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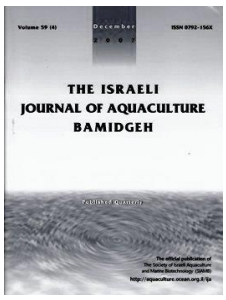

Effects of Sustainable Diets containing Fish-Trim Waste, on Growth Performance of Juvenile Sablefish (*Anopoploma fimbria*)

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Keywords: sustainable diets, hydrolysate, fish trim waste, sablefish

Abstract

A feeding trial was conducted to investigate the use of alternative fish feeds produced from fish-trim waste in high plant protein diets for juvenile sablefish (*Anopoploma fimbria*). Fish meal in the control diet (CD) was replaced in the experimental diets by low molecular weight hydrolysate (LMWH) protein derived from Pacific whiting (*Merluccius productus*) processing waste, or by Atlantic salmon (*Salmo salar*) processing trim (ST), which was incorporated into the feed with an experimental heated ball mill. The feeding trial was conducted in an indoor recirculating seawater system and each feed was randomly assigned to triplicate groups of 50 fish (average initial body weight of 57.5 g). The results showed that compared to the control diet, the diets containing LMWH or ST significantly increased feed consumption and fish weight gain. The ST group showed a significantly higher weight gain than the LMWH group. The ST feed, but not the LMWH feed, had a significantly lower feed conversion ratio than the CD feed. The ST feed increased lipid retention efficiency and lipid content in whole body tissue. These results demonstrate that salmon fish trim waste and enzyme hydrolyzed whiting trim in high plant protein diets can increase the performance in sablefish.

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Introduction

Fish meal is an indispensable feed ingredient for efficient growth of many commercial fish species (Miles and Chapman, 2006). For the aquaculture industry to expand, utilization of fish meal must be reduced by lowering the amount used in fish feeds. This can be achieved by use of waste such as heads, viscera, and backbones from fish processing as a substitute for fishmeal in fish feed. Fish waste from small or remote fish processing industries is often discarded due to a lack of available, economic processing facilities. Supplies could be extended by reducing the amount of fish meal used in feeds, but this requires developing the use of protein from plants or terrestrial animals. Substitution of fish meal with plant protein in the diets of many fish species is currently limited due to decreased feed acceptability, reduced feed efficiency, and nutritional imbalance (Aksnes et al., 2006).

Almost all fish meal is produced by wet reduction (Pigott and Stansby, 1967). The economics of the wet reduction process requires continuous operation and is dependent on large volumes of fish. These constraints are satisfied by large industrial fisheries that capture small fish of limited market value as human food. Some small fish processors located near a wet reduction facility can realize a small return by selling their processing waste to the facility however this becomes more problematic when distance to the processing facility increases.

To improve the economic value of adding fish processing waste into fish meal or fish feeds at small or remote fish processing locations, a heated ball mill was built and tested. All the ingredients for fish feed consisting of plant ingredients, fish processing waste, vitamins, minerals, and other trace nutrients were processed in a single batch to produce a complete dry feed mash for storage or pelleting. With this system, the heated ball mill grinds, dries, pasteurizes, and mixes all the ingredients at low temperatures, in a single operation, reducing labor and equipment costs. The process is scalable and produces a value added feed from fish processing waste. Fresh raw material from fish processing has been shown to increase alternative feed consumption and growth in shrimp (Ricque-Marie et al., 1998), European catfish (Havasi et al., 2015) sea bass (Altan et al., 2010), red seabream (Mamauag, 2014) as well as in salmonids (Clancy et al., 1995). The processing temperature for fish meal is also important for optimal protein quality. Growth of Atlantic salmon is higher when fish meal is processed between 70-100°C than when fish meal is dried at temperatures exceeding 100°C (Opstvedt and Mundheim, 1988). The heated ball mill system facilitates processing fish meal at these lower temperatures.

