t_0, and Δt is the number of days.

Statistical analysis

Experimental data were subjected to a single factor analysis of variance (ANOVA) or t- test (analytical data) using Sigma Stat 3.5 to detect statistically significant differences between treatment means. Levenes test was used to check for homogeneity of variance within the treatment groups, and Holm Sidak all pairwise multiple comparison of means test was applied for testing significance of mean differences between the treatment groups where applicable. Data expressed in percentages were arcsine transformed prior to analysis. The significance level was set at $P < 0.05$.

Results & Discussion

Fig. 1a illustrates the Specific growth rate, SGR. Results showed a significantly decline in growth rate (P≤0.016), when CONV was substituted by the highest dietary inclusion levels of JM75 or JM90. FCR is illustrated as net feed intake /biomass weight gain (Fig. 1b, P=0.095) and also as FCR based on gross energy intake /weigh gain (Fig. 1c, P=0.474), thus was not significantly different between diets or inclusion level.

Fig. 1.a.- 1.c Growth performance of rainbow illustrated as SGR and FCR by substitution of conventional mussel meal with experimental meals at various dietary inclusion levels.

Fig.1.a

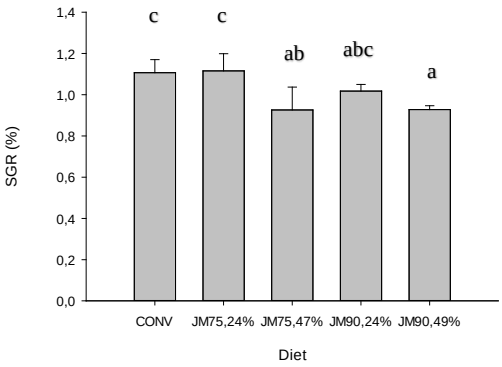


Fig. 1.b

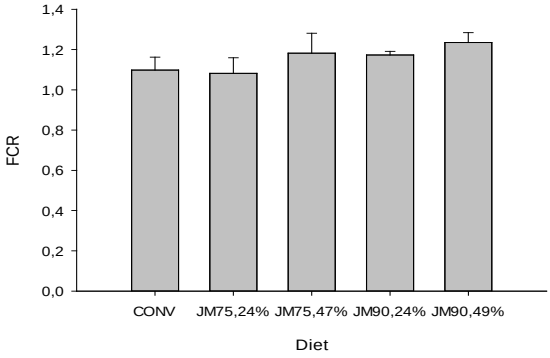
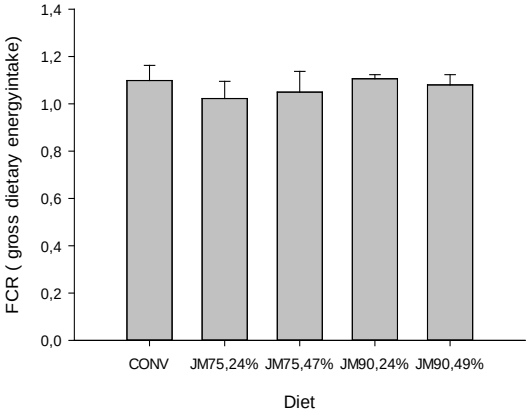


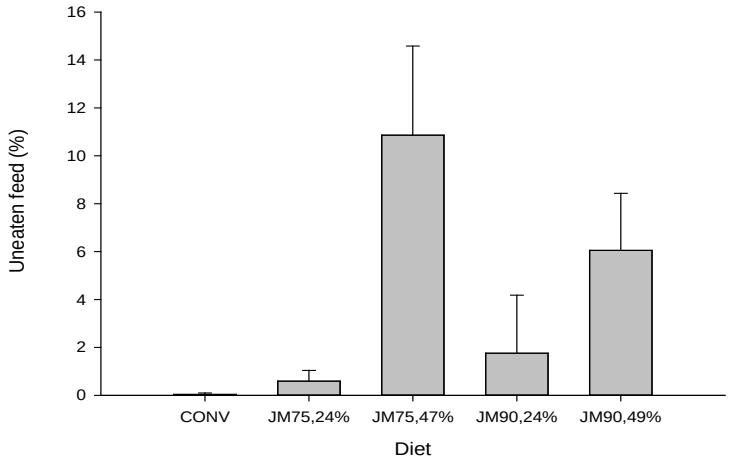
Fig.1.c



Performance results indicate some inferior growth rate (SGR) by inclusion of whole mussel meals, but as FCR illustrate, this was likely caused by a lower feed intake and energy intake in diets with the highest inclusion level of the experimental mussel meal, as FCR was not significantly different (i.e. differences tended to level out, when FCR was calculated based on gross energy intake Fig. 1.c).

From Fig.2. Palatability decreased with an increased inclusion of JM75 and JM90 (Fig.2), and it required prolonged weaning time to accustom fish to the taste of these diets before accepting. The off taste may refer to parts attached to the mussel such as byssus threads, sponges; barnacles etc., but was not examined further.

