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Short communication

Effect of dietary inclusion of Atlantic snow crab, *Chionoecetes opilio* and Northern pink shrimp, *Pandalis borealis* processing by-products on nutrient digestibility by juvenile haddock, *Melanogrammus aeglefinus* L.

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ABSTRACT

A study was conducted to evaluate the effect of dietary inclusion of Atlantic snow crab, *Chionoecetes opilio* (Crab) and Northern pink shrimp, *Pandalis borealis* (Shrimp) by-product meals on nutrient digestibility in haddock, *Melanogrammus aeglefinus*. The study provides coefficients of total tract apparent digestibility (CTTADs) essential for diet formulations aimed at further growth performance and nutrient utilization studies with gadoids using these waste streams as potential replacements for high-cost fish meals and poorly digestible wheat by-products. Organic matter (OM) CTTAD was significantly improved (P=0.005) with Shrimp150 (0.82) relative to the Control (0.79), while Shrimp300 was similar (0.79) (P=1.000). No significant difference in OM CTTAD was found between the Control and Crab150 (P=0.110), Shrimp150 (P=0.473) or Shrimp300 (P=0.144) at an average of 0.80. However, OM CTTAD was significantly reduced (P<0.001) for Crab300 (0.76). Inclusion of Shrimp at 150 g/kg significantly improved (P=0.003) gross energy (GE) CTTAD (0.85 on average) relative to the Control (0.83), while Crab150 (P=0.081), Crab300 (P=0.134) and Shrimp300 (P=0.986) were statistically equal to the Control (average, 0.82). Crude protein (CP) CTTAD of Crab150 (P=0.803) and Shrimp150 (P=0.980) were similar to the Control at an average of 0.90 while Crab300 (P<0.001) and Shrimp300 (P=0.005) were significantly reduced (average 0.86). Dietary inclusion of either Atlantic snow crab or Northern pink shrimp processing by-product meal at 150 g/kg, concomitant with a 50% reduction in wheat middlings and 10% reduction in fish meal, resulted in OM, GE and CP CTTADs equal to or exceeding that of the fish meal-based Control diet.

1. Introduction

To meet nutritional requirements, gadoid feeds contain 650–700 g/kg fish meal, 80–100 g/kg marine fish oil and 20–30 g/kg vitamins and trace elements (Lall and Nanton, 2002; Lall et al., 2003). The remaining 150–250 g/kg is typically carbohydrate-rich wheat by-products that are poorly digested by cold-water gadoids (Tibbetts et al., 2005; NRC, 2011). Therefore, in addition to lowering the reliance on costly fish meal, it is also important to identify local alternative ingredients with higher digestibility than wheat by-products. Traditionally, crustacean by-product meals have not been used in diets for

Abbreviations: OM, organic matter; GE, gross energy; CP, crude protein; FCR, feed conversion ratio; DP, digestible protein; DE, digestible energy; IFN, international feed number; CTTAD, coefficient of total tract apparent digestibility.

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farmed salmonids like Atlantic salmon, *Salmo salar* and rainbow trout, *Oncorhynchus mykiss*. Unlike other animal and plant protein sources, the major by-product of crustacean processing is the shell which contain high ash (>25%) and chitin (>15%), both of which can negatively affect digestibility, growth and skeletal development in farmed fish (Fox et al., 1994; NRC, 2011). Unlike salmonids, gadoids have shown better utilization of ash- and chitin-rich ingredients (Danulat, 1987; Danulat and Kausch, 1984; Toppe et al., 2006). It is also well known that crustacean by-product meals enhance diet palatability for fish due to their excellent chemo-attractant properties (Hertrampf and Piedad-Pascual, 2000). Crustacean by-products are abundantly available and under-utilized in Atlantic Canada. Commercial landings of Atlantic snow crab and Northern pink shrimp in Atlantic Canada were 76,726 and 121,600 t, respectively, in 2010. Since only ~30% of the crab body and ~60% of the shrimp body is suitable for human consumption (De Groot, 1995) with the remainder composed of head, shell, viscera and unextractable meats, annual quantities of these processing by-products could exceed 53,000 and 48,000 t, respectively. The primary objective of this study is to determine the CTTADs essential for diet formulations aimed at further growth performance and nutrient utilization studies with gadoids using these valuable waste streams as potential replacements for high-cost fish meals and poorly digestible wheat by-products.

2. Materials and methods

2.1. Crustacean by-product meals and experimental diets

Live Atlantic snow crab and Northern pink shrimp caught off the coast of Newfoundland and Labrador (NL) in January was iced on board, size graded on the wharf and delivered to the processing plant (Quin-Sea Fisheries, Old Perlican, NL). Crab was immediately cooked in freshwater (100 °C for 11 min) and edible meats extracted by hand while shrimp pre-soaked in sodium metaphosphate-based maturing agent and then mechanically peeled to obtain edible meats. Shells and other by-products were dried in an industrial dryer (140 °C for 20 min) and delivered to us by overnight courier. Initial moisture content was higher than desired (110–130 g/kg) so was further dried (80 °C for 90 min) to reduce moisture content to <100 g/kg. Products were finely ground (500 μm screen) using a laboratory hammer mill (Model 3100, Perten Instruments, Huddinge, Sweden). Analyzed proximate composition, gross energy concentration and chitin content are shown in Table 1. Five isonitrogenous (530 g/kg CP) experimental diets (Table 2) were formulated according to DP and DE values of practical feed ingredients for juvenile haddock (Tibbetts et al., 2004). Diets consisted of four test diets containing either crab by-product (Crab) or shrimp by-product (Shrimp) at either 150 or 300 g/kg (w/w basis) and a fish meal-based control diet (Control). All diets (4.0 mm pellets) were prepared as described in Tibbetts et al. (2006).

2.2. Experimental procedures

Fish were cared for in accordance with the Canadian Council on Animal Care's Guidelines on the Care and Use of Fish in Research, Teaching and Testing (CCAC, 2005). One hundred and ninety juvenile haddock were randomly allotted to ten 120 L cylindro-conical fiberglass tanks equipped with fecal collection columns (Tibbetts et al., 2006). To ensure positive growth, fish were bulk-weighed and counted at the beginning and end of the experiment and they had an initial mean weight of 123.1 ± 0.4 g (range, 122.5–123.6 g) and a final mean weight of 144.0 ± 7.2 g (range, 130.3–153.6 g). Fish were acclimated for 8 days prior to the digestibility trial and each of 5 diets was fed to tanks containing 19 fish for 25 days. Filtered (30 μm) UV-treated seawater (salinity, 28–30 ppt) was supplied at 2 L/min in a flow-through system and continuously aerated (11.0 ± 0.3 mg/L dissolved oxygen; 99.2 ± 2.3% saturation). Water temperature was maintained thermostatically (11.6 ± 0.1 °C) and recorded daily. Fish were hand-fed to apparent satiety 3 times daily Monday to Friday (09:00, 13:00, 16:00 h) and twice daily Saturday and Sunday (09:00, 13:00 h). Each week-day, after the final feeding of the day (16:00 h), the tanks and fecal collection columns were thoroughly cleaned with a brush to remove any residual particulate matter (feces and uneaten feed). Fecal samples were collected each morning (08:30 h) into 250 mL plastic bottles, centrifuged (2750 × *g* for 35 min at 5 °C) and the supernatant gently decanted and discarded. A minimum of 60 g of wet fecal material was collected from 2 replicate tanks, comprised of 30 g at each of 2 sub-sampling periods per tank, and each sample was stored in a sealed

Table 1
Proximate composition (g/kg), gross energy concentration (MJ/kg) and chitin content (g/kg) of Atlantic snow crab (Crab) and Northern pink shrimp (Shrimp) by-product meals (dry matter basis).

	Crab	Shrimp
Analysis		
CP	323	378
Lipid	7	28
Ash	463	431
GE	9.3	11.2
Carbohydrate[a]	123	101
Chitin	247	214

[a] Carbohydrate was calculated as 1000 – (crude protein + lipid + ash).

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container at −20 °C. Fecal samples were lyophilized at a low (<5 °C) shelf temperature (model 50-SRC-6, The Virtis Company, Gardiner, NY, USA), finely ground and stored at −20 °C.

2.3. Analytical techniques

Crustacean by-product meals, experimental diets and lyophilized fecal samples were analyzed in triplicate for proximate composition, gross energy concentration and chromic oxide content according to Tibbetts et al. (2006). Chitin content of Crab and Shrimp was determined according to Black and Schwartz (1950). Briefly, ~0.5 g of sample is hydrolyzed for 1 h in boiling HCl (1 M) and the residues retained (40–60 μm). Residues are then digested for 2 h in boiling NaOH (5%, w/v) and then washed with boiling dH_2O and acetone. Residues are then dried (110 °C) and incinerated (600 °C) and then chitin content estimated as: ([dry residue weight − incinerated residue weight]/[sample weight]) × 100%.

2.4. Statistical procedures

The experimental design was a randomized block and CTTADs were calculated from the average of 2 replicate tanks receiving each experimental diet and 2 consecutive sub-sampling periods from each tank. Statistical analyses were performed using ANOVA and in the case of a significant difference, treatment means were differentiated using the Tukey's multiple range test (SigmaStat® v. 3.5). Raw data was confirmed to have a normal distribution and constant variance using the Kolmogorov–Smirnov test (SigmaStat® v. 3.5). A 5% level of probability ($P<0.05$) was chosen to sufficiently demonstrate a statistically significant difference.