Manufacture of acidic fish hydrolysate is an inexpensive solution for producing animal feed from fish processing waste. Adding acid to fish waste inhibits spoilage and allows proteases in the fish waste to digest the protein and separate it from the bones to produce liquid slurry (Kristinsson and Rasco, 2000). The acid hydrolysate process is limited to local use for animal feeds due to transportation costs and inadequate protein quality. Fish hydrolysates can also be prepared with commercial enzymes (Aksnes et al., 2006; Chotikachinda et al., 2013). This process can be controlled to produce consistent protein hydrolysates based on peptide and free amino acid composition. There is evidence that at low levels of fish meal replacement with enzyme hydrolysate, growth performance, and feed utilization increases in carnivorous fish, especially when high levels of plant protein are used (Zheng et al., 2011). Removal of small molecular weight compounds from fish hydrolysates or fish meal solubles has resulted in reduced growth and feed efficiency for carnivorous fish (Aksnes et al., 2006). Inclusion of small molecular weight compounds from fish hydrolysates in fish feeds appear to increase feed efficiency and could further reduce the use of fish meal.

The purpose of this study was to assess the effect of the incorporation of fish processing waste. Two different technologies were used in order to increase intake and performance of alternative fish feeds using the heated ball mill process to address the economics of processing at small or remote fish processing facilities. In addition, an enzyme hydrolysate was prepared from fish processing waste to evaluate its potential as a feed ingredient in these feeds.

Materials and Methods

Experimental diets. Three isonitrogenous and isoenergetic diets were prepared. The targeted variable was the source of fish protein in each diet (Table 1).

Table 1. Experimental feeds formulations and proximate composition

Ingredients (g/kg dry diet)	*Diets*		
	CD	*LMWH*	*ST*
Soy protein isolate	242.6	242.6	242.6
Wheat flour	139	139	139
Corn gluten	243.4	243.4	243.4
Fishmeal	134	94	-
Salmon Trim	-	-	304
Hake Hydrolysate	-	40	-
Salmon Oil	160	170	-
Dicalcium phosphate	20	20	20
Choline	5	5	5
L-Methionine	2	2	2
L-Lysine	2	2	2
Taurine	5	5	5
Vitamin pre-mix	15	15	15
Mineral pre-mix	1	1	1
Stabilized Vitamin C	1	1	1
PermaPel	20	20	20
Cellulose	10	-	-
Proximate Analysis (g/kg)			
Crude protein	527.7	539.2	530.3
Crude lipid	182.8	173.2	174.7
Ash	65.5	63.8	70.1
CHO by difference	224	223.8	224.9

A: The control diet (CD) included fish meal as source of fish protein.

B: The hydrolysate diet (LMWH) substituted 4.0% of the fish meal with low molecular weight fish hydrolysate (LMWH). LMWH was prepared from fresh Pacific whiting (*Merluccius productus*) processing waste consisting of heads, viscera, and trims. Processing waste was collected from a food company in Oregon and frozen at -28°C until time of use. Thawed fish were minced with a cutter (Hobart VCM 25, Troy, OH) and water was added at a ratio of 8:10 (w/w). After heating at 90°C for 20 min to inactivate the endogenous enzymes and kill bacteria, the mince was hydrolyzed at 50°C for 3 h (pH=8.5) using alcalase (0.6% v/w) and flavourzyme (0.48% v/w) (Sigma, St. Louis, MO). At the end of hydrolysis, the mince was heated again (90 °C, 20 min) to inactivate the added enzymes. The hydrolysate was filtered through a 150 micron screen to remove bone particulate and the liquid was centrifuged at 3500 × g (Sorval RC, Wilmington, DE) to remove fine particles and lipid. A sample of supernatant was filtered with a Microcon centrifugal filter 3000 MWCO (Centricon YM-3 Millipore, Bedford, MA). The protein content of the filtrate was determined spectroscopically as described previously (Nicklason and Johnson, 2008) and contrasted against the source hydrolysate to confirm that >90% of the hydrolyzed protein was under 3000 Da molecular weight.

