



NRC Publications Archive Archives des publications du CNRC

Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758)

Tibbetts, Sean M.; Milley, Joyce E.; Lall, Santosh P.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:

<https://doi.org/10.1016/j.aquaculture.2006.08.052>

Aquaculture, 261, 4, pp. 1314-1327, 2006-12

NRC Publications Record / Notice d'Archives des publications de CNRC:

<https://nrc-publications.canada.ca/eng/view/object/?id=f63bedb5-90c2-436c-aa10-437dfe58c5d2>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=f63bedb5-90c2-436c-aa10-437dfe58c5d2>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.



Provided for non-commercial research and educational use only.
Not for reproduction or distribution or commercial use.



This article was originally published in a journal published by Elsevier, and the attached copy is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research and educational use including without limitation use in instruction at your institution, sending it to specific colleagues that you know, and providing a copy to your institution's administrator.

All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

<http://www.elsevier.com/locate/permissionusematerial>

Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758)

Sean M. Tibbetts, Joyce E. Milley, Santosh P. Lall *

National Research Council of Canada, Institute for Marine Biosciences, 1411 Oxford Street, Halifax, Nova Scotia, Canada B3H 3Z1

Received 13 June 2006; received in revised form 28 August 2006; accepted 29 August 2006

Abstract

Studies were conducted with Atlantic cod, *Gadus morhua* (L.), to determine the apparent digestibility coefficients (ADCs) of protein and energy and the digestible energy (DE) content in feed ingredients widely available in Canada. We also tested the assumption of “independency” used in digestibility studies. The feed ingredients included two fish meals (herring, anchovy), three crustacean by-product meals (whole krill, crab, shrimp), two animal by-product meals (poultry by-product, hydrolyzed feather), six oilseed meals (soybean, soy protein concentrate, soy protein isolate, canola, canola protein concentrate, flaxseed), two pulse meals (white lupin, pea protein concentrate) and two cereal grain meals (corn gluten, wheat gluten). Protein ADCs were high for wheat gluten meal (99.9%), soy protein concentrate (98.6%), soy protein isolate (97.4%), whole krill meal (96.3%), herring meal (93.3%), soybean meal (92.3%), anchovy meal (92.2%), pea protein concentrate (89.8%), white lupin meal (89.7%), crab meal (89.4%), canola protein concentrate (88.8%) and corn gluten meal (86.3%); mid-range for poultry by-product meal (80.2%) and canola meal (76.0%); and low for shrimp meal (66.7%), hydrolyzed feather meal (62.4%) and flaxseed meal (50.2–55.0%). Energy ADC was high for whole krill meal (96.3%), wheat gluten meal (95.4%), soy protein concentrate (94.9%), herring meal (92.8%), soy protein isolate (92.1%), soybean meal (88.1%) and anchovy meal (86.4%); mid-range for canola protein concentrate (83.3%), corn gluten meal (82.7%), crab meal (82.4%), pea protein concentrate (76.7%) and white lupin meal (75.3%); and low for poultry by-product meal (71.0%), canola meal (60.6%), hydrolyzed feather meal (58.9%), shrimp meal (41.4%) and flaxseed meal (21.2–37.4%). From the protein ADC data, results clearly showed that the basal diet and test feed ingredients were digested independently of one another in nearly all cases, the only exceptions being for those diets containing test ingredients of very high (>99%, wheat gluten) or very low (<67%, hydrolyzed feather and flaxseed) protein ADCs. In the case of DE, the basal diet and test feed ingredients were digested independently in all test diets without exception.

© 2006 Crown Copyright. Published by Elsevier B.V. All rights reserved.

Keywords: Cod; Digestibility; Marine by-products; Animal by-products; Plant proteins

1. Introduction

In recent years, marine culture of gadoids has expanded in Eastern Canada and Western Europe. The

production of species like Atlantic cod is expected to reach 140–180,000 tonnes by the year 2010 (Rosenlund and Skretting, 2006). These fish are known to have a high protein requirement (50–60%) (Lall et al., 2003; Rosenlund et al., 2004) but limited information is available on digestion of major nutrients and energy from various feed ingredients (Tibbetts et al., 2004; Kim et al., 2006). Selection of potential ingredients for feed

* Corresponding author. Tel.: +1 902 426 6272; fax: +1 902 426 9413.

E-mail address: santosh.lall@nrc-cnrc.gc.ca (S.P. Lall).

formulation for any fish species requires knowledge of the apparent digestibility coefficients (ADCs) of energy-yielding nutrients (starch and sugars, fat, protein, non-starchy polysaccharides). Fish meal provides the main source of protein in salmonid and marine fish diets. The nutritional value of various fish meals for salmonids grown in Canada has been investigated extensively (Anderson et al., 1997; Lall and Anderson, 2005). World-wide fish meal use for aquafeeds will reach 4 million tonnes by 2015, representing >66% of the expected global supply (New and Wijkström, 2002). With this ever-growing demand for high-quality fish meals, fish feeds must increasingly be formulated with alternate protein sources from marine, animal or plant origin that are both economical and highly digestible (see review of Hardy, 1996). The use of these alternatives in on-growing diets must still be able to support similar fish performance and, concurrently, have little or no adverse effects upon fish health and the environment.

Several factors can affect protein quality and the nutrient profile of fish, crustacean and animal by-product meals. These include characteristics of the raw material (species, freshness, whole animal or scraps), processing of the raw ingredients such as the drying process and temperature, lipid peroxidation and storage conditions of the meal (Pike, 1991). The major by-product of crustacean processing is the shell which contains 50–80% chitin, an amino polysaccharide (poly- β -(1 \rightarrow 4)-*N*-acetyl-glucosamine). The natural diet of cod consists of >37% chitin-rich crustaceans and echinoderms including crabs, shrimps and brittle stars (see Lall and Nanton, 2002). Cod naturally produce significant concentrations of the digestive enzyme chitinase (Danulat and Kausch, 1984) and *in vivo* chitin digestibility may be as high as 90% for cod (Danulat, 1987). Accordingly, crustacean by-products have been identified as good candidates to replace fish meal in diets for Atlantic cod (Toppe et al., 2006). At the same time, crustacean by-product meals are usually high in ash content (>20%), which can adversely affect digestibility of fish feeds (NRC, 1993).

Poultry by-product and hydrolyzed feather meals are produced from the wastes generated by the poultry processing industry. Production processes are similar to that of fish meal with an extra $\text{Ca}(\text{OH})_2$ digestion in the production of hydrolyzed feather meal. These animal by-product meals are generally high in crude protein (60–80%); however, they tend to be methionine deficient. Poultry by-product meal can also be high in ash (>15%) and is often variable in proximate composition. Protein digestibility can be quite low for hydrolyzed feather meal due to high levels of keratin (Dong et al., 1993; Hardy and Barrows, 2002).

Partial replacement of fish meal with plant protein supplements or complete replacement with concentrates from these products has been successful in several commercially important salmonid species (Higgs et al., 1995; Kaushik et al., 1995) and turbot (Regost et al., 1999). Factors limiting the use of plant protein sources include low protein content, high fiber content, an amino acid imbalance, poor palatability and the presence of anti-nutritional factors or toxicants (e.g. protease inhibitors, lectins, phytic and/or erucic acid, sinapin, saponins, phytoestrogens, alkaloids, tannins, cyanogens, glucosinolates). These factors adversely affect digestion, absorption, physiological utilization of protein and amino acids, lipids and fatty acids and minerals and cause several other undesirable effects when incorporated into fish feeds (see review of Francis et al., 2001). Plant-based protein sources, however, can provide high nutritional value in fish diets when properly incorporated into feed formulations, supplemented with purified amino acids and feed attractants and properly heated during feed processing. Unfortunately, many of the modified plant-based feed ingredients (protein concentrates, isolates and glutes) become cost-prohibitive in least-cost ration formulations (Hardy, 1996).

The objectives of the present study were to: (1) determine the apparent digestibility coefficients (ADCs) of protein and energy and the digestible energy (DE) content of a wide range of feed ingredients available in Canada including fish meals, crustacean by-product meals, animal by-product meals and plant-based meals when included at 30% in the diet for Atlantic cod and (2) test the assumption that the basal mix portion of the test diet (70%) and the test feed ingredient (30%) are digested independently of one another (Cho et al., 1982).