Fig.2 Uneaten feed collected during the experiment

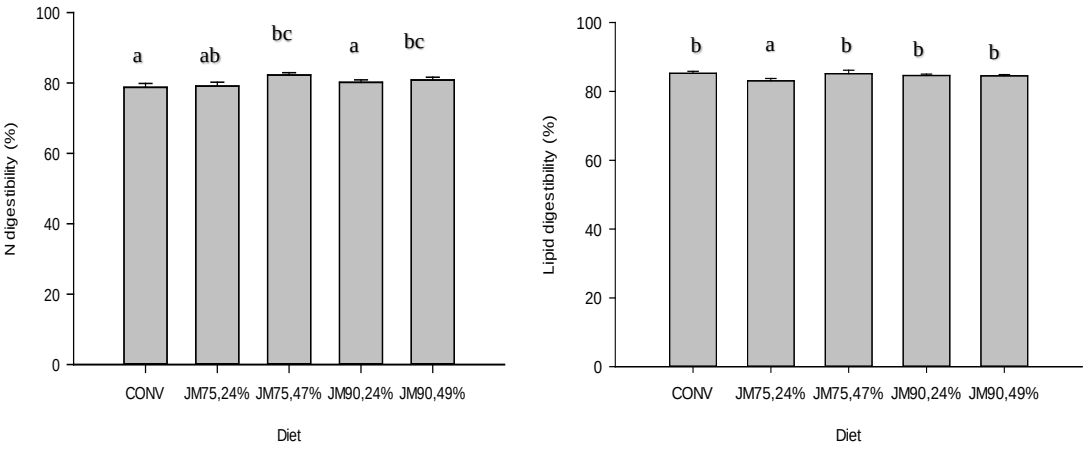


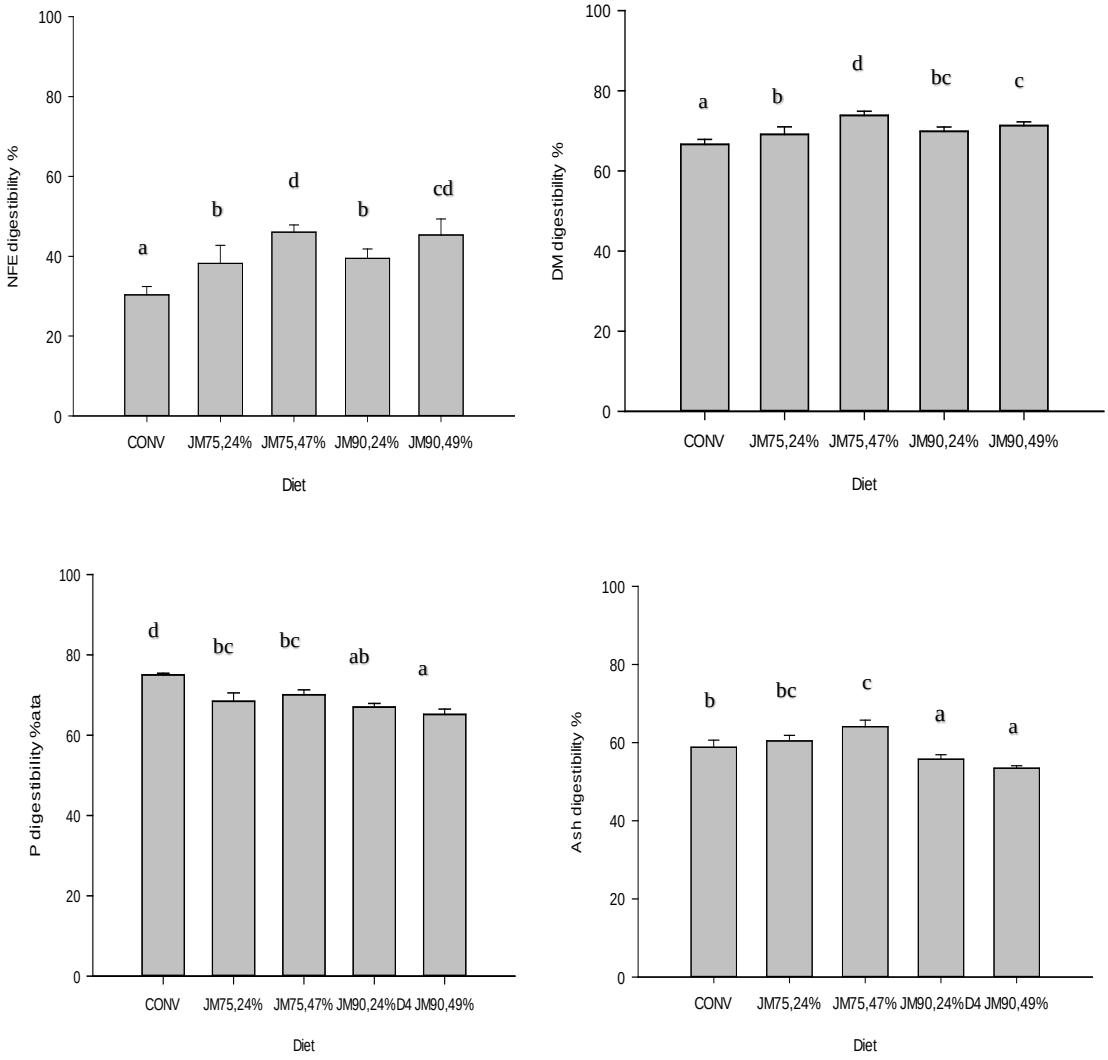
The growth and feed utilization of the fish were registered for a period of 12 days + acclimation, which may have masked some more profound effects on performance and fish quality, which could be evaluated further in prolonged performance experiments.

Digestibility & Mass balance

The results of the nutrient digestibility is illustrated in Figure 3.

Fig. 3. Digestibility of N, Fat (lipid), NFE, DM , P and Ash during 12 days of experimental testing. A different letter above the graphs indicates significantly differences between diets.