3. Results

Proximate composition, GE concentration and chitin content of Crab and Shrimp are presented in Table 1. Lipid contents were 7 and 28 g/kg, respectively. Chitin content of Crab (247 g/kg) was higher than Shrimp (214 g/kg). Ash content of Crab and Shrimp was high (>400 g/kg) with Crab higher (463 g/kg) than Shrimp (431 g/kg). The largest difference between Crab and Shrimp was CP content, which was higher for Shrimp (378 g/kg) than for Crab (323 g/kg). Since lipid content of Crab and Shrimp were very low (<30 g/kg) and CP content of Shrimp was more than 50 g/kg higher than Crab, GE content in Shrimp (11 MJ/kg) was higher than Crab (9 MJ/kg). Formulation and composition of experimental diets is presented in Table 2. Diets were isonitrogenous (average, 532 g/kg CP) and ranged in ash (114–221 g/kg) and chitin content (0–74 g/kg). Lipid,

Table 2
Formulation and composition of the experimental diets including crustacean by-product meals.

	Control	Crab 150	Crab 300	Shrimp 150	Shrimp 300
Ingredients (g/kg of diet)					
Fish meal[a]	515	470	455	460	414
Soybean meal[a]	80	80	49	80	80
Crab by-product[b]	-	150	300	–	–
Shrimp by-product[b]	–	–	–	150	300
Corn gluten meal[a]	100	100	100	100	100
Wheat middlings[c]	204	104	0	104	0
Chromic oxide[d]	5	5	5	5	5
Choline chloride[e]	6	6	6	6	6
Vitamin mixture[f]	10	10	10	10	10
Mineral mixture[f]	10	10	10	10	10
Fish oil[a]	70	65	65	75	75
Analysis (g/kg or MJ/kg dry matter basis)					
CP	534	526	531	534	533
Lipid	176	120	131	185	161
Ash	114	164	221	162	206
Carbohydrate[g]	176	190	118	118	100
Chitin	0	37	74	32	64
GE	22.0	20.1	18.7	20.7	19.4
DP[h]	485	472	457	483	463
DE[i]	18.3	17.0	15.3	17.7	16.1

[a] Corey Feed Mills Ltd., Fredericton, NB, Canada.
[b] Quin-Sea Fisheries Ltd., Old Perlican, NL, Canada.
[c] Walker's Livestock Feeds, Dartmouth, NS, Canada.
[d] EM Science, Gibbstown, NJ, USA.
[e] United States Biochemical, Cleveland, OH, USA.
[f] Vitamin and mineral mixtures according to Tibbetts et al. (2004).
[g] Carbohydrate calculated as 1000 − (crude protein + lipid + ash).
[h] Digestible protein.
[i] Digestible energy.

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Table 3
Coefficients of total tract apparent digestibility (CTTADs)[a] of organic matter (OM), gross energy (GE) and crude protein (CP) of experimental diets including crustacean by-product meals.[b]

Diet	OM	GE	CP
Control	0.789 ± 0.008^{b}	0.831 ± 0.002^{ab}	0.909 ± 0.004^{b}
Crab150	0.811 ± 0.006^{bc}	0.846 ± 0.004^{bc}	0.898 ± 0.004^{b}
Crab300	0.764 ± 0.004^{a}	0.817 ± 0.002^{a}	0.861 ± 0.010^{a}
Shrimp150	0.824 ± 0.003^{c}	0.855 ± 0.004^{c}	0.904 ± 0.004^{b}
Shrimp300	0.790 ± 0.006^{b}	0.828 ± 0.005^{ab}	0.869 ± 0.008^{a}
Pooled SEM	0.005	0.004	0.006
P value	<0.001	<0.001	<0.001

[a] Calculated according to Tibbetts et al. (2006).
[b] Mean ± SEM ($n = 2$); values within the same column with different superscripts are significantly different (P<0.05).

carbohydrate and GE ranged from 120–185 g/kg, 100–190 g/kg and 19–22 MJ/kg, respectively. Fish fed experimental diets showed positive growth with average growth rate of 3.13 ± 0.03%/day (range, 3.07–3.17%/day) and survival was 100%. CTTAD of OM, GE and CP are presented in Table 3. CTTADs of OM for the Control, Crab150, Crab300, Shrimp150 and Shrimp300 diets were 0.79, 0.81, 0.76, 0.82 and 0.79, respectively. CTTADs of GE were 0.83, 0.85, 0.82, 0.86 and 0.83, respectively. CTTADs of CP were 0.91, 0.90, 0.86, 0.90 and 0.87, respectively.

4. Discussion

The various diet formulations reduced fish meal use by 45–101 g/kg and wheat middlings by 100 g/kg to total replacement (Table 2). Based on CP and GE CTTAD, inclusion of Crab or Shrimp at 150 g/kg were the only formulations that maintained dietary DP and DE levels close to the Control at 472–485 g/kg DP and 17–18 MJ/kg DE. OM CTTAD for Crab300 (0.76) was statistically lower than all other diets which ranged 0.79–0.82 (average, 0.80) and this is consistent with this diet containing the highest ash (221 g/kg) and chitin (74 g/kg). This OM CTTAD (0.80) agrees with previous work with juvenile haddock fed similar diets (Tibbetts et al., 2005). OM CTTAD of Crab150 (P=0.110) and Shrimp300 (P=1.000) (0.79–0.81) were statistically not different from the Control (0.79), while Shrimp150 was significantly higher (0.82; P=0.005). This indicates that replacing fish meal and wheat middlings with Crab (up to 150 g/kg) and Shrimp (up to 300 g/kg) is possible without compromising OM CTTAD. Shrimp at 150 g/kg is preferable to 300 g/kg as OM CTTAD was significantly higher (P=0.006) for Shrimp150 (0.82) than for Shrimp300 (0.79), which was not different from the Control (0.79). Inclusion of Crab at 150 (P=0.081) and 300 g/kg (P=0.134) and Shrimp at 300 g/kg (P=0.986) had no significant impact on GE CTTAD relative to the Control (average, 0.83) while Shrimp150 significantly improved (P=0.003) GE CTTAD (0.86), which is consistent with previous findings (0.86) in juvenile haddock fed similar diets (Tibbetts et al., 2005). Again, inclusion of Shrimp at 150 g/kg is preferable to 300 g/kg as GE CTTAD was significantly higher (P=0.001) for Shrimp150 (0.86) than Shrimp300 (0.83), which was not different from the Control (0.83). With regards to significantly lower OM and GE CTTADs of Shrimp300 relative to Shrimp150, OM and GE portions of feeds are derived from CP, lipid and carbohydrate. Since carbohydrate digestibility in cold-water fish is low (NRC, 2011) and differences in lipid digestibility between diets is unlikely (having identical levels of fish oil and little oil contribution from Shrimp itself), reduced OM and GE CTTADs is likely due to lower CTTAD of CP at the higher Shrimp inclusion (300 g/kg) as a result of higher dietary ash and chitin. Indeed, CP CTTAD was significantly lower (P=0.014) for Shrimp300 (0.87) than for Shrimp150 (0.90). CP CTTAD for Crab and Shrimp was, in fact, significantly reduced (P<0.05) at 300 g/kg (0.86–0.87) relative to Crab150 (P=0.007), Shrimp150 (P=0.014) and Control (0.90–0.91) which were statistically not different (P=0.803 and 0.980, respectively). CP CTTAD range is similar but slightly lower than previously reported for juvenile haddock (0.93) fed similar diets (Tibbetts et al., 2005). Difference is likely due to higher ash content of diets used in this study (114–164 g/kg) than the previous study (92–112 g/kg) and diets used in the previous study had a much lower inclusion of crustacean meal (50 g/kg). CP CTTAD in this study for Crab150, Shrimp150 and Control (0.90–091) agree well with those of Atlantic cod (0.91), a related gadoid fish (Jobling et al., 1991). Significant improvements in OM and GE CTTAD of Shrimp150 (0.82 and 0.86, respectively) relative to Control (0.79 and 0.83, respectively) may be related to efficient utilization of chitin provided at a moderate level (32 g/kg) in Shrimp150. This is supported by similar findings with Atlantic cod showing efficient chitin utilization at moderate inclusion levels (Danulat, 1986, 1987). Unlike fish by-products, the major by-product of crustacean processing is the shell, which contains 150–300 g/kg chitin (poly-β-(1 → 4)-*N*-acetyl-glucosamine), representing 500–800 g/kg of total organic components of the shell (Garzón et al., 1998). Although chitinase enzyme activity has been found in the digestive tracts, *in vivo* chitin CTTAD in salmonids is low (<0.05) for rainbow trout (Lindsay et al., 1984) and Atlantic salmon (0.13–0.40) (Olsen et al., 2006). Danulat (1986, 1987), who also reported significant chitinase enzyme activity in the digestive tracts of Atlantic cod, found high *in vivo* chitin digestibility (>0.90). The same may be true of haddock since they are highly related, have similar feeding habits and the diet of wild haddock consists of 48% echinoderms and crustaceans which are highly chitinous (Lall et al., 2003).

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5. Conclusion

Dietary inclusion of either Atlantic snow crab or Northern pink shrimp processing by-product meal at 150 g/kg, concomitant with a 50% reduction in wheat middlings and 10% reduction in fish meal, resulted in OM, GE and CP CTTADs equal to or exceeding that of the fish meal-based Control diet. This study provided CTTADs essential for diet formulations aimed at further studies on growth performance and nutrient utilization efficiency of gadoids fed diets including these crustacean by-product meals.

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