2. Materials and methods

2.1. Fish

Atlantic cod juveniles were cultured at the NRC Institute for Marine Biosciences, Marine Research Station (Halifax, Nova Scotia) for use in this study. Three hundred and sixty of these fish (89.9 ± 4.0 g average weight) were temperature acclimated in a single 2000 L circular fiberglass tank with flow-through (30 L/min), filtered (30 μm) seawater (salinity, 28–30 ppt). Temperature acclimation involved a gradual increase in water temperature (0.5 °C per day) from 4 to 12 °C over a 3-week period. During this period, the fish were hand-fed EWOS™ 5.0 mm Marine Feed (EWOS Canada, Surrey, BC, Canada) twice daily (0900 and 1600 h) to

apparent satiation. The proximate composition (*as-fed* basis) of this diet was: moisture 63 g/kg, crude protein 551 g/kg, lipid 119 g/kg, ash 106 g/kg, and gross energy 21 MJ/kg.

2.2. Experimental diets

A practical, fish meal-based basal diet (Table 1) was formulated according to digestible protein (DP) and digestible energy (DE) values of feed ingredients for haddock (Tibbetts et al., 2004). Seventeen experimental diets were subsequently produced containing a mixing ratio (w/w basis) of basal diet (69.75%) and test feed ingredient (29.75%). One additional diet containing 99.5% basal diet with no test feed ingredient was also produced and served as the reference diet. All 18 experimental diets were supplemented with chromic oxide (Cr₂O₃, 5 g/kg) as the inert digestion indicator (Austreng, 1978).

The test feed ingredients consisted of two fish meals (herring, anchovy), three crustacean by-product meals (whole krill, crab, shrimp), two animal by-product meals (poultry by-product, hydrolyzed feather), six oilseed

meals (soybean, soy protein concentrate, soy protein isolate, canola, canola protein concentrate, flaxseed), two pulse meals (white lupin, pea protein concentrate) and two cereal grain meals (corn gluten, wheat gluten). Their international feed number, proximate composition, gross energy content and supplier are given in Table 2. Dry ingredients of the basal diet and all test feed ingredients were finely ground (<800 µm) using a Perten Laboratory Mill (Model 3100, Perten Instruments, Huddinge, Sweden). Micronutrients (vitamins and minerals) were pre-mixed with ground wheat as a base, using a twin-shell blender (Paterson-Kelly, East Stroudsburg, PA, USA) prior to being added to the main ingredient mixture. All ingredients were mixed in a Hobart mixer (Model H600T, Rapids Machinery Co., Troy, OH, USA) and steam-pelleted into 4.0 mm pellets (California Pellet Mill Co., San Francisco, CA, USA). The pellets were dried in a forced-air drier at 80 °C for 90 min to form dry, sinking pellets and stored in air-tight containers at –20 °C until use. Diets were screened to remove fines prior to feeding.

2.3. Digestibility system and fecal collection

After the 3-week temperature acclimation, the fish were randomly distributed into a digestibility system consisting of 12 tanks (120 L capacity) each equipped with a fecal collection column (Fig. 1), which was a modification of the Guelph system (Cho et al., 1982). The modifications were made in order to (1) utilize a single, circular fiberglass tank as the experimental unit rather than triple, grouped rectangular tanks and (2) increase the rate and quantity of fecal recovery by re-positioning the fecal collection column directly below the drain at the bottom of the tank. This modification increased the efficiency of fecal settlement by eliminating any requirement for horizontal flow. A gate valve was installed at the connection between the tank and the fecal collection column so that the column could be isolated from the effluent water and removed from the system for cleaning at the end of each day without any disruption in water flow to the fish.

The fish were acclimated to these tanks and the experimental diets for 2 weeks prior to beginning the trial. The experiment was conducted according to a randomized block design and replicated twice. Each of the 18 experimental diets was fed to two tanks, each containing 30 fish with an initial mean weight of 89.9±4.0 g. Filtered (30 µm), UV-treated seawater (salinity, 28–30 ppt) was supplied to each tank at a flow rate of 3 L/min in a flow-through system and continuously aerated (8.6±0.8 mg/L dissolved oxygen; 91±6% gas saturation). The water temperature was maintained thermostatically (11.9±

Table 1
Formulation and proximate composition of the basal diet (*as-fed* basis)

Ingredient	(g/kg)
Herring meal (76.9% CP) ^a	480.0
Wheat gluten meal (80.1% CP) ^b	50.0
CPSP-G (73.2% CP) ^c	50.0
Wheat middlings (17.9% CP) ^d	168.0
Whey powder (10.4% CP) ^e	70.0
Krill hydrolysate (57.7% CP) ^f	20.0
Corn starch (pre-gel) ^g	56.0
Vitamin mixture ^h	19.5
Mineral mixture ^h	19.5
Choline chloride ⁱ	3.0
Herring oil ^j	64.0
Proximate composition (n=2)	
Moisture (g/kg)	100.5
Crude protein (g/kg)	487.5
Lipid (g/kg)	120.6
Ash (g/kg)	63.1
Carbohydrate ^k (g/kg)	228.3
Gross energy (MJ/kg)	20.5

^a St. Laurent Gulf Products Limited (Caraquet, NB, Canada).

^b Roquette UK Limited (Northants, UK).

^c Concentre proteique soluble de poisson (*soluble fish protein concentrate*) (Sopropêche, France).

^d Dover Mills Limited (Halifax, NS, Canada).

^e Farmers Co-operative Dairy (Truro, NS, Canada).

^f SD-KH2, MaraVision Marine Products (Vancouver, BC, Canada).

^g National Starch and Chemical Company (Bridgewater, NJ, USA).

^h Vitamin and mineral premixes according to Tibbetts et al. (2004).

ⁱ USB Corporation (Cleveland, OH, USA).

^j Corey Feed Mills Limited (Fredericton, NB, Canada).

^k Calculated as 1000 – (moisture + crude protein + lipid + ash).

Table 2
Proximate composition and gross energy content (*as-fed* basis) of the test feed ingredients (n=2)

	International feed number	Moisture (g/kg)	Crude protein (g/kg)	Lipid (g/kg)	Ash (g/kg)	Carbohydrate ^a (g/kg)	Gross energy (MJ/kg)
Fish meals							
Herring meal ^b	5-02-000	70.8	745.4	101.3	104.4	0.0	20.8
Anchovy meal ^c	5-01-985	77.8	683.2	95.8	157.6	0.0	19.1
Crustacean by-product meals							
Whole krill meal ^d	5-16-423	47.7	723.9	52.9	175.5	0.0	18.8
Crab meal ^e	5-01-663	91.3	540.4	57.1	227.3	83.9	15.8
Shrimp meal ^f	5-04-226	62.3	372.3	34.8	383.8	146.8	12.4
Animal by-product meals							
Poultry by-product meal ^g	5-03-798	50.2	663.4	145.7	107.6	33.1	22.0
Hydrolyzed feather meal ^g	5-03-795	58.0	835.0	79.4	38.1	0.0	22.7
Oilseed meals							
Soybean meal ^h	5-04-612	113.7	473.1	20.4	59.8	333.0	17.4
Soy protein concentrate ⁱ	5-08-038	79.0	686.6	3.1	51.1	180.2	19.0
Soy protein isolate ⁱ	–	76.4	855.7	44.0	44.7	0.0	21.2
Canola meal ^j	5-06-145	63.1	389.1	26.5	71.0	450.3	18.2
Canola protein concentrate ^j	–	47.5	614.5	27.3	103.5	207.2	19.4
Flaxseed meal ^k	–	120.5	309.9	95.1	46.3	428.2	18.8
Pulse meals							
Pea protein concentrate ^l	–	72.1	489.8	40.7	49.0	348.4	18.5
White lupin meal ^m	–	74.5	384.9	62.1	34.2	444.3	18.9
Cereal grain meals							
Corn gluten meal ^h	5-28-242	110.1	616.2	42.6	9.9	221.2	20.9
Wheat gluten meal ⁿ	–	73.9	793.1	19.0	5.0	109.0	22.6

^a Calculated as 1000 – (moisture + crude protein + lipid + ash).

^b Scotia Garden Seafood Incorporated (Yarmouth, NS, Canada).

^c Sindicato SA, Grupo Sipesa (Lima, Peru).

^d Aqion (Colorado Springs, CO, USA).

^e St. Laurent Gulf Products Limited (Caraquet, NB, Canada).

^f Island Fisherman's Co-Op (Lemeque, NB, Canada).

^g Rothsay (Dundas, ON, Canada).

^h Bunge Canada (Oakville, ON, Canada).

ⁱ Soycomil[®] and Pro-Fam[®], respectively; Archer Daniels Midland (Decatur, IL, USA).

^j MCN BioProducts Incorporated (Saskatoon, SK, Canada).

^k Bioriginal Food and Science Corporation (Saskatoon, SK, Canada).

^l Parrheim Foods (Portage La Prairie, MB, Canada).

^m Alberta Department of Agriculture (AB, Canada).

ⁿ Roquette UK Limited (Northants, UK).

0.2 °C) and monitored daily. The rearing temperature of 12 °C is within the preferred zone of 9–17 °C for Atlantic cod where gastric evacuation rate, appetite and feeding rates are maximized (Jobling, 1988). During the 10-week experimental period, fish were hand-fed to apparent satiety 3 times daily during the week (0900, 1300, 1600 h) and twice daily on weekends (0900, 1300 h). Any dead or moribund fish were collected, weighed and recorded on a daily basis. Each week-day, after the final feeding (1600 h), the tanks and fecal collection columns were thoroughly cleaned with a brush to remove any residual particulate matter (feces and uneaten feed). There were no fecal collections made on weekends. Fecal samples were collected each morning (0830 h) into 250 mL plastic bottles, centrifuged (4000 rpm [2750 ×g]

for 20 min at 4 °C) and the supernatant carefully decanted and discarded. Approximately 17–18 h elapsed between the last feeding and the fecal collection. A minimum of 40 g of wet material was collected from each tank (20 g at each of 2 consecutive collection periods) and each sample was stored in a sealed container at –20 °C for the duration of the collection period. Fecal samples were lyophilized, finely ground and stored at –20 °C until further analyses.

2.4. Analytical techniques, calculations and data analyses

Test feed ingredients, experimental diets and lyophilized fecal samples were analyzed in duplicate using the same procedures. Moisture was determined by drying in an oven

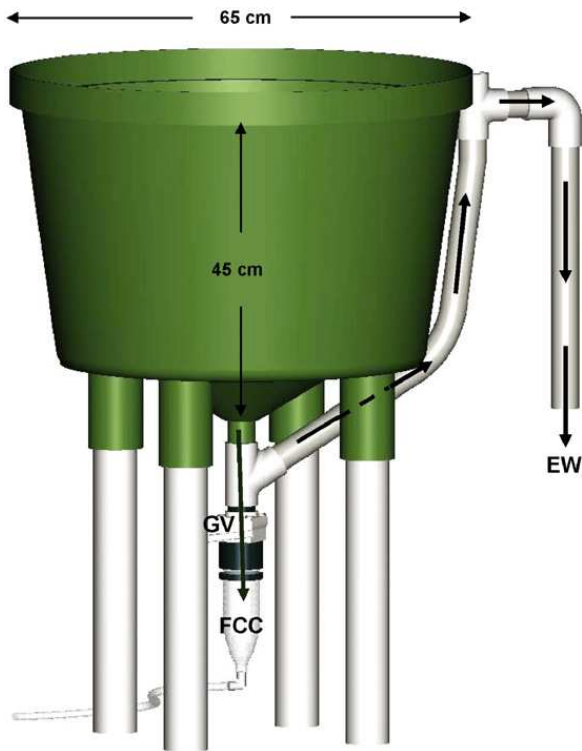


Fig. 1. Modified digestibility system used in this study (GV = gate valve; FCC = fecal collection column; EW = effluent water).

at 105 °C for 18 h and ash by incineration in a muffle furnace at 550 °C for 18 h (Woyewoda et al., 1986). Crude protein (% nitrogen $\times 6.25$) was measured by the Dumas method (Ebeling, 1968) using a Leco nitrogen determinator (Model FP-528, Leco Corporation, St. Joseph, MI, USA). Total lipid was determined using a modified Bligh and Dyer (1959) method. Organic matter was calculated by difference (100 – [moisture + ash]) and carbohydrate was calculated by difference (100 – [moisture + ash + protein + lipid]). Gross energy was measured using an isoperibol oxygen bomb calorimeter (model 6200, Parr Instrument Company, Moline, IL, USA) equipped with a Parr 6510 water handling system for closed-loop operation. Chromic oxide content of experimental diets and fecal samples was determined by flame atomic absorption spectrophotometry using an AAnalyst 300 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) following a microwave acid digestion procedure as described by Peach (2005, pp. 52–54) using a Multiwave sample preparation platform system (Perkin-Elmer, Norwalk, CT, USA).

Diet digestibility (% dry matter digestibility) for the reference and test diets was calculated as follows:

$$\text{Diet digestibility}(\%) = 100 - (100 \times [\text{Cr}_2\text{O}_3 \text{ diet} / \text{Cr}_2\text{O}_3 \text{ feces}])$$

Apparent digestibility coefficients (ADCs) of protein and energy for the reference and test diets were calculated according to Maynard et al. (1979 p. 41) as follows:

$$\% \text{ADC} = 100 - (100 \times [\text{Cr}_2\text{O}_3 \text{ diet} / \text{Cr}_2\text{O}_3 \text{ feces}] \times [\text{nutrient feces} / \text{nutrient diet}])$$

Using these data, protein and energy ADCs for the single test feed ingredients were calculated according to Forster (1999).

$$\% \text{ADC} = ([a + b] \times \text{ADC test diet} - [a] \times \text{ADC reference diet}) \times b^{-1}$$

- a* nutrient contribution of reference diet to nutrient content of test diet
b nutrient contribution of test ingredient to nutrient content of test diet

To calculate the predicted test diet ADC, the following formula was used:

$$\begin{aligned} \text{Test diet protein ADC or DE} \\ = ([0.7 \times \text{reference diet protein ADC or DE}] \\ + [0.3 \times \text{test ingredient protein ADC or DE}]) \end{aligned}$$

Mean protein and energy ADC (or DE) \pm standard error (SE) were calculated from the average of 2 replicate tanks receiving each experimental diet. Statistical analyses were performed using analysis of variance, ANOVA (SYSTAT® 8.0) with a 5% level of probability ($P < 0.05$) selected in advance to sufficiently demonstrate a statistically significant difference.

3. Results and discussion

3.1. Composition of test feed ingredients

The proximate composition and gross energy content of the 17 test feed ingredients are reported in Table 2 along with their international feed numbers. The moisture content of the feed ingredients ranged between 5 and 12%. The crude protein (68 and 75%) and lipid (10%) content of the fish meals are in the typical range of 55–75% and 5–10%, respectively (Hardy, 1996). The ash values were as expected with herring meal at 10% and anchovy meal at 16% (NRC, 1993). Since herring meal contains higher protein and lower ash than anchovy meal, the gross energy content of the herring meal was about 2 MJ/kg higher than anchovy meal (21 vs. 19 MJ/kg).

The krill meal used in this study was produced by finely grinding (<800 µm) whole freeze-dried krill (*Euphausia superba*) and thus the proximate composition was quite different from that found in commercially produced krill meals. The earlier work of Storebakken (1988) reported a proximate composition of 62% crude protein, 12% lipid, 16% ash and 5% chitin in krill. Typically, krill meals produced from various species contain in the range of 33–55% protein, 15–20% lipid and 15–28% ash (Hardy and Barrows, 2002). The whole krill meal used here contained considerably higher protein (72%), lower lipid (5%) and had an ash content within the range reported (17%). The crab meal used in this study was provided by a local company that has made significant improvements in processing of Atlantic snow crab (*Chionoecetes opilio*) over the years. Crab meals typically contain 32% protein and 41% ash (NRC, 1993) while the crab meal used in this study had a much higher protein (54%) and lower ash (23%) content. The crude protein (37%) and lipid (3.5%) contents of the shrimp meal were close to expected (Hardy, 1996; NRC, 1993), whereas the ash content was very high (38%). Most shrimp meals typically contain 18–27% ash (Hardy, 1996; NRC, 1993).

The poultry by-product meal used in this study contained 15% lipid, 11% ash and 66% crude protein. Typically, poultry by-product meals contain 58–60% protein and 14–16% ash (Hardy, 1996; Hardy and Barrows, 2002). The hydrolyzed feather meal contained the expected (80–85%) protein level (83%) but higher levels of lipid (8%) and ash (4%) where typical levels are 5 and 3%, respectively (NRC, 1993; Hardy and Barrows, 2002).

The composition of soybean meal and canola meal were as expected at 47 and 39% protein, 2 and 3% lipid and 6 and 7% ash, respectively (Hardy, 1996). Canola and soy protein concentrates are typically high (55–80%) in protein (Hardy, 1996) and the products used in this study were in that range (61 and 69%, respectively). As expected, the protein content of the soy protein isolate was much higher at 86%. Further processing of these plant-based ingredients increased the gross energy (MJ/kg) contents (soybean meal [17], soy protein concentrate [19], soy protein isolate [21] and canola meal [18], canola protein concentrate [19]).

The pea protein concentrate used in this study was an air-classified protein concentrate and contained higher protein (49%) than regular pea meals which contain <25% protein (Hardy, 1996). The white lupin meal contained 38% protein, which is in the typical range (35–43%) for dehulled lupin seeds (Hardy, 1996). Both pulse meals contained relatively high lipid (4 and 6%),

low ash (3 and 5%) and high gross energy (19 MJ/kg), which is comparable to some fish meals and other plant protein concentrates.

Crude protein and lipid content of the corn gluten meal were slightly higher than typically reported (62 and 4%) and may be the result of the slightly lower ash (1%) content (NRC, 1993). The wheat gluten meal used in this study was typically high (79%) in protein (Hardy, 1996) and very low in lipid (2%) and ash (0.5%). The flaxseed meal was produced by finely grinding (<800 µm) flaxseed press-cake and it contained relatively low amounts of protein (<31%) and high carbohydrate (43%), which was similar to canola meal (45%).

It should be noted that differences in proximate composition of test feed ingredients do exist from batch to batch given the variations in the season of harvest/catch of the raw materials and processing conditions used by various production plants. In addition to differences in their proximate composition, differences in digestibility also occur in feed ingredients that appear to be the same. These effects and also the effect of fecal collection method on ADC values will be discussed further.

3.2. Survival and feed acceptance

Over the 10-week experimental period, fish survival was high on all diets (96–100% survival). It was observed that all diets were accepted equally well by the fish with the exception of diets containing crustacean by-product meals and pea protein concentrate. The crustacean meals induced a positive feeding response. The diet containing pea protein concentrate was not readily accepted by the fish. This can likely be attributed to the presence of soyasaponin 1 which occurs naturally in peas and is described as having a bitter, astringent and metallic flavor (Price et al., 1985).

3.3. Test diet composition and digestibility

The proximate composition, gross energy content and dry matter diet digestibility of the experimental diets are shown in Table 3. All diets had moisture contents in the range of 8 to 10%. Protein and energy content ranged from 44 to 61% and from 18 to 21 MJ/kg, respectively and reflected the protein and energy contents of the test ingredients. The ash content was in the range of 5 to 11% for the experimental diets with the exception of the diet containing shrimp meal (15%).

Digestibility of the reference diet was 76% and most test diets were similar to or higher than that value (range,

Table 3
Proximate composition, gross energy content (*as-fed* basis, $n=2$) and diet digestibility (mean \pm SE, $n=4$, ranked highest to lowest) of the reference and test diets

	Moisture (g/kg)	Crude protein (g/kg)	Ash (g/kg)	Gross energy (MJ/kg)	Diet ADC (%)
Wheat gluten meal	90.7	595.3	50.1	21.1	81.1 \pm 0.5
Whole krill meal	89.3	560.8	98.4	20.0	80.2 \pm 0.4
Soy protein isolate	94.2	611.2	63.9	20.6	79.8 \pm 1.1
Herring meal	91.7	570.9	80.4	20.5	79.0 \pm 0.1
Soy protein concentrate	91.1	559.6	65.3	20.0	77.4 \pm 0.4
Anchovy meal	94.4	566.9	95.2	19.8	77.3 \pm 0.6
Corn gluten meal	100.2	535.6	53.1	20.5	77.0 \pm 0.4
Reference	100.1	493.9	69.7	20.4	76.0 \pm 0.7
Soybean meal	100.4	483.7	68.5	19.6	75.5 \pm 0.6
Canola protein concentrate	83.5	542.2	80.8	20.0	74.9 \pm 0.4
Crab meal	94.8	507.8	109.3	19.2	74.5 \pm 0.2
Poultry by-product meal	81.8	548.5	82.9	20.8	73.3 \pm 1.2
Pea protein concentrate	87.9	495.1	65.9	19.9	72.7 \pm 0.3
White lupin meal	89.6	456.0	60.1	20.0	70.8 \pm 0.6
Hydrolyzed feather meal	83.0	599.3	60.4	21.1	68.4 \pm 0.7
Canola meal	88.4	468.6	70.7	19.8	66.8 \pm 0.6
Shrimp meal	85.4	463.3	154.1	18.2	60.9 \pm 0.5
Flaxseed meal (period 2)	102.7	439.5	63.2	19.9	58.8 \pm 0.1
Flaxseed meal (period 1)					52.7 \pm 0.3

73–81%), with the exceptions of test diets containing white lupin meal, hydrolyzed feather meal, canola meal, shrimp meal and flaxseed meal (range, 53–71%). This is likely due to high levels of ash ($>38\%$) in shrimp meal, carbohydrate ($>40\%$) in canola, flaxseed and white lupin meals and keratin protein in hydrolyzed feather meal.

There were 2 consecutive fecal collection periods for fish fed all experimental diets and ADCs of each diet at the 2 collection periods were compared by ANOVA. No significant differences ($P>0.05$) between collection periods, with the exception of the diet containing flaxseed meal were observed; accordingly, data for periods 1 and 2 were pooled for the remaining 17 experimental diets. For the diet containing flaxseed meal, there was a significant period effect ($P<0.05$) where the diet ADC for period 1 was 53% but had significantly improved to 59% by period 2. As a result,

all further data analysis for this diet was treated separately and denoted as flaxseed meal (period 1) and flaxseed meal (period 2), respectively. The flaxseed meal used in this study was not a commercial product, rather it was prepared in our lab by finely grinding press-cake after oil extraction and was not dehulled. This product likely was quite high in indigestible fiber (essentially “bulk”), which promoted a laxative effect and had a pronounced effect on fecal output, as has been observed with European seabass (Dias et al., 1998). Thus, it is not surprising that diet digestibility was low. The significant increase in diet ADC from 53% in period 1 to 59% in period 2 indicates that the fish gut microflora may have adapted to this dietary stressor by increasing in population in the presence of the elevated level of dietary fiber, however, there is no evidence in the literature to support this claim. If these fish were kept on this diet for a longer period of time, it is doubtful that the diet ADC would continue to improve significantly given the cold-water, carnivorous nature of Atlantic cod.

3.4. Fish meals

Protein ADCs for the fish meals were high (Table 4). The value for herring meal (93%) is similar to that previously reported for haddock (*Melanogrammus aeglefinus*) (94–96%) (Tibbetts et al., 2004; Kim et al., 2006) and salmonids such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*) and Chinook salmon at 89–96% (Anderson et al., 1997; Hajen et al., 1993; Sugiura et al., 1998; Burel et al., 2000; Cheng and Hardy, 2002). The value for anchovy meal (92%) is similar to those reported for salmonid species, which is in the range of 86–94% (Anderson et al., 1995; Hajen et al., 1993; Sugiura et al., 1998, 2000; Thiessen et al., 2004; Glencross et al., 2005). Protein ADCs of fish meals measured with cod are also similar to those reported for turbot (*Psetta maxima*), seabass (*Dicentrarchus labrax*) and Atlantic halibut (*Hippoglossus hippoglossus*) at 91–96% (Gomes da Silva and Oliva-Teles, 1998; Burel et al., 2000; Peach, 2005). Energy ADCs for the fish meals were also high (herring meal, 93% and anchovy meal, 86%) and are in the same range as those reported for the species mentioned above (88–99%).

As noted previously, differences in ADC values of feed ingredients do occur frequently and are usually the result of species differences, variations in the season of harvest/catch of the raw materials and processing conditions used by various production plants. We have no control over these factors in the present study as only one sample of each feed ingredient was used. In

Table 4
Apparent digestibility coefficients (%) for protein and energy and the DE content (MJ/kg) of 17 common and alternate test feed ingredients and the reference diet for Atlantic cod

Ingredient	Protein ADC	Energy ADC	DE ^a
Reference diet	91.2	80.7	16.5
Fish meals			
Herring meal	93.3±0.6	92.8±0.1	19.3±0.0
Anchovy meal	92.2±0.5	86.4±0.7	16.5±0.1
Crustacean by-product meals			
Whole krill meal	96.3±0.6	96.3±0.6	18.1±0.1
Crab meal	89.4±0.7	82.4±0.7	13.0±0.1
Shrimp meal	66.7±1.4	41.4±4.0	5.1±0.5
Animal by-product meals			
Poultry by-product meal	80.2±0.7	71.0±1.1	15.6±0.2
Hydrolyzed feather meal	62.4±0.3	58.9±0.3	13.3±0.1
Oilseed meals			
Soybean meal	92.3±1.5	88.1±0.3	15.3±0.1
Soy protein concentrate	98.6±0.6	94.9±0.3	18.0±0.1
Soy protein isolate	97.4±0.6	92.1±0.8	19.5±0.2
Canola meal	76.0±1.6	60.6±1.7	11.0±0.3
Canola protein concentrate	88.8±0.4	83.3±0.3	16.1±0.1
Flaxseed meal (period 1)	50.2±1.6	21.2±0.3	4.0±0.1
Flaxseed meal (period 2)	55.0±1.1	37.4±0.1	7.0±0.0
Pulse meals			
Pea protein concentrate	89.8±0.8	76.7±0.3	14.2±0.1
White lupin meal	89.7±3.8	75.3±1.3	14.3±0.2
Cereal grain meals			
Corn gluten meal	86.3±1.0	82.7±0.7	17.2±0.1
Wheat gluten meal	99.9±0.3	95.4±0.7	21.5±0.2

Values are mean±SE ($n=4$ except for flaxseed meal where $n=2$).