C: The third diet (ST) included salmon trim (ST) as the source of fish protein and fat. Fresh farmed Atlantic salmon (*Salmo salar*) waste consisting of heads, viscera, backbones, and trim were collected from a local processor and frozen at -28°C. An experimental heated ball mill (Fig. 1) was used to make a finished feed in one step. All the feed ingredients consisting of the fish waste, plant ingredients, vitamins, and minerals were processed by the ball mill to a dry feed mash in a single operation. Unground bone particulate was removed by screening through an 8 mesh screen. Salmon oil, rendered from additional salmon trim, was used to balance the lipid content of the control and LMWH diets. All diets were extruded through a 3 mm die using a pellet mill

(California Pellet Mill, San Francisco, CA). The fatty acid and amino acid compositions of the diets are summarized in Tables 2 and 3, respectively.

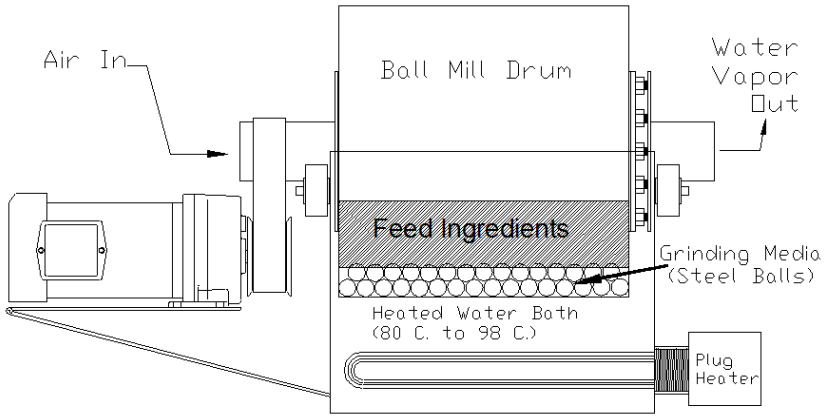

Fig. 1. Heated ball mill for grinding, mixing, and drying fish feeds

Table 2. Simplified fatty acid composition of sablefish feeds. (Results are expressed as % total fatty acids. ARA=arachidonic acid; EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid)

Fatty acid	*Diets*		
	CD	*LMWH*	*ST*
14:0	1.8	1.7	2.0
16:0	14.2	13.1	14.0
18:0	4.1	3.9	3.9
ΣSFAs[1]	20.6	19.0	20.2
16:1	4.1	3.8	4.1
18:1[2]	42.3	41.8	40.7
20:1[2]	1.7	1.8	1.7
22:1[2]	0.2	0.5	0.5
ΣMUFAs[3]	48.5	48.1	47.2
18:2(n-6)	15.9	16.5	16.1
20:2(n-6)	0.8	0.9	0.8
20:3(n-6)	0.4	0.4	0.4
20:4(n-6)	0.6	0.5	0.6
Σn-6[4]	17.7	18.3	17.9
18:3(n-3)	2.6	2.9	2.8
18:4(n-3)	0.4	0.1	0.0
20:5(n-3)	2.5	2.7	2.9
22:5(n-3)	1.1	1.1	1.1
22:6(n-3)	3.7	3.4	3.9
Σn-3[5]	11.0	10.8	11.5
Σn-3/n-6	0.62	0.59	0.64
EPA/DHA	0.68	0.79	0.74
EPA/ARA	4.2	5.4	4.8

[1]Sum of fatty acids (SFAs): 14:0, 16:0, 18:0, 20:0, 22:0, 22:0, and 24:0.
[2]Sum of n-7, n-9, and n-11 isomers.
[3]sum of monounsaturated fatty acids (MUFAs): 14:1, 16:1, 18:1, 20:1, 22:1, and 24:1.
[4]Sum of n-6 fatty acids: 18:2, 20:2, 20:3, AND 20:4.
[5]Sum of n-3 fatty acids: 18:3, 18:4, 20:3, 20:4, 20:5, 21:5, 22:5, and 22:6.

Table 3. Amino acid composition of feeds. Results are expressed as g amino acid per 100 g feed.

Amino acids	*Diets*		
	CD	*LMWH*	*ST*
Taurine	0.49	0.51	0.45
Hydroxyproline	0.18	0.18	0.31
Aspartic Acid	4.33	4.37	4.20
Threonine	1.89	1.91	1.83
Serine	2.33	2.40	2.34
Glutamic Acid	9.69	9.88	9.40
Proline	3.85	3.97	3.83
Lanthionine	0.00	0.00	0.00
Glycine	2.02	2.10	2.40
Alanine	3.29	3.43	3.32
Cysteine	0.74	0.75	0.70
Valine	2.43	2.43	2.21
Methionine	1.18	1.17	1.06
Isoleucine	2.26	2.27	2.11
Leucine	6.03	6.17	5.70
Tyrosine	2.20	2.25	2.08
Phenylalanine	2.80	2.89	2.69
Hydroxylysine	0.04	0.04	0.08
Ornithine	0.04	0.04	0.04
Lysine	2.14	2.10	1.68
Histidine	1.17	1.15	1.04
Arginine	2.70	2.69	2.58
Tryptophan	0.51	0.52	0.40
Total	52.31	53.22	50.45

Fish Growth Trial. Sablefish (*Anoplopoma fimbria*) were hatched and raised to an average weight of 0.5 g at the NOAA Manchester Laboratory (Manchester, WA) and transported to the indoor recirculating seawater system at the Northwest Fisheries Science Center (Seattle, WA) in April 2013. Fish were reared to a weight of 57.5 g at 14°C at 28 psu salinity under normal photoperiod and fed a fishmeal based salmon feed in November 2013. At this time, 450 fish were equally distributed into nine 4 foot × 4 foot (1.20m x 1.20m) tanks. The three experimental feeds were randomly assigned to three tanks each. Fish were fed to apparent satiation every other day for 4 weeks. Fish weights were recorded every two weeks. Net feed weight was recorded after each feeding for the calculation of feed conversion ratios.

Three composite samples (4 fish each) were collected initially at the beginning of the feeding study and frozen at -28°C for the analysis of whole body proximate composition. After four weeks, fish were weighed and 25 fish from each tank were saved at -28°C for comparison of whole body proximate composition.

Sample Analysis. Solid content and dry matter proximate composition were determined for each feed and whole body composite. Unless stated otherwise, results are expressed as mean and standard deviation of three replicates per treatment. Moisture and ash were determined according to standard methods (AOAC, 1980). Protein was estimated by determining nitrogen concentration by Dumas combustion methodology with a LECO FP-2000 nitrogen analyzer (LECO Corp., St. Joseph, MI), and multiplying the result by 6.25. Fat content was determined by Soxhlet extraction. Amino acid analysis of the three feeds was conducted at the Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, MO. Fatty acid methyl esters were prepared from extracted feed lipids using boron trifluoride as a catalyst and were chromatographically separated on a Hewlett-Packard Model 5890 gas chromatograph

with a flame ionization detector employing a DB-225 polysiloxane column (Agilent Technologies, Wilmington, DE).

Calculation and Data Analysis. The following variables were calculated:

Fish weight gain (WG %) = 100 × (final fish weight – initial fish weight)/(initial fish weight)

Feed conversion ratio (FCR) = (dry matter feed used)/(fish weight gain)

Lipid retention efficiency (LRE %) =100 × (lipid gain)/(lipid consumed)

Protein retention efficiency (PRE %) =100 × (protein gain)/(protein consumed)

All statistical analyses were performed with R version 2.13.0 statistical software (The R Foundation for Statistical Computing, Palo Alto, CA, USA). Differences were deemed significant when $P<0.05$. Treatment means were computed from tank means (n=3) and reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to detect significant differences in growth, feed intake, feed conversion, whole body proximate composition, and protein retention attributable to treatment. When significant differences were detected by ANOVA, the Tukey HSD test was subsequently employed to assess the significance of differences between treatment means.

