
VOL. 310 NOS. 1–2, 22 DECEMBER 2010
ISSN 0044-8486
ELSEVIER
Aquaculture

Aquaculture 310 (2010) 240–243

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Short communication

Evaluation of apparent digestibility coefficient of energy of various vegetable feed ingredients in Florida pompano, *Trachinotus carolinus*

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A R T I C L E I N F O

Article history:
Received 2 July 2010
Received in revised form 10 October 2010
Accepted 12 October 2010

Keywords:
Trachinotus carolinus
Apparent digestibility coefficient (ADC) of energy
Vegetable feed ingredients
Carbohydrates

A B S T R A C T

The apparent digestibility coefficient (ADC) of energy of various vegetable feed ingredients with relatively high levels of carbohydrate was determined for Florida pompano, *Trachinotus carolinus*. A practical fish meal and soybean meal based reference diet was formulated to contain 54% protein, 11% lipid, and 1% chromic oxide. Using the 70:30 replacement strategy seven test diets were produced that included corn grain, sorghum grain, whole wheat flour, wheat bran, wheat middlings, full fat rice bran, and defatted rice bran. Each of the diets were offered to juvenile pompano (111.6 g mean weight) maintained in a recirculation system. After one week of conditioning to the diets, fecal samples were obtained by manually stripping the fish. Chromium content and gross energy of the diets and fecal samples was then determined and digestibility coefficients calculated. The ADC of energy of the evaluated feedstuffs showed a wide variation, ranging from 8.2% for wheat middlings, to 55.4% for whole wheat flour. Knowing the contribution of common vegetable feed ingredients to the digestible energy content of production diets is critical to achieve cost-effective formulations that may lead to a reduction in the overall feeding cost of Florida pompano production, providing useful information to improve feed formulations for this species.

1. Introduction

In the United States, Florida pompano, *Trachinotus carolinus*, is among the marine fish species with aquaculture potential that has caught the interest of the industry for being a truly euryhaline species. It can be raised in marine or low-salinity environments, tolerates low dissolved oxygen and handling stress, accepts balanced feeds and can be reproduced in captivity (Riche, 2009; Riche and Williams, 2010). However, commercial formulated feeds for this species need to be optimized for production in order to run a profitable aquaculture operation.

Carbohydrates are included in production diets as an inexpensive energy source to help spare the use of protein as an energy source, as well as to take advantage of their binding properties to increase the stability of feeds. Carbohydrates supplied in fish diets are generally from cereal grains. The ability of fish to utilize carbohydrates varies with the source of carbohydrate, the processing of the carbohydrate, in addition to species specific differences (Hemre et al., 2002; Erfanullah, 1999; Lin and Shiau, 1995). Physiological differences related to the feeding habits of the species (*e.g.*, enzyme activity), along with the chemical characteristics of the carbohydrate (*e.g.*, molecular complexity) results in variable digestibility. Additional factors will also affect the digestibility of carbohydrates, for instance, the degree of heat and moisture during processing, the level of dietary inclusion and the way they bind to other ingredients, as well as other physical and biochemical changes they may go through while crossing the gastrointestinal tract (Weurding et al., 2001). According to Williams et al. (1985) carbohydrate digestibility in Florida pompano is about 50%, emphasizing its limited availability and consequently, limited energy digestibility. *In vivo* digestibility evaluation of the energy provided by various plant feedstuffs with relatively high levels of carbohydrate can provide useful information for developing an efficient commercial diet for this species, by accurately replicating the conditions that carbohydrates are subjected to within the gastrointestinal tract. The aim of this study was to evaluate the apparent digestibility coefficient (ADC) of energy of various vegetable feed ingredients in a diet for *T. carolinus*.

2. Materials and methods

A reference diet containing 1% chromic oxide, the non-digestible marker, was formulated to contain 54% crude protein and 11% lipid (Table 1). Seven additional diets containing 30% of the test ingredient and 70% of the reference diet were used to determine the ADC of energy (Cho et al., 1982; Wilson and Poe, 1985). The feed ingredients evaluated were corn grain (Grain Processing Corporation, Muscatine,

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doi:10.1016/j.aquaculture.2010.10.016

Table 1
Ingredient composition of the reference diet.

Ingredient	g kg^{-1} dry diet
Fishmeal[a]	600.0
Soy concentrate[b]	226.0
Whole wheat flour[c]	105.5
Menhaden fish oil[a]	38.0
Chromic oxide sesqui[d]	10.0
Trace mineral premix[e]	2.5
Vitamin premix w/o choline[f]	5.0
Choline cloride[c]	2.0
Stay C 35%[g]	1.0
Lecithin (soy refined)[c]	10.0
Total	1000.0

[a] Omega Protein Inc., Reedville, VA, USA.
[b] Soycomil P, Soy protein concentrate, 63% protein, Archer Daniels Midland, Decatur, IL. USA.
[c] MP Biochemicals Inc., Solon, OH, USA.
[d] Fisher Scientific, