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Nutritional Analysis of Whole Green Crab, *Carcinus maenas*, for Application as a Forage Fish Replacement in Agrifeeds

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Abstract

Nutritional composition of a composite sample of whole green crabs, *Carcinus maenas* (L.), was undertaken to evaluate efficacy as a forage fish replacement for seafood-meal manufacture. Whole green crabs sampled from New Hampshire waters were ground together and analyzed for proximate profile (moisture, lipid, protein, fiber, and ash), fatty acid profile, amino acid profile, mineral composition, and mercury content. Green crab mince contained 16.55 ± 0.29% ash, 12.27 ± 0.25% protein, and 0.21 ± 0.07% lipid, and comprised all amino acids essential for chickens and most species of fish. Fatty acid composition of ground green crab was 67.98% unsaturated, and 23.29% saturated, and was richer in eicosapentaenoic acid (EPA) than docosahexaenoic acid (DHA). Levels of mercury in green crab mince were below testable limits. The nutritional profile of green crab mince was evaluated relative to the nutritional profile of menhaden from the literature, and possible agrifeed applications for whole green crab were considered. Green crab showed great potential as a forage fish replacer in seafood-meal applications for chickens and ash tolerant species of fish.

Keywords: DHA, menhaden, proximate, fishmeal, crabmeal, mercury, fatty acid, amino acid

1. Introduction

Fishmeal is the primary protein content in feed formulated for finfish and is the largest variable expenditure in the global aquaculture industry (Naylor et al., 2009). Its use in the aquaculture industry has increased dramatically in the past decade - from 33% in 2000 to 73% in 2010 (Shepherd, 2011). However as of 2010, a significant proportion of world fishmeal also is used in terrestrial agriculture, particularly in feed for chickens (5%) and swine (20%; Shepherd, 2011). The wild-caught forage fisheries (e.g. menhaden, anchovies, sardines, capelin; Naylor et al., 2009) from which this fishmeal is produced are highly volatile (McCoy, 1990; Tacon & Metian, 2008; Bimbo, 2009; Naylor et al., 2009), and seventy-five percent are fully exploited or even over-exploited (McCoy, 1990; FAO, 2002; Tacon & Metian, 2008; Bimbo, 2009). The Fisheries and Agriculture Organization of the United Nations estimates that by 2020, existing fishmeal/fish oil resources will no longer be able to support industry demand (FAO, 2002). Numerous fishmeal subsititutes (either whole or partial replacements), including plant derivatives and seafood by-products, already are being tested in aquaculture and agriculture as forage fish prices increase. However, plant and byproduct aquafeeds are often incomplete sources of protein and fatty acids for crustaceans, finfish, and livestock. Widespread invasive marine species represent another potential fishmeal replacement group, especially those that do not support commerical fisheries.

The objective of this study was to analyze the nutritional profile of whole green crab and compare it to reported nutritional profiles of forage fish commonly used for the production of fishmeal in order to assess green crab as a possible agrifeed and aquafeed ingredient. Green crabs, *Carcinus maenas* (L.), are an invasive and globally-dispersed species present on both the east and west coasts of North America, with severe negative ecological impacts on native species (Glude, 1954; Welch, 1968; Carlton & Cohen, 2003; DeGraff & Tyrell, 2004). Green crabs also represent a plentiful, easily-harvested, and underutilized nutrient-rich biomass, although their biomass is largely unquantified as their use in human diets is limited. A fishery for *C. maenas* as a delicacy for humans and as a scent for seafood-based products (Pascoal et al., 2009) occurs in Portugal, where the majority of the crabs is exported live to Spain for consumption or re-export (Gomes, 1988). However, because green crabs are relatively small-bodied, shelling by hand is too labor intensive for a green crab meat product to

be profitable, especially in countries where labor is expensive (Skonberg & Perkins, 2002). In addition, soft-shell green crab is unlikely to be cost-effective either due to high costs associated with harvesting premoult crabs and the labor-intensive operation of shedder facilities (Gaudé & Anderson, 2011). The nutritional composition of a representative sample (as caught in the field) of whole green crabs has not yet been published, and this is a crucial first step for evaluating whole green crab as a forage fish replacement. Both proximate and fatty-acid/amino-acid composition analysis provide information needed to formulate, test, and cost-analyze potential agriculture (feed additive or partial fish meal replacement) markets for this invasive species. Ash content and composition can be a limiting factor in some aquaculture applications because of intestinal and visual ailments associated with high ash contents in freshwater fish diets (Richardson et al., 1985). For these reasons, calcium, zinc, and potassium content of the crabs also need to be quantified. Many heavy metals associated with anthropogenic activities are present in estuarine sediments and it is known that *C. maenas* accumulates As, Cu, Zn, Fe, Cd, Mn, Cu, and Hg from the environment (Andersen & Depledge, 1994; Bjerregaard & Depledge, 2002; Elumalai et al., 2007). However, there is an absence of data to support biomagnification of many of these metals, with the exception of Hg (ie. methylmercury; Kennish, 1992). In this study, crabs were harvested from a moderately developed area, therefore, green crab mince was tested for Hg as an indicator of industrial contamination. Previously published nutritional analyses of green crabs include proximate analysis, fatty acid profile, and amino acid profile of claw and leg meat separately (Skonberg & Perkins, 2002), and of leg meat mince (Naczk et al., 2004). Chitin, total carotenoids, total fatty acid, and total nitrogen content of *C. maenas* shell (Naczk et al., 2004), and fatty acid profile of *C. maenas* hepatopancreas (Styrishave & Andersen, 2000) also have been reported. However, none of these studies used a representative random sample of the range of crabs (size, sex, etc.) that would be caught in a commercial crab trap, and none of them considered the nutritional profile of the whole animal. The current analysis provides information omitted from previous studies regarding the nutritional composition of whole green crabs. In this study, the nutritional profile of whole, ground green crab was analyzed in order to determine if green crabs would be appropriate for partial replacement of fish meal in agriculture and aquaculture applications.

2. Method

Green crabs were collected from the Hampton-Seabrook Estuary (HSE), New Hampshire, U.S.A. in March 2010 (Figure 1). The HSE, located at the coastal border of New Hampshire and Massachusetts, is a temperate, shallow, sandy and muddy-bottom basin fed by five rivers and two smaller streams (Fairchild et al., 2008). Crabs were caught in rectangular plastic-coated wire mesh traps measuring 61 x 28 x 31 cm with a single vertical chute in the top measuring 15 x 5 x 10 cm deep, and baited with a single cod rack each weighing approximately 454 g. From the center of three separate traps, 1.86kg of whole crabs (± 0.05 kg) were selected, snap-frozen on dry ice, and sent directly to New Jersey Feed Labs (Ewing, New Jersey, U.S.A.) for nutritional analyses.

Samples first were finely pulverized in a Mikro-pul sample mill and 14g of each sample was reserved for testing. Proximate analysis of these samples was accomplished according to the Association of Analytical Chemists (AOAC) methods 990.03, 930.15, 920.39, 978.10, and 942.05. Calcium, phosphorus, and zinc concentrations were assessed via AOAC methods 985.01 and 984.27. Mercury content was assessed via AOAC method 975.08, amino acid profiles were assessed via AOAC methods 994.12, 985.28, 988.15, and 994.12, and fatty acid profiles were determined via AOAC method 963.22 (Horwitz & Latimer, 2011).

For consideration as a forage fish replacement, all nutritional parameters for whole green crab mince (GCM) were compared to values published in the literature of whole ground menhaden (WGM), *Brevoortia spp.*, which comprises approximately 90% of forage fish material for U.S. fishmeal production, annually, by weight (IFFO, 2009; IFFO, 2011).Values, averages, and standard deviations of the three ground green crab composites are reported.

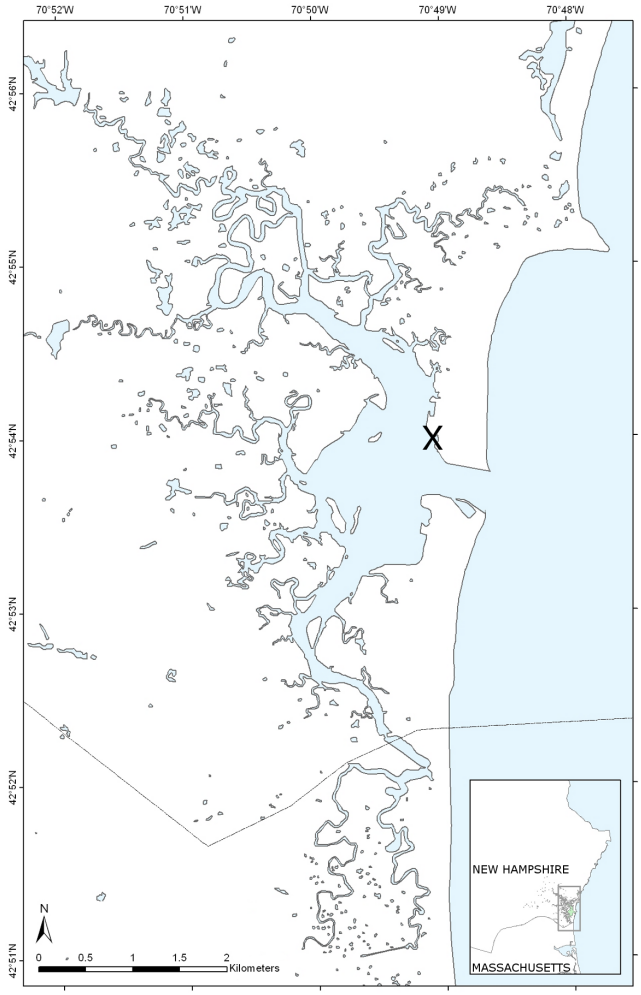

Figure 1. Location of sampling site, as denoted by 'X', in the Hampton-Seabrook Estuary (HSE), New Hampshire, U.S.A., with inset showing the relative location of the HSE

3. Results

The proximate composition of GCM is reported in Table 1. Green crab mince contained all amino acids assayed, including those essential to fish and chickens (Table 2). The largest amino acid components were glutamic acid 1.26% wwb, valine 0.74% wwb, aspartic acid 0.72% wwb, and glycine 0.66% wwb. Because whole GCM contains shell material, a discrepancy exists between the higher calculated "protein content", 12.27%, (Table 1) and the "amino acid content", 7.45%, of GCM (Table 2).

Mineral content of green crab mince was mostly calcium, accounting for 5.70% of wet sample mass, followed by potassium (0.22%), then zinc ($3.778*10^{-4}$%). Mercury was below testable limits (< $5.00*10^{-6}$%) in all three samples (Table 3).

Fatty acid composition of GCM was 67.98% unsaturated and 23.29% saturated (Table 4). The primary saturated fatty acid in GCM was palmitic acid (16:0), contributing 15.56% of total fatty acids, or 66.81% of total saturated fat content. The largest sources of unsaturated fatty acid were oleic (18:1ω9 and 18:1ω7), comprising 16.59% of total fatty acid and 22.27% of unsaturated fatty acids, eicosapentanoic (EPA, 20:5ω3), comprising 9.56% of total fatty acid and 12.84% of unsaturated fatty acids, and docosahexanoic (DHA, 22:6ω3), comprising 8.43% of total fatty acid and 11.31% of unsaturated fatty acids. Lipid of whole GCM was 3.06% linoleic acid (18:2 ω6), and 0.98% linolenic acid (18:3ω6 and 18:3ω3; Table 4).

Table 1. Proximate composition of whole green crab mince (GCM), reported as % of sample (wet weight basis) ± 1 standard deviation (θ). N=3

Proximate component	GCM Average ±θ
Moisture	67.96 ±0.46
Ash	16.55 ±0.29
Protein	12.27 ±0.25
Fiber	2.87 ±0.15
Fat	0.21 ±0.07

Table 2. Amino acid content of whole green crab mince (GCM), reported as % of sample (wet weight basis) ± 1 standard deviation (θ). N=3. Amino acids essential to fish[f] and chickens[c] are denoted