^a As-fed basis.

addition, differences can occur due to procedures used by various laboratories including fecal collection method, ADC equation used and variations in the formulation of the reference diet. With regard to the fecal collection method and ADC equation used, it is well documented that procedures involving manually stripping, anal suction or dissection cause significant stress to the animal and likely result in fecal samples contaminated with non-fecal nutrients (digestive enzymes, bodily fluids, sloughed epithelial cells, etc.). Fecal samples obtained by these methods tend to underestimate ADC while methods involving settlement, siphoning or screening may overestimate ADC due to leaching losses. The method we chose to use involved the use of a settlement column like the one used on the original Guelph system where Cho et al. (1982) reported no significant losses due to leaching. In addition, our modified tank design further reduced the likelihood of leaching losses by increasing fecal recovery time. Variability in ADC values is also due to the use of different equations to calculate ADC. Recently, Forster (1999) concluded that the traditional

equation used to calculate ADC (Cho et al., 1982) is flawed and, thus, the ADC literature for fish contains values calculated by various equations. In a preliminary work, we have confirmed the use of Forster's equation for our work with cod (Tibbetts et al., 2006). While much of the data cited in this paper for comparison would likely have been calculated using the traditional equation, the differences are typically very small and not significant, but may partly explain some of the variation presented especially for feed ingredients of low digestibility.

3.5. Crustacean by-product meals

Protein ADCs were high for whole krill (96%) and crab (89%) meals and low for shrimp meal (67%). Although little published information exists for krill meal digestibility in fish, a lower value (87%) has been reported for rainbow trout (Vens-Capel and Horstmann, 1978 in Storebakken, 1988) and is likely due to differences in product quality. Although a different product, the protein ADC of krill hydrolysate was found to be almost the same (98%) in Atlantic halibut (Peach, 2005). The 2% higher protein ADC observed in halibut may be due to the lack of chitin present in krill hydrolysates, regardless, the protein ADC of whole krill meal by cod is very high. Protein ADC of crab meal measured in this study with cod (89%) is similar to that of Atlantic halibut (88%) and both are higher than reported previously in our lab with haddock (82%) (Tibbetts et al., 2004). This is likely the result of improved production protocols now employed by the crab meal manufacturer as mentioned earlier. The low protein ADC reported here for shrimp meal (67%) is similar to our previous report with haddock (74%) and both are lower (82%) than that reported for Atlantic halibut (Tibbetts et al., 2004; Peach, 2005). The discrepancy between haddock/cod and other species may be due to the unusually high ash content of the shrimp meal sample used in these studies. As such, digestibility of shrimp meal by gadoids may have to be re-examined with alternate shrimp meal sources. Energy ADC was high for whole krill meal (96%), mid-range for crab meal (82%) and low for shrimp meal (41%). The value reported for whole krill meal (96%) is consistent with that reported for krill hydrolysate (97%) by Atlantic halibut (Peach, 2005). The energy ADC for crab meal in cod fully agrees with that reported for haddock (83%) but the value for shrimp meal (41%) is significantly lower than those reported for haddock and halibut at 70–75% (Tibbetts et al., 2004; Peach, 2005).

3.6. Animal by-product meals

Protein ADCs were mid-range for poultry by-product meal (80%) and low for hydrolyzed feather meal (62%). Animal by-product meals are highly variable in proximate composition based upon several factors (raw material source and freshness, production processes and storage) and, as such, the reported values for protein ADC are also highly variable in fish studies. Protein ADC values reported for poultry by-product meal for salmonids (Hajen et al., 1993; Sugiura et al., 1998; Bureau et al., 1999; Cheng and Hardy, 2002; Cheng et al., 2004) and Atlantic halibut (Peach, 2005) are in a wide range of 74–96%. Our value reported for cod (80%) is within this range and also consistent with that reported (80%) for gilthead seabream (*Sparus aurata*) (Lupatsch et al., 1997). Protein ADC of hydrolyzed feather meal is higher for salmonids at 71–87% (Hajen et al., 1993; Sugiura et al., 1998, 2000; Bureau et al., 1999; Cheng et al., 2004) than that reported here for cod (62%) but similar to that reported for Atlantic halibut (58%) (Peach, 2005). The highly variable nature of animal by-product meals is also reflected in energy ADC where the values reported for the species listed above are also highly variable for poultry by-product meal (65–91%) and hydrolyzed feather meal (57–85%). Our values for poultry by-product meal (71.0%) and hydrolyzed feather meal (58.9%) are consistent with those reported for Chinook salmon at 72% and 57%, respectively (Hajen et al., 1993). The energy ADC of hydrolyzed feather meal is also similar to that of Atlantic halibut at 62% (Peach, 2005).

3.7. Oilseed meals

Protein ADC was high for soybean meal (92%), soy protein concentrate (99%) and soy protein isolate (97%). Digestibility of soybean meal has been extensively studied with various fish species and although there is a broad range reported on the protein ADC (76–98%), the value found here for cod (92%) is consistent with those reported for rainbow trout (92%), coho salmon (93%) and haddock (92%) (Glencross et al., 2005; Sugiura et al., 1998; Tibbetts et al., 2004). Similarly, there is a wide range of values (61–92%) reported for energy ADC for the above species (Hajen et al., 1993; Lupatsch et al., 1997; Gomes da Silva and Oliva-Teles, 1998; Morales et al., 1999; Lee, 2002; Cheng and Hardy, 2003; Peach, 2005; Glencross et al., 2005; Tibbetts et al., 2004) although the value found for cod (88%) agrees with haddock (88%) (Kim et al., 2006). The protein ADC of soy protein concentrate for cod (99%) is

consistent with those reported for rainbow trout (98%) and Atlantic halibut (100%) while the energy ADC (95%) is slightly higher than those of rainbow trout (87%) and Atlantic halibut (92%) (Glencross et al., 2005; Peach, 2005). The protein ADC of soy protein isolate for cod (97%) is close to that reported for rainbow trout (98%) while the energy ADC (92%) is slightly lower than that of rainbow trout (96%) (Glencross et al., 2005). Clearly, concentrating soybean meal into concentrates/isolates has a positive effect on digestibility and may be attributed to a reduction in anti-nutritional factors associated with raw soybean meal. This has been confirmed with rainbow trout, Atlantic salmon and Atlantic halibut where no negative effects on fish growth performance were observed with diets containing relatively high levels of soy protein concentrate (Kaushik et al., 1995; Storebakken et al., 1998a,b; Berge et al., 1999). However, given that protein and energy digestibility of soybean meal is already high for cod (92 and 88%, respectively), further processing significantly increases cost of the products and therefore may not provide any additional benefit on a price per digestible nutrient basis. The use of these ingredients in commercial cod feeds will require growth studies and a full economic evaluation in a least-cost ration formulation. Interestingly, it was recently found that, in contrast to salmon, cod do not develop enteritis when soybean meal is included at high levels in the feed, which is very promising, given the high dietary protein requirement of cod (Rosenlund and Skretting, 2006).