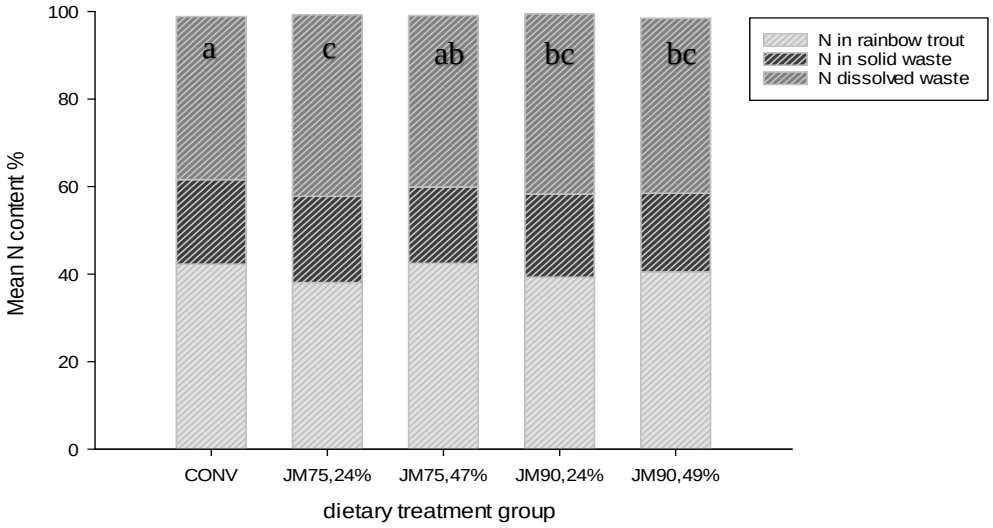


The N (protein) digestibility varied between diets from 78.8 - to 82.3 %, highest for JM 75,47% followed by JM90,49%, which were both significantly higher ($P<0.023$) than N digestibility of the CONV and JM90,24% diets. Fat digestibility were in the range 83.3%- to 85.2 % and significantly lower ($P\leq0.014$) for JM75,24%, than the other diets.
The NFE digestibility was quite different and in the range from 30- to 46 %, lowest for the CONV mussel diet. All diets contained wheat starch as the main carbohydrate source with inclusion levels from 5-21 %, which should be readily digestible. However, digestibility of NFE decreased significantly with an increase in inclusion level, i.e. lowest for fish fed the CONV diet ($P<0.004$), followed by fish fed the JM75,24% and JM90,24% diets, The significantly highest digestibility was observed in fish fed diets JM75,47% and JM90,49% for which starch inclusion was lowest (7.3%-5.1 %) Overall NFE digestibility values were within previous values observed in carnivorous fish, that have a limited ability to digest and utilize carbohydrates.
DM digestibility gave an overall significantly higher nutrient digestibility for diet JM75,47% as compared with the other 4 diets ($P\leq0.027$), caused by high protein, NFE and ash digestibilities. The higher DM digestibility was not directly reflected in the observed FCR

values for which there were no significant differences between diets, indicating that nutrients digested was not used into conversion of new tissue, but perhaps in other metabolism. However, results may be masked by the fact, that feed rejection was highest for this code giving some uncertainties in utilization of the feed.
Fish fed the CONV diet showed the highest P digestibility ($P<0.001$) of all diets, followed by the two JM75 diets, for which P digestibility was significantly higher ($P<0.001$) than the JM90,49% diet. Analyses of the mussel CONV meal revealed a higher total P content than for the JM meals. With an increased dietary inclusion of juiced mussel meal sources of inorganic P (monosodium phosphate (MSP), monocalcium phosphate (MCP) and monoammonium phosphate (MAP), may be present, for which the uptake and digestibility may be different. Additionally the higher ash content in the experimental diets, as due to shell fraction remainings, may also have had an influence, as inorganic P shell content would most likely not be available for digestion in the fish.
Ash digestibility was significantly higher for fish fed the CONV diet; the JM75,24% and the JM75,47% diet than for the two JM90 diets ($P\leq0.034$), in addition ash digestibility was higher for JM75,47% as compared with the CONV diet ($P\leq0.024$). The higher ash digestibility for the JM diets spray dried at 75 °C as compared with the diets spray dried at 90° C is interesting. High levels of ash (as present in the experimental diets) may affect dry matter digestibility and results in higher waste output in rainbow trout (Bureau et al. 1999), but no clear evidence of this in the present study.

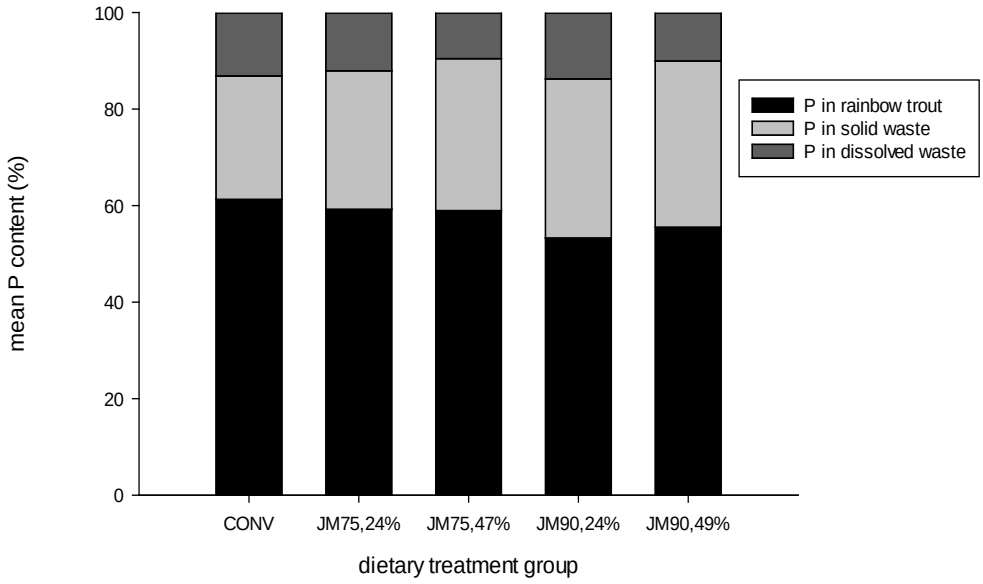
The complete N and P mass balance figures (Fig. 4 & 5) illustrate the fate of these nutrients and have been standardized to a recovery rate of 100 % to ease interpretation , the residual N and TP values are stated in figure notes.

Fig. 4 Mean nitrogen (N) mass balance (n=3) for juvenile rainbow trout fed 5 exp. diets. Data are adjusted to 100% of the nitrogen feed intake. Residual nitrogen (%): -10.5±2.7; -7.7 ± 4.7; -9.5 ± 2.0; -7.3 ± 1.0; -11.5 ± 6.2 for the 5 diets respectively. A different lower case letter between dietary treatments indicate, that the amount of N recovered was significantly different ($P<0.05$).



There were no significant differences for N retained in fish (38.1-42.4 %, i.e. corresponding to 27.6-31.0 g N kg feed^{-1}) or solid waste 12.6-14.3 % (i.e. 12.6-14.3 g N kg feed^{-1}), but a significant difference in dissolved waste N (37.3-41.5 % (i.e. 27.3-30.1 g N kg feed^{-1}). The amount of dissolved N waste was significantly higher ($P<0.03$) for JM75,24% than for both JM75,47% and the CONV diet, and likewise significantly higher for JM90,24% and JM90,49% than the CONV ($P\leq0.01$). The apparently lower dissolved N waste for the CONV diet than most of the exp. diets may likely indicate a higher part of the N source not available as protein (see D.6.3)

Fig. 5. Mean phosphorus (P) mass balance (n=3) for juvenile rainbow trout fed 5 exp. diets. Data are adjusted to 100% of the nitrogen feed intake. Residual phosphorus (%): -17.47±6.39; -23.41±15.9; -17.67±11.7; -15.78±7.15;±-13.42±6.47 for the 5 diets respectively. A different lower case letter between dietary treatments indicate, if the amount of P recovered was significantly different ($P<0.05$).



There was a relative high residual difference for P (i.e. % difference between P fed to fish and P analysed in fish, in solid waste and dissolved waste). As P is present in feeds in relatively low quantities as compared with N, small analytical differences will give rise to larger deviations. Despite not significant, P mass balance calculations showed a trend ($P\geq0.105$) towards a lower P retention in fish with the inclusion of JM90,24% and JM90,49%, (i.e. values from 61.1-53.3 %, i.e. corresponding to 4.3-3.9 g P kg feed^{-1}). For the solid P waste fraction this appeared to increase in the experimental diets, while the lowest content was observed with CONV (i.e. values from 34.5-25.5 % i.e corresponding to 2.8-1.8 g P kg feed^{-1}),however not significantly different ($P\geq0.19$). P in dissolved waste appeared lower for JM75 and JM90 at the highest experimental inclusion level (i.e. values from 13.7-9.5 %, i.e. corresponding to 0.97-0.76 g P kg feed^{-1}).

Results indicate a higher P uptake in fish, than in the experimental diets, which support the digestibility data. The reason may be a better utilization of the dietary phosphorus or

unavailability in the experimental diets (i.e. inorganic P source; or a higher P contribution by small shell fractions as part of the higher ash content in these diets).