Amino Acid	GCM Average ±θ
Methionine[f,c]	0.17 ±0.01
Cystine[c]	0.06 ±0.00
Lysine[f,c]	0.36 ±0.01
Phenylalanine[f,c]	0.30 ±0.01
Leucine[f,c]	0.46 ±0.02
Isoleucine[f,c]	0.33 ±0.01
Threonine[f,c]	0.28 ±0.00
Valine[f,c]	0.74 ±0.06
Histidine[f,c]	0.15 ±0.00
Arginine[f,c]	0.46 ±0.01
Glycine	0.66 ±0.02
Aspartic Acid	0.72 ±0.01
Serine	0.23 ±0.00
Glutamic Acid	1.26 ±0.02
Proline	0.47 ±0.02
Hydroxyproline	0.01 ±0.00
Alanine	0.47 ±0.01
Tyrosine	0.30 ±0.02
Tryptophan[f,c]	0.02 ±0.00
TOTAL	7.45 ±0.11

Table 3. Content of selected minerals in whole green crab mince (GCM) ± one standard deviation (θ), reported as percent of sample (wet weight basis). N=3

Mineral	GCM Average ± θ
Calcium	5.70 ±0.08
Zinc	$3.778*10^{-4}$ ±$1.460*10^{-4}$
Potassium	0.22 ±0.01
Hg	<$5.00*10^{-6}$ ±0.00

Table 4. Fatty acid composition (% of total oils) of whole green crab mince (GCM) ± one standard deviation (θ). N=3. Fatty acids essential to chickens[c] and some species of fish[f] are noted

	Fatty Acid	GCM Average ± θ
Saturated	12:0	0.28 ±0.08
	14:0	1.90 ±0.11
	15:0	1.04 ±0.01
	16:0	15.56 ±0.74
	17:0	0.90 ±0.05
	18:0	3.56 ±0.30
	20:0	0.05 ±0.09
Unsaturated	14:1	0.24 ±0.03
	16:1	8.06 ±0.29
	16:2	0.26 ±0.04
	17:1	0.95 ±0.14
	18:1ω9	15.14 ±1.40
	18:1ω7	4.88 ±0.25
	18:2ω6[c]	3.06 ±0.84
	18:3ω6	0.19 ±0.06
	18:3ω3[c]	0.79 ±0.05
	18:4ω3	0.28 ±0.24
	20:1ω11	2.08 ±0.15
	20:1ω9	3.65 ±0.26
	20:1ω7	2.23 ±0.21
	20:2ω6	1.57 ±0.08
	20:3ω3	0.18 ±0.21
	20:4ω6	2.58 ±0.19
	20:5ω3[f]	8.73 ±0.57
	22:1ω11	2.99 ±0.40
	22:1ω9	0.28 ±0.24
	22:4ω6	0.51 ±0.06
	22:5ω3	1.28 ±0.14
	22:6ω3[f]	7.69 ±0.52
	24:1	0.37 ±0.12
	Other	8.72 ±1.23
	Total %ω3	18.95 ±1.64
	Total %ω6	7.91 ±0.51
	Total% Saturated	23.29 ±1.37
	Total % Unsaturated	67.98 ±6.47

4. Discussion

Because green crabs sampled in this study were drawn from one location within one estuary at one time of year, it is expected that nutritional composition of green crabs from a different estuary, location, or season would vary (Styrishave & Andersen, 2000). Moisture content of whole GCM resembles that of WGM reported by several

researchers previously (Hale & Bauersfield, 1978; Lanier et al., 1983), but GCM is lower in protein and fat and higher in ash. Protein content was determined by AOAC 990.03, a combustion method in which protein content is back-calculated with a conversion factor from total nitrogen present in the sample. The shells of crustaceans are rich in chitin, a nitrogenous, long-chain polymer of 2-acetoamido-2-deoxy-β-D-glucopyranose ($C_8H_{15}NO_6$; Manni et al., 2010). Green crab shell contains 12.6-14.5% chitin, accounting for 2.6-3.11% of total nitrogen in the sample (Naczk et al., 2004), explaining most of the discrepancy between calculated protein content and protein content by amino acid analysis. For amino acids essential to fish and chickens, GCM was much lower in lysine and tryptophan and much higher in valine than reported values for menhaden hydrolysate (Hale & Bauersfield, 1978; Table 2). Levels of all other essential amino acids were comparable to menhaden reference values from literature (Hale & Bauersfield, 1978). Green crab mince contained 5.70% calcium (wwb), compared to a menhaden reference value of 1.29% (Scott & Latshaw, 1993) and also was much lower in zinc than menhaden reference values (37.78 ppm vs. 67.00 ppm; Scott & Latshaw, 1993; Table 3). The potassium content of GCM (0.22%) was roughly identical to that previously reported for menhaden (0.21%; Scott & Latshaw, 1993; Table 3). GCM mercury levels were below testable limits (< 0.05 ppm; Table 3); 1.98 ppm is typical for menhaden (Scott & Latshaw, 1993). GCM was richer in DHA (7.69 vs. 7.00%) and lower in EPA (8.73% vs. 13.50%) than WGM literature values, and slightly higher in arachidonic acid (ARA, 20:4ω6; 2.58 vs. 1.00%; Joseph, 1985). Lipid of whole GCM was 3.06% linoleic acid (18:2 ω6) compared to 1.10% for WGM literature values, and 0.98% linolenic acid (18:3ω6 and 18:3ω3) compared to 1.40% based from literature values for WGM (Joseph, 1985; Table 4).

Without further processing, the relatively high calcium content of GCM renders it a poor replacement for forage fish in diets for several species. For example, Chinook salmon, *Oncorhynchus tshawytscha*, are susceptible to cataracts, nephrocalcinosis, suppressed appetite, general decline in growth rates, and increased mortality on high (51 g/kg) calcium diets; the putative cause is diminished zinc bioavailability (Richardson et al., 1985). Juvenile Atlantic salmon, *Salmo salar*, show reduced growth on dietary ash levels above 17.5%, but tolerate ash up to this inclusion as long as minimal zinc requirements are met (100ppm; Shearer et al., 1992). Channel catfish, *Ictalurus punctatus*, fingerlings also present reduced growth rates on diets supplemented with 0.5 and 2.0% $CaCO_3$ and $CaCl_2$, respectively (Richardson et al., 1985; Gatlin & Scarpa, 1993). Despite its high ash content, GCM could be demineralized easily with appropriate chelators, but that would result in an additional expense to processing. An alternate option is to use GCM as a fishmeal replacement for ash-tolerant cultured species like cod, *Gadus morhua*, flatfishes, and cobia, *Rachycentron canadum*. It is typical for 30% of wild summer flounder, *Paralichthys dentatus*, wild cod gut contents, and 78% of wild cobia gut contents (by weight) to consist of crustaceans (Latour et al., 2008; Fines & Holt, 2010; Krumsick & Rose, 2012). Cod show excellent growth without reduced feed efficiency when fed crab by-product meal at inclusions of up to 176g/kg diet, the highest level tested (Toppe et al., 2006). Crab meal has been explored as a diet finisher for southern flounder, *Paralichthys lethostigma*, on speculation that it would change sensory properties of the final fillet just prior to slaughter to enhance product value (González et al., 2006). After southern flounder were fed a high-ash diet consisting of 5% crab meal for fishmeal replacement, the fillets had a similar proximate composition but less fishy flavor (considered a positive sensory attribute) than fish fed a standard fishmeal diet (González et al., 2006). Unfortunately, feed efficiency was not considered. For cobia, organic matter of crab byproduct meal has greater apparent digestibility (94.1 ± 8.9%) than both shrimp meal (64.1 ± 26.2%) and fish meal (56.7 ± 9.9%) at the same level of inclusion (30%). Protein, lipid, and total energy showed no significant difference in apparent digestibility among the three cobia diets (Fines & Holt, 2010).

For terrestrial applications, chickens may be suitable for GCM replacement or supplementation, as chickens have high dietary ash requirements and also consume large amounts of fish meal (Delgado et al., 2003; IFFO, 2007; Leeson & Summers, 2009). Broiler chickens have shown good growth on fishmeal replacement diets utilizing crab mussel, *Mytulis edulis*, and bone meals, while laying hens have demonstrated a good tolerance for red crab, *Pleuronectes planipe*s, and shrimp, *Litopenaeus spp.*, meals, causing no organoleptic differences in eggs (Adesehinwa et al., 2005; Jönsson & Elwinger, 2009; Carranco et al., 2011; Etuk et al., 2012).

Chickens, cobia, some flatfish species, and cod possess endogenous digestive chitinases which may provide energetic benefit from GCM (Danulat & Kausch, 1984; Suzuki et al., 2002; Kurokawa et al., 2004; Fines & Holt, 2010). Because it contains the whole crab, GCM is a richer source of protein than many shellfish and fish byproducts. Fish and chickens fed a whole GCM as a fishmeal replacer would be expected to have better condition factors than those fed seafood byproduct meals.

Although GCM contains all essential amino acids for fish and chickens, GCM protein may not be complete for fish and chickens due to the relatively low amount of lysine and tryptophan (as compared to menhaden).

Complete replacement of fishmeal in formulated diets may not be possible without tryptophan and lysine supplementation, depending on the target farmed species and processing conditions used in producing green crab meal. The largest amino acid components of whole green crab protein were glutamic acid 1.26% wwb, valine 0.74% wwb, aspartic acid 0.72% wwb, and glycine 0.66% wwb; these amino acids have all been implicated as important finfish feeding stimulants in aquaculture (Carr et al., 1996). Whole green crab mince in fact may improve the palatability of a formulated diet for fish. Chickens are also an excellent test market for GCM because they can self-select for the correct ration of protein if provided with grain and protein food resources (Forbes & Shariatmadari, 1994).

Fish and chickens have similar essential fatty acid requirements. In vertebrates, ω-unsaturated fatty acids help maintain membrane fluidity and are involved in enzyme activation and neurotransmission (Stickney, 1994). Vertebrate metabolism generally allows for desaturation and elongation of short-chain ω3 fatty acids to long-chain ω3 fatty acids and short-chain ω6 fatty acids to long-chain ω6 fatty acids, but not from ω3 to ω6 or vice versa. Both fish and chickens need ω-unsaturated fatty acids for normal growth and development. For poultry, linoleic acid (18:2ω6) and linolenic acid (18:3ω3) are essential fatty acids. Chickens and chicks fed a diet deficient in linoleic acid (generally regarded to be < 1% of the diet) will suffer retarded growth, an increased water requirement, fatty liver, reproductive difficulties, and immune insufficiency (Watkins, 1991). Laying hens can require up to 5% dietary linoleic acid for egg production (Watkins, 1991; Zornig et al., 2001). Chickens also require linolenic acid in their diet for production of EPA and DHA (Watkins, 1991). Some fish are able to desaturate and elongate linolenic and linoleic acid to longer chain polyunsaturated fatty acids, but in many species this is not done with any efficiency (Stickney, 1994). Most fish require both EPA and DHA from their diets. For juvenile turbot, *Scophthalmus maximus*, and perhaps other flatfish species, it has been recommended to enrich feed with high levels of EPA and DHA and to keep levels of arachidonic acid (ARA; 20: 4ω6) low in order to prevent malpigmentation (Hamre, 2006). For juvenile cobia, DHA is required for optimal growth performance, but EPA appears to be only a trace requirement (Trushenski et al., 2011). Data regarding fixed DHA and/or EPA requirements for cod is lacking, although some larval studies have examined fatty acid supplementation relative to normal rotifer and *Artemia* diets (Cutts et al., 2006). Unfortunately, the requirements for juveniles (which would be candidates for a pelletized feed) are often dissimilar to those of larvae. This is a promising area for future research.

The overall ratio of EPA+DHA to ARA in green crab lipid is lower than that of menhaden but may not be low enough to merit fortification. The notably high linoleic acid level in GCM would make it a good candidate for chicken feed. The exact lipid content requirement for the diets of most commercially-raised species of fish is usually assumed to be around 10% of the dry weight of the diet, which is assumed to spare all protein intake for anabolism (Halver & Hardy, 2002). Lipid only accounts for about 2.0% of the dry weight of GCM, but separation of solids from liquids during the cooking and pressing stages of processing GCM into meal would allow processors to enrich the meal to the desired fat content by the addition of fat separated from a larger starting volume of GCM. Protein and ash left over as a result of this process could be utilized in some other capacity, such as fertilizer.

In conclusion, GCM has favorable nutritional characteristics for some applications in forage fish replacement. Continuation of this work in the form of a diet study (in fish or chickens) should be considered, as green crabs are plentiful and easy to capture (Fulton et al., 2013), and could form the basis of a new fishery in New England. In addition, controlling green crabs via harvest could perform a valuable ecological service and may increase yield in other local fisheries by limiting the effects of this invasive predator.

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