Results

Fish growth and feed performance. Feed intake was significantly influenced by the diet formulation (Fig. 2). Feed made with fish trim (ST) or fish hydrolysate (LMWH) were more aggressively eaten than the control feed.

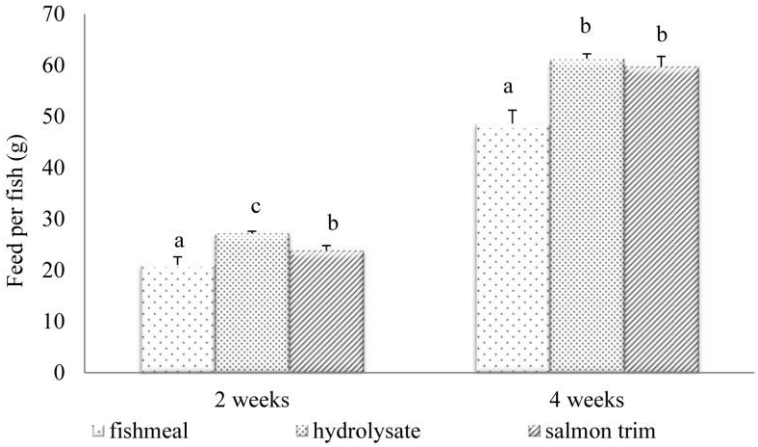

Fig. 2. Feed consumption. Mean + SD (n=3) of feed consumed by the fish in each treatment. Different lowercase letters denote significant difference at $P<0.05$.

Fish weight gain (Fig. 3) was significantly higher for the treatments with LMWH and ST as all or part of the fish protein component in the formulation compared to the fish meal control (CD).

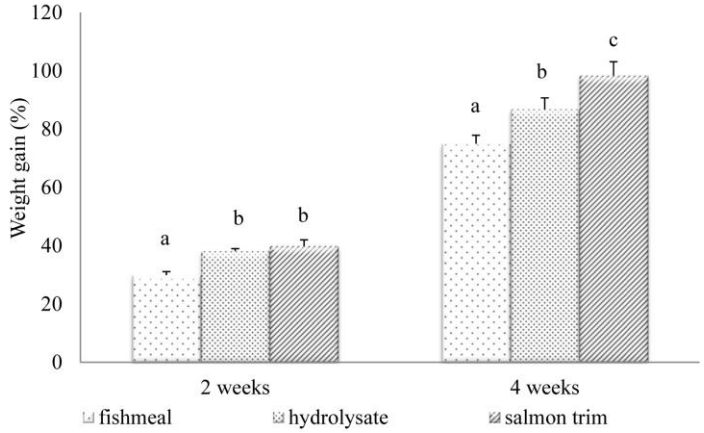

Fig. 3. Fish weight gain, mean + SD (n=3), for each feed treatment as the percent of beginning fish weight. Different lowercase letters denote significant difference at $P<0.05$.

At week four, weight gain of ST fish was significantly higher ($P<0.05$) than the CD and LMWH fish. At week two and week four, the ST diet showed a significantly lower feed conversion ratio than the other treatments (Fig. 4).

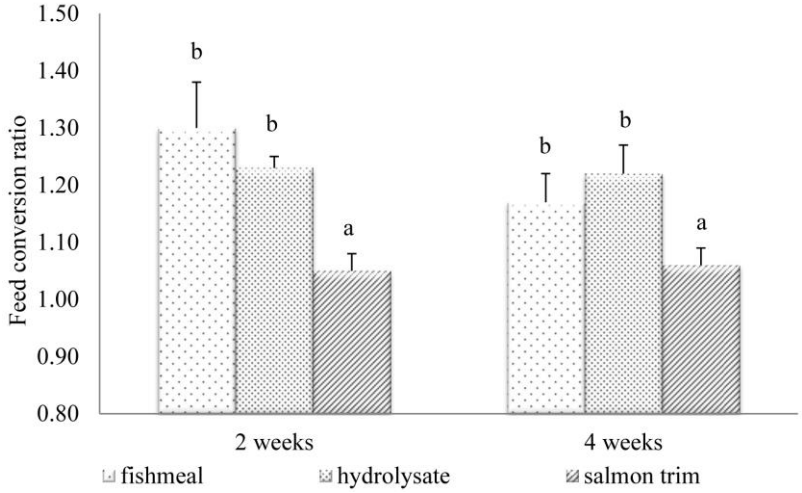

Fig. 4. Feed conversion ratio of the three feeds, mean + SD (n=3). Different lowercase letters denote significant difference at $P<0.05$.

Whole body composition. There was a significant difference in whole body moisture content between all treatments at week four of the study (Fig. 5).

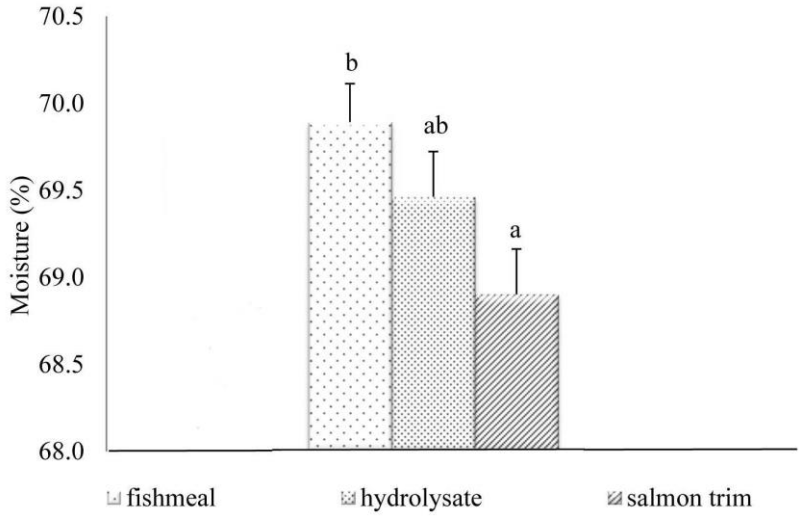

Fig. 5. Week four whole body moisture content, mean + SD (n=3). Different lowercase letters denote significant difference at $P<0.05$.

Control diet fish were highest in moisture and ST fish were lowest. Lipid content was significantly higher in ST fish and the protein content was significantly lower (Fig. 6). There was no significant difference in the protein and lipid content between the control diet fish and the LMWH fish.

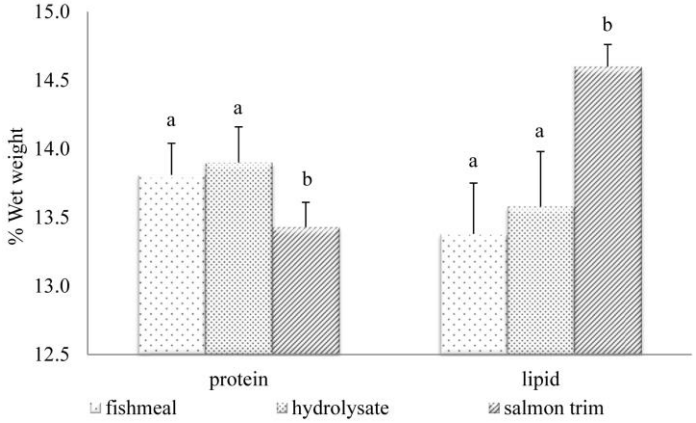

Fig. 6. Week four protein and lipid content, mean + SD (n=3). Different lowercase letters denote significant difference at $P<0.05$.

Lipid and protein efficiency. Lipid and protein retention was significantly affected by the diet (Fig. 7). Retention of protein from the CD and ST feeds was equal and significantly greater than the retention of LMWH feed protein. Lipid retention was significantly higher for the ST feed.

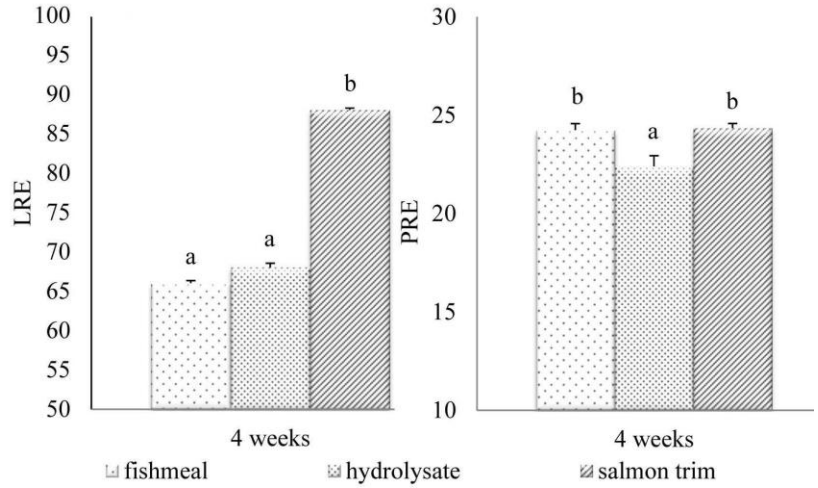

Fig. 7. Week four lipid retention efficiency (LRE) and protein retention efficiency (PRE), mean + SD (n=3). Different lowercase letters denote significant difference at $P<0.05$.

Discussion

Sablefish are a piscivorous marine species (Laidig et al., 1997). The high level of plant ingredients in the experimental feeds (625g in 1 kg dry diet, see Table 1) were expected to affect palatability and performance. Replacing a portion of the fish meal with hake hydrolysate (LMWH feed) improved feed intake. Increases in feed intake with the addition of fish hydrolysates have been observed previously in salmonids (Refsie et al., 2004). Feed intake of the ST feed group was also significantly higher than that of the control group which was fed the fish meal based feed. This could be explained by the high quality of the raw material used in the ST feed and the processing method. Unlike the wet rendering process, which removes a soluble fraction by pressing the fish meal, the ball mill process retains the low molecular weight soluble proteins. These soluble proteins have been shown to increase the palatability and performance for salmon fed the diets high in plant ingredients (Espe et al., 2006). The fresh raw material used in the ST diet was of high quality and in all probability had a minimal amount of decomposition products, such as volatile basic nitrogen, histamine and malondialdehyde, which are often found in fish meal. Decomposition of raw fish material and the higher temperatures used in the wet rendering process relative to the ball mill process has been shown to lower performance and palatability in fish fed fish meal based feed (Aksnes and Mundheim, 1997).

Along with the improved feed intake, the fish fed the LMWH and ST feeds had better growth than the CD fish group. The results from this study may encourage the use of alternative, plant based feeds for sablefish culture. In addition, a major goal for sustainable fish culture is to lower the "fish in to fish out" (FI/FO) ratio of aquaculture feeds. The "fish in" is the weight of whole wild caught fish needed to provide fish meal and fish oil needed to produce a kilogram of whole aquaculture fish. For the control diet, 0.157 kg of fishmeal is required in order to produce 1 kg of sablefish. The general yield of fish meal from targeted industrial whole fish is 20% (FAO, 2014). Based on these figures, this study showed that 0.785 kg of whole wild fish was required to grow 1 kg of sablefish, i.e., the FI/FO was 0.785. Compared to the CD feed, the LMWH feed reduced the FI/FO by 27%, i.e., 0.57 kg of whole fish was required to grow 1 kg of whole sablefish. Fishmeal and fish oil are the most expensive ingredients in fish feed. The current price of fishmeal is over $2.10/kg delivered, which is a 200% increase in price over the past 10 years. Replacing fish meal and oil in aquaculture feeds with processing trim lowers FI/FO ratios as well as the costs of these feeds. Additionally, lowering the FI/FO allows aquaculture producers to explore new markets, driven by consumers who prefer sustainable fish production.

The ST feed produced the best performance. There is no FI/FO for ST since no fish meal was used. The ST feed also showed the lowest feed conversion ratio (1.06), significantly lower than the CD (1.17) and LMWH (1.22) feeds. The goal of the ST treatment was to evaluate the potential of the new heated ball mill process to convert fish processing waste to a value added feed in locations that are currently not served by existing technology due to their small scale of operation or remoteness. For aquaculture farms that raise two or more species and process fish on site, the potential exists to make feed from the processing waste of one species into feed for another species using the ball mill process. This can be repeated with the second species, by processing their waste into feed for the first species. In the American Pacific Northwest, sablefish can be raised with Atlantic salmon, providing an opportunity to test this approach. In the production of salmon fillets, 1 kg of whole salmon will generate 0.48 kg of heads, viscera, backbone and trim. This is enough scrap to make 0.62 kg of ST feed on a dry matter basis. Based on the feed conversion ratio of ST feed (1.06) in the current study, the waste left over from processing 1 kg of salmon to fillet is sufficient to grow0.59 kg of sablefish. Using CD feed to raise the remaining 0.41 kg of whole sable fish to balance the input of scrap from 1 kg of whole salmon, results in a combined FI/FO of 0.32. As a follow-up, a study is planned to incorporate processing waste from sablefish produced from the ST feed into an alternative feed for Atlantic salmon.

Results from this study demonstrate two processing technologies that can reduce the amount of fish meal in aquaculture feeds while increasing feed intake. The FI/FO was reduced by 27% by substituting protein hydrolysate from fish processing waste. Feed made with incorporated processing scrap using the heated ball mill process further reduced FI/FO. However, the protein retention efficiency in fish fed the LMWH feed was significantly lower than fish fed the other feeds. This could be explained by the high levels of free amino acids in the LMWH feed and the demonstrated lower bio-efficacy values of free amino acids found in protein hydrolysates (Zarate et al., 1999; Ambardekar et al., 2009; Dabrowski et al., 2010; Bodin et al., 2012). Free amino acids appear to have more rapid gastrointestinal absorption rates than protein-bound amino acids. As such, plasma concentrations of free amino acids are higher than those observed for the protein-bound amino acids. Some of the plasma free amino acids may not be rapidly taken up by the tissues when the capacity of the cells to suitably metabolize absorbed amino acids for protein synthesis is exceeded (Espe et al., 1993). Consequently, it is possible that some of these free amino acids will be used for other catabolic purposes such as donating the carbon chain during the oxidation process as an energy source (Nunes et al., 2014).

In this study the significantly higher lipid retention and whole body lipid observed in ST fish is also of importance. Sablefish are a fatty fish and are valued for the high lipid content of their flesh. Producers of farmed sablefish must ensure that flesh lipid levels

are similarly high and comparable to those of wild fish. In this study, improvements in lipid retention and feed conversion associated with the ST feed resulted in improved growth and significantly higher whole body lipids. These results are similar to those observed in red drum *Sciaenops ocellatus* when fishmeal was substituted by low temperature fish protein meals (Moon and Gatlin, 1994). In the previous study, improved feed performance was attributed to higher protein digestibility and perhaps better amino acid balance of low temperature fish protein meals. As the price of industrial fish meal and fish oil has dramatically increased, and will most likely continue to increase in the near future, alternative feeds such as the ST feed that support superior growth and increased retention of dietary lipids should be highly valued by producers of sablefish and other fatty fish.

Acknowledgements

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