Protein ADC was mid-range for canola meal (76%) and high for canola protein concentrate (89%). For canola meal, this value is lower than other fish species which are in the range of 83–95% (Hajen et al., 1993; Mwachireya et al., 1999; Burel et al., 2000; Cheng and Hardy, 2002; Tibbetts et al., 2004; Peach, 2005) but the value for canola protein concentrate (89%) is consistent with rainbow trout (90%) reported by Thiessen et al. (2004). The energy ADC of canola meal for cod (61%) is in the range (52–76%) reported for salmonids and halibut (Anderson et al., 1992; Hajen et al., 1993; Mwachireya et al., 1999; Burel et al., 2000; Cheng and Hardy, 2002; Peach, 2005) and was similar (60%) to haddock (Tibbetts et al., 2004). The energy ADC for canola protein concentrate is relatively unknown for most fish species with the exception of rainbow trout (reported value of 86%, Thiessen et al., 2004), which is higher than the value obtained for cod (83%). Like soybean meal, further processing of canola meal to produce canola protein concentrate had a positive effect on both protein ADC (canola protein concentrate 89% > canola meal 76%) and energy ADC (canola protein concentrate 83% > canola meal 61%). However, it appears

that ash is also concentrated to a relatively high level (>10%) which is roughly double that of the soy products and, hence, the digestibility of energy of canola protein concentrate is marginal. The use of canola products in cod and haddock (Tibbetts et al., 2004) diets agrees with those of Burel et al. (2000) on rainbow trout and turbot, that despite much progress in genetic engineering and processing technologies, the potential use of rapeseed- and canola-derived meals at higher levels in carnivorous fish feeds may not be feasible.

Protein and energy ADCs of flaxseed meal by cod were low. Although there is little data for comparison among cold-water fish species, the values are better for protein (81%) and energy (63%) for rohu (*Labeo rohita*) (Hossain et al., 1997), which is not surprising given the warm water preference of that species. The product used in that study was a commercial product with a higher protein and lower fiber and carbohydrate content, while the flaxseed meal we used was produced in our lab by finely grinding flaxseed press-cake after oil extraction. This product contained seed hulls which contributed high levels of indigestible fiber to the experimental diet. When incorporated at 30% of the diet, it likely increased the dietary fiber (bulk) concentration to a level that induced a laxative effect. As a result of the increased gut transition rate, a pronounced effect on fecal output was observed with the flaxseed diet. Increased dietary “bulk” content caused a significantly increased fecal egestion time in European seabass as well (Dias et al., 1998). Undoubtedly, this was the cause of poor digestibility of other nutrients and energy, an observation supported by Mwachireya et al. (1999) who found that high levels of dietary fiber had an adverse effect on nutrient digestibility.

3.8. Pulse meals

Protein ADC was high for pulse meals (90% for both) and mid-range for energy ADC (pea protein concentrate, 77% and white lupin meal, 75%). The protein ADCs of the pulse meals (90%) are consistent with those reported for rainbow trout (Morales et al., 1999; Burel et al., 2000; Glencross et al., 2003, 2005; Thiessen et al., 2003). The protein ADC of pea protein concentrate is also similar to turbot (93%) but lower for white lupin meal where a higher value (98%) has been reported (Burel et al., 2000). The higher protein digestibility is likely due to the fact that the lupin meal used by Burel et al. (2000) was finely ground and then extruded, whereas, lupin meal used here was finely ground but not processed. Energy ADC of pea protein concentrate was highly variable (54–87%) for rainbow

trout (Burel et al., 2000; Thiessen et al., 2003) but there is good agreement between the value for cod (77%) and that of turbot (78%) by Burel et al. (2000). Like pea protein concentrate, the reported energy ADC values for white lupin meal are highly variable (52–77%) for rainbow trout (Morales et al., 1999; Burel et al., 2000; Glencross et al., 2003, 2005) but the value for cod (75%) falls within this range. The extruded lupin meal used by Burel et al. (2000) also led to higher energy ADC by turbot (85%) as compared to cod (75%). There appears to be some potential for the use of pulse meals in marine fish diets, but they should be pre-extruded to increase the digestibility of non-protein components and, in the case of pea protein concentrate, should be produced by wet-milling to reduce the levels of soyasaponin 1 that may present off-flavors in the diet. In a comprehensive review of pea proteins, Owusu-Ansah and McCurdy (1991) noted that the major drawback of pea protein supplemented products was the objectionable flavor and that further investigation was needed, especially with the concentrates. Since feed intake was reduced in fish receiving the pea protein concentrate diet and it is well-known that a reduction in feed intake can elevate the level of metabolic fecal nitrogen, overcoming the palatability problems may reveal the protein ADC to be even higher than reported here (90%).

3.9. Cereal grain meals

Protein ADC was high for corn gluten meal (86%) and mid-range for energy ADC (83%). The reported protein ADC values for salmonids (87–97%) are slightly higher than our value (86%) for cod (Anderson et al., 1992; Yamamoto et al., 1997, 1998; Sugiura et al., 1998; Morales et al., 1999; Cheng and Hardy, 2003; Thiessen et al., 2004) while it was similar to those reported for other marine fish (79–94%) like haddock, seabream and Atlantic halibut (Yamamoto et al., 1998; Tibbetts et al., 2004; Peach, 2005). Although there is some variation in the reported energy ADC values (76–91%) for rainbow trout (Morales et al., 1999; Cheng and Hardy, 2003; Thiessen et al., 2004), our value for cod (83%) was within that range and similar to those recently reported for haddock (81%) and Atlantic halibut (85%) (Tibbetts et al., 2004; Peach, 2005). It has been reported that corn gluten meal can effectively replace up to one-third of the fish meal in diets for turbot (Regost et al., 1999) and there is good potential for its use in cod diets, provided there are no adverse effects of xanthophylls present to pigment the flesh.

Protein ADC was high for wheat gluten meal (100%) as was energy ADC (95%). These values are consistent

Table 5
Apparent digestibility coefficients (ADC) for protein of the test diets — comparison of measured vs. predicted values for determination of independency

Test diet	Diet protein ADC		P
	Measured	Predicted	
Fish meal diets			
Herring meal	92.0±0.2	91.8±0.2	0.55
Anchovy meal	91.6±0.2	91.5±0.2	0.79
Crustacean by-product meal diets			
Whole krill meal	93.2±0.2	92.7±0.2	0.20
Crab meal	90.6±0.2	90.7±0.2	0.90
Shrimp meal	85.2±0.3	83.9±0.4	0.05
Animal by-product meal diets			
Poultry by-product meal	87.8±0.7	88.4±0.6	0.51
Hydrolyzed feather meal	78.2±0.8 ^a	82.0±0.6 ^b	0.01
Oilseed meal diets			
Soybean meal	91.5±0.4	91.5±0.4	0.99
Soy protein concentrate	94.0±0.2	93.4±0.2	0.08
Soy protein isolate	92.8±1.1	92.3±0.8	0.74
Canola meal	87.3±0.4	86.6±0.5	0.32
Canola protein concentrate	89.9±0.5	90.1±0.4	0.77
Flaxseed meal (period 1)	79.7±1.7 ^a	75.1±2.4 ^b	0.03
Flaxseed meal (period 2)	83.5±0.2 ^a	80.4±0.3 ^b	0.02
Pulse meal diets			
Pea protein concentrate	90.3±0.6	90.3±0.6	1.00
White lupin meal	90.8±1.0	90.8±1.1	0.96
Cereal grain meal diets			
Corn gluten meal	89.5±0.4	89.8±0.3	0.62
Wheat gluten meal	94.8±0.1 ^a	93.8±0.1 ^b	0.00

Values are mean±SE ($n=4$ except for flaxseed meal where $n=2$); values within same row having different superscript letters are significantly different ($P<0.05$).

with those reported for Atlantic salmon, coho salmon, rainbow trout and European seabass with protein ADC of 100–101% and energy ADC of 98% (Sugiura et al., 1998; Robaina et al., 1999; Storebakken et al., 2000). The use of wheat gluten meal in the diet for Atlantic salmon has proven, not only to be equal to that of fish meal, but in many cases, superior to using fish meal alone. In a comprehensive study with Atlantic salmon, Storebakken et al. (2000) found no differences in growth of fish fed diets containing 17% wheat gluten meal (35% of total dietary protein) compared to a diet containing fish meal as the only protein source. They showed that partial replacement of fish meal with wheat gluten meal led to increased protein, fat and energy ADCs as well as availability of amino acids (except alanine and lysine). With such high digestibility, lack of anti-nutritional factors and no offensive taste, wheat gluten meal, properly supplemented with certain amino acids, shows significant potential as a fish meal replacement in cod diets. However, like all plant protein concentrates, economics of feed production will need to be considered.

3.10. Test diet independency

For digestibility data of single feed ingredients to be useful in least-cost ration formulations, it is assumed that the protein ADC or DE content of the single feed ingredient and the protein ADC or DE content of the basal mix portion of the diet are independent of one another (Cho et al., 1982). If this assumption is true, then the calculated (or predicted) protein ADC or DE content of a test diet and the actual measured protein ADC or DE content of the test diet would always be the same. This assumption has been tested and validated for other species like rainbow trout, channel catfish, carp, tilapia, ayu, seabass, Australian silver perch and Australian short-finned eel (Cho et al., 1982; Wilson and Poe, 1985; Cho and Kaushik, 1990; Watanabe et al., 1996a,b; da Silva and Oliva-Teles, 1998; Allan et al., 1999; Engin and Carter, 2002) but yet to be validated for Atlantic cod. We compared the predicted and measured values in order to test this assumption using a wide range of test feed ingredients (Tables 5 and 6). For the protein ADC data, our results clearly show that this assumption was true for virtually all test diets, with the only exceptions being for those diets containing test ingredients of very high

Table 6
Digestible energy (DE) content of the test diets — comparison of measured vs. predicted values for determination of independency

Test diet	Diet DE		P
	Measured	Predicted	
Fish meals			
Herring meal	19.0±0.0	19.0±0.0	0.34
Anchovy meal	18.1±0.1	18.2±0.0	0.79
Crustacean by-product meals			
Whole krill meal	18.6±0.1	18.5±0.0	0.28
Crab meal	17.2±0.0	17.1±0.0	0.38
Shrimp meal	14.5±0.2	14.4±0.2	0.88
Animal by-product meals			
Poultry by-product meal	17.8±0.2	17.7±0.1	0.63
Hydrolyzed feather meal	16.8±0.1	17.0±0.0	0.21
Oilseed meals			
Soybean meal	17.8±0.1	18.0±0.0	0.20
Soy protein concentrate	18.6±0.0	18.7±0.0	0.63
Soy protein isolate	19.2±0.2	19.1±0.1	0.80
Canola meal	16.3±0.1	16.3±0.1	0.89
Canola protein concentrate	17.7±0.1	17.9±0.0	0.13
Flaxseed meal (period 1)	14.2±0.0	14.1±0.0	0.74
Flaxseed meal (period 2)	15.2±0.0	15.2±0.0	0.21
Pulse meals			
Pea protein concentrate	17.3±0.1	17.4±0.0	0.34
White lupin meal	17.3±0.1	17.4±0.1	0.42
Cereal grain meals			
Corn gluten meal	18.6±0.1	18.6±0.0	0.74
Wheat gluten meal	19.9±0.1	19.8±0.1	0.33

Values are mean±SE ($n=4$ except for flaxseed meal where $n=2$).

(>99%, wheat gluten) or very low (<67%, hydrolyzed feather and flaxseed) protein ADCs. In terms of DE, the assumption was true for all test diets without exception. The correlation between measured and predicted values was very high (Pearson correlations of 0.95 for protein ADC and 0.99 for DE). It would appear that for the rare feed ingredient where independency does not hold true, the poor digestibility of that particular ingredient would warrant its exclusion from diet formulation.

4. Conclusions

This study has identified several highly digestible (>92% protein ADC and >85% energy ADC) feed ingredients for Atlantic cod on-growing diets, including fish meals, soy-based products, whole krill and wheat gluten meal. Other ingredients with some potential include pulse meals, crab meal, corn gluten meal and canola protein concentrate (85–90% protein ADC and 75–85% energy ADC). Due to high levels of poorly digestible components (ash, fiber, carbohydrate and keratin), poultry and feather by-products, canola, shrimp and flaxseed meals have limited value as feed ingredients for Atlantic cod diets.

Acknowledgements

Assistance by Audrey Butt and Ryan Gibbs with sample preparation and analyses, Laura Garrison with fish husbandry and Glen Farrell for the schematic of the digestibility system is gratefully appreciated. We thank the following companies for their generous donation of test feed ingredients: St. Laurent Gulf Products Ltd., Caraquet, NB, Shur-Gain Feeds, Truro, NS, Skretting Canada, Vancouver, BC, MCN BioProducts Inc., Saskatoon, SK and Bioriginal Food and Science Corp., Saskatoon, SK. The critical review and valuable suggestions of Drs. Harry Murray and John van der Meer are greatly appreciated.

References

- Allan, G.L., Rowland, S.J., Parkinson, S., Stone, D.A.J., Jantrarotai, W., 1999. Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. *Aquaculture* 170, 131–145.
- Anderson, J.S., Lall, S.P., Anderson, D.M., Chandrasoma, J., 1992. Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo salar*) reared in seawater. *Aquaculture* 108, 111–124.
- Anderson, J.S., Lall, S.P., Anderson, D.M., McNiven, M.A., 1995. Availability of amino acids from various fish meals fed to Atlantic salmon (*Salmo salar*). *Aquaculture* 138, 291–301.
- Anderson, J.S., Higgs, D.A., Beames, R.M., Rowshandeli, M., 1997. Fish meal quality assessment for Atlantic salmon (*Salmo salar* L.) reared in sea water. *Aquac. Nutr.* 3, 25–38.
- Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture* 13, 265–272.
- Berge, G.M., Grisdale-Helland, B., Helland, S.J., 1999. Soy protein concentrate in diets for Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* 178, 139–148.
- Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Biophys.* 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, 345–358.
- Burel, C., Boujard, T., Tulli, F., Kaushik, S.J., 2000. Digestibility of extruded peas, extruded lupin and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). *Aquaculture* 188, 285–298.
- Cheng, Z.J., Hardy, R.W., 2002. Apparent digestibility coefficients of nutrients and nutritional value of poultry by-product meals for rainbow trout, *Oncorhynchus mykiss*, measured *in vivo* using settlement. *J. World Aquac. Soc.* 33, 458–465.
- Cheng, Z.J., Hardy, R.W., 2003. Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.* 9, 77–83.
- Cheng, Z.J., Hardy, R.W., Huige, N.J., 2004. Apparent digestibility coefficients of nutrients in brewer's and rendered animal by-products for rainbow trout (*Oncorhynchus mykiss* (Walbaum)). *Aquac. Res.* 35, 1–9.
- Cho, C.Y., Kaushik, S.J., 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Rev. Nutr. Diet.* 61, 132–172.
- Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid species: energy intake, expenditure and productivity. *Comp. Biochem. Physiol.* 73B, 25–41.
- da Silva, G.J., Oliva-Teles, A., 1998. Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. *Aquat. Living Resour.* 11, 187–191.
- Danulat, E., 1987. Digestibility of chitin in cod, *Gadus morhua*, *in vivo*. *Helgol. Meeresunters.* 41, 425–436.
- Danulat, E., Kausch, H., 1984. Chitinase activity in the digestive tract of the cod, *Gadus morhua* (L.). *J. Fish Biol.* 24, 125–133.
- Dias, J., Huelvan, C., Dinis, M.T., Métailler, R., 1998. Influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass (*Dicentrarchus labrax*) juveniles. *Aquat. Living Resour.* 11, 219–226.
- Dong, F.M., Hardy, R.W., Haard, N.F., Barrows, F.T., Rasco, B.A., Fairgrieve, W.T., Forster, I.P., 1993. Chemical composition and protein digestibility of poultry by-product meals for salmonid diets. *Aquaculture* 116, 149–158.
- Ebeling, M.E., 1968. The Dumas method for nitrogen in feeds. *J. Assoc. Off. Anal. Chem.* 51, 766–770.
- Engin, K., Carter, C.G., 2002. Ingredient apparent digestibility coefficients for the Australian short-finned eel (*Anguilla australis australis*, Richardson). *Anim. Sci.* 75, 401–413.
- Forster, I., 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquac. Nutr.* 5, 143–145.
- Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199, 197–227.
- Glencross, B.D., Boujard, T., Kaushik, S.J., 2003. Influence of oligosaccharides on the digestibility of lupin meals when fed to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 219, 703–713.

- Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R., Sipsas, S., 2005. Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. *Aquaculture* 245, 211–220.
- Gomes da Silva, J., Oliva-Teles, A., 1998. Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. *Aquat. Living Resour.* 11, 187–191.
- Hajen, W.E., Higgs, D.A., Beames, R.M., Dosanjh, B.S., 1993. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in seawater. 2. Measurement of digestibility. *Aquaculture* 112, 333–348.
- Hardy, R.W., 1996. Alternate protein sources for salmon and trout diets. *Anim. Feed Sci. Technol.* 59, 71–80.
- Hardy, R.W., Barrows, F.T., 2002. Diet formulation and manufacture. In: Halver, J.E., Hardy, R.W. (Eds.), *Fish Nutrition*, 3rd Edition. Academic Press, New York, USA. 824 pp.
- Higgs, D.A., Dosanjh, B.S., Prendergast, A.F., Beames, R.M., Hardy, R.W., Riley, W., Deacon, G., 1995. Use of rapeseed/canola protein products in finfish diets. In: Sessa, D., Lim, C. (Eds.), *Nutrition and Utilization Technology in Aquaculture*. AOCS Press, Champaign, FL. 130–156 pp.
- Hossain, M.A., Nahar, N., Kamal, M., 1997. Nutrient digestibility coefficients of some plant and animal proteins for rohu (*Labeo rohita*). *Aquaculture* 151, 37–45.
- Jobling, M., 1988. A review of the physiological and nutritional energetics of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. *Aquaculture* 70, 1–19.
- Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fishmeal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout. *Aquaculture* 133, 257–274.
- Kim, J.-D., Tibbetts, S.M., Milley, J.E., Lall, S.P., 2006. Effect of the incorporation level of herring meal into test diet on apparent digestibility coefficients for protein and energy by juvenile haddock, *Melanogrammus aeglefinus* L. *Aquaculture* 258, 479–486.
- Lall, S.P., Nanton, D., 2002. Nutrition of Atlantic cod. *Bull. Aquac. Assoc. Can.* 102-1, 23–26.
- Lall, S.P., Nanton, D.A., Tibbetts, S.M., Roy, P.K., Milley, J.E., 2003. Nutrient requirements and feeding of haddock. In: Aiken, D.E. (Ed.), *Early Rearing of Haddock: State of the Art*. Special Publication Number, vol. 7. Aquaculture Association of Canada, pp. 79–86.
- Lall, S.P., Anderson, S., 2005. Amino acid nutrition of salmonids: dietary requirements and bioavailability. In: Montero, D., Basurco, B., Nengas, I., Alexis, M., Izquierdo, M. (Eds.), *Proceedings of the Workshop on Mediterranean Fish Nutrition*, 1–2 June, 2002, Rhodes, Greece, vol. 63, pp. 73–90.
- Lee, S.M., 2002. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). *Aquaculture* 207, 79–95.
- Lupatsch, I., Kissil, G.W., Sklan, D., Pfeffer, E., 1997. Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. *Aquac. Nutr.* 3, 81–89.
- Maynard, L.A., Loosli, J.K., Hintz, H.F., Warner, R.G., 1979. *Animal Nutrition*, 7th Edition. McGraw-Hill, New York. 603 pp.
- Morales, A.E., Cardenete, G., Sanz, A., de la Higuera, M., 1999. Re-evaluation of crude fibre and acid-insoluble ash as inert markers, alternatives to chromic oxide, in digestibility studies with rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 179, 71–79.
- Mwachireya, S.A., Beames, R.M., Higgs, D.A., Dosanjh, B.S., 1999. Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of commercial canola meal in rainbow trout, *Oncorhynchus mykiss* (Walbaum), held in fresh water. *Aquac. Nutr.* 5, 73–82.
- National Research Council, 1993. *Nutrient Requirements of Fish*. National Academy Press, Washington, D.C. 114 pp.
- New, M.B., Wijkström, U.N., 2002. Use of Fishmeal and Fishoil in Aquafeeds: Further Thoughts on the Fishmeal Trap. *FAO Fish Circ. No. 975*. FAO, Rome, Italy.
- Owusu-Ansah, Y.J., McCurdy, S.M., 1991. Pea proteins: a review of chemistry, technology of production, and utilization. *Food Rev. Int.* 7, 103–134.
- Peach, R.W., (2005). Protein and energy utilization by juvenile Atlantic halibut (*Hippoglossus hippoglossus*). M.Sc. Thesis. Dalhousie University, Halifax/Nova Scotia Agricultural College, Truro, Nova Scotia, 133 p.
- Pike, I.H., 1991. Freshness of fish for fish meal — effect of growth on salmon. In: Kaushik, S.J., Luquet, P. (Eds.), *Fish Nutrition in Practice*. Les Colloques, vol. 61. INRA, Paris, pp. 843–846.
- Price, K.R., Griffiths, N.M., Curl, C.L., Fenwick, G.R., 1985. Undesirable sensory properties of the dried pea (*Pisum sativum*). The role of saponins. *Food Chem.* 17, 105–115.
- Regost, C., Arzel, J., Kaushik, S.J., 1999. Partial or total replacement of fish meal by corn gluten meal in diet for turbot (*Psetta maxima*). *Aquaculture* 180, 99–117.
- Robaina, L., Corraze, G., Aguirre, P., Blanc, D., Melcion, J.P., Kaushik, S., 1999. Digestibility, postprandial ammonia excretion and selected plasma metabolites in European sea bass (*Dicentrarchus labrax*) fed pelleted or extruded diets with or without wheat gluten. *Aquaculture* 179, 45–56.
- Rosenlund, G., Karlsen, Ø., Tveit, K., Mangor-Jensen, A., Hemre, G.-I., 2004. Effect of feed composition and feeding frequency on growth, feed utilization and nutrient retention in juvenile Atlantic cod, *Gadus morhua* L. *Aquac. Nutr.* 10, 371–378.
- Rosenlund, G., Skretting, M., 2006. Worldwide status and perspective on gadoid culture. *ICES J. Mar. Sci.* 63, 194–197.
- Storebakken, T., 1988. Krill as a potential feed source for salmonids. *Aquaculture* 70, 193–205.
- Storebakken, T., Shearer, K.D., Roem, A.J., 1998a. Availability of protein, phosphorous and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein concentrate based diets to Atlantic salmon, *Salmo salar*. *Aquaculture* 161, 365–379.
- Storebakken, T., Kvien, I.S., Shearer, K.D., Grisdale-Helland, B., Helland, S.J., Berge, G.M., 1998b. The apparent digestibility of diets containing fish meal, soybean meal or bacterial meal fed to Atlantic salmon (*Salmo salar*): evaluation of different faecal collection methods. *Aquaculture* 169, 195–210.
- Storebakken, T., Shearer, K.D., Baeverfjord, G., Nielsen, B.G., Åsgård, T., Scott, T., De Laporte, A., 2000. Digestibility of macronutrients, energy and amino acids, absorption of elements and absence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. *Aquaculture* 184, 115–132.
- Sugiura, S.H., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* 159, 177–202.
- Sugiura, S.H., Babbitt, J.K., Dong, F.M., Hardy, R.W., 2000. Utilization of fish and animal by-product meals in low-pollution feeds for rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquac. Res.* 31, 585–593.

- Thiessen, D.L., Campbell, G.L., Adelizi, P.D., 2003. Digestibility and growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with pea and canola products. *Aquac. Nutr.* 9, 67–75.
- Thiessen, D.L., Maenz, D.D., Newkirk, R.W., Classen, H.L., Drew, M.D., 2004. Replacement of fishmeal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.* 10, 379–388.
- Tibbetts, S.M., Lall, S.P., Milley, J.E., 2004. Apparent digestibility of common feed ingredients by juvenile haddock, *Melanogrammus aeglefinus* L. *Aquac. Res.* 35, 643–651.
- Tibbetts, S.M., Milley, J.E., Lall, S.P., 2006. Protein digestibility of common and alternative feed ingredients by juvenile Atlantic cod, *Gadus morhua*. XII International Symposium on Fish Nutrition and Feeding, 28 May– 1 June 2006, Biarritz, France, p. 245.
- Toppe, J., Aksnes, A., Hope, B., Albrektsen, S., 2006. Inclusion of fish bone and crab by-products in diets for Atlantic cod, *Gadus morhua*. *Aquaculture* 253, 636–645.
- Watanabe, T., Takeuchi, T., Satoh, S., Kiron, V., 1996a. Digestible crude protein contents in various feedstuffs determined with four freshwater fish species. *Fish. Sci.* 62, 278–282.
- Watanabe, T., Takeuchi, T., Satoh, S., Kiron, V., 1996b. Digestible energy: methodological influences and a mode of calculation. *Fish. Sci.* 62, 288–292.
- Wilson, R.P., Poe, W.E., 1985. Apparent digestible protein and energy coefficients of common feed ingredients for channel catfish. *Prog. Fish-Cult.* 47, 154–158.
- Woyewoda, A.D., Shaw, S.J., Ke, P.J., Burns, B.G., 1986. Recommended laboratory methods for assessment of fish quality. Canadian Tech Report of Fisheries and Aquatic Sciences, vol. 1448ISSN :0706-6473. Fisheries and Oceans Canada.
- Yamamoto, T., Ikeda, K., Unuma, T., Akiyama, T., 1997. Apparent availabilities of amino acids and minerals from several protein sources for fingerling rainbow trout. *Fish. Sci.* 63, 995–1001.
- Yamamoto, T., Akimoto, A., Kishi, S., Unuma, T., Akiyama, T., 1998. Apparent and true availabilities of amino acids from several protein sources for fingerling rainbow trout, common carp, and red seabream. *Fish. Sci.* 64, 448–458.

Author's personal