Conclusion:

Mitigation mussels harvested and processed by crushing, juicing and filtering and subsequent spray drying to obtain a meal is (..apart from the spray drying) a simple and cheap methodology for obtaining meals to be used for nutritional studies. The present studies have shown, that the methodology could be optimized as due to very small crushed shell remainings passing the filtration process giving an unwanted high ash content in the meal. A high ash content will take up "space" in a modern energy dense fish feed and is therefore unwanted.
Harvested mitigation mussels contain other less nutritive parts such as byssus threads, barnacles etc. which apart from a low nutritional value may likely have influenced the palatability and growth performance, as rainbow trout needed a longer weaning period for accepting the diet based on these meals especially at high inclusion levels of the meals (i.e. 47-49%). The feed utilization and nutrient digestibility of the experimental juiced meals were in the same range regardless of spray drying at 90 °C or at 60 °C, and thus seemed to have minor effects on nutrient utilization. Apparently N digestibility increased with an increased inclusion of JM, but this was not reflected in the feed utilization (FCR), and could reflect N compounds that was not protein. This was supported by an observed increase in N dissolved waste for all experimental diets as compared with the conventional mussel meal diet. Lipid digestibility was not significantly affected while NFE digestibility was surprisingly low for all diets. The P retention in fish tended to be lowest for fish fed on JM diets dried at 90 °C. N and P mass balance data indicate a tendency for a higher waste either on a solid or dissolved form. Aquaculture waste should preferably be on solid form rather than on susp./dissolved form, as it is generally easier to treat/remove solid waste.

References:

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bureau, D. P. ; Harris, A. M. ; Cho, C. Y., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 180 (3-4): 345-358.

Dalsgaard, A.J., Pedersen, P.B., 2011. Solid and suspended/dissolved waste (N, P, O) from rainbow trout (Oncorynchus mykiss). Aquaculture 313, 92-99.

DS (Danish Standards), 1975. Water analysis. Determination of ammonia-nitrogen, DS224:1975. Dansk Standardiseringsråd, Denmark. 6 pp.

ISO, 1986. Water quality—determination of nitrate. Part 1: 2.6-Dimethylphenol Spectrometric Method, ISO 7890–1:1986. International Organization for Standardization, Geneva. 15 pp.

ISO, 1997. Water Quality—Determination of Nitrogen. Part 1: Method Using oxidative Digestion with Peroxodisulfate, ISO 11905–1:1997. International Organization for Standardization, Geneva. 13 pp.

ISO, 1998. Animal Feeding Stuffs—Determination of Phosphorus Content—Spectrometric Method, ISO 6491:1998. International Organisation for Standardization, Geneva. 16 pp.

ISO, 2004. Water Quality—Determination of Phosphorus–Ammonium molybdate Spectrometric Method, ISO 6878:2004. International Organisation for Standardization, Geneva. 21 pp.

ISO, 2005. Animal Feeding Stuffs—Determination of Nitrogen Content and Calculation of Crude Protein Content—Part 2: Block Digestion/Steam Distillation Method, ISO5983–2:2005. International Organisation for Standardization, Geneva. 14 pp.

Guillaume, 2001 Guillaume, J., 2001. Terminology and methodology. In: Guillaume, J., Kaushik, S., Bergot, P.,Métailler, R. (Eds.), Nutrition and Feeding of Fish and Crustaceans. Springer-Praxis,Chichester, UK, pp. 9–16.

Hopkins, K.D., 1992. Reporting fish growth: a review of the basics. Journal of the World Aquaculture Society 23, 173–179.

Jobling, M., 1994. Fish Bioenergetics, first ed. Chapman & Hall, London

Jobling, M., 2001. Feed composition and analysis. In: Houlihan, D.F., Boujard, T., Jobling,M. (Eds.), Food Intake in Fish. Blackwell Science Ltd., Oxford, pp. 1–24

NMKL (Nordic Committee on Food Analysis), 1991. Moisture and ash. Gravimetricdetermination in meat and meat products, NMKL 23. Nordic Committee on FoodAnalysis, Oslo, Norway. 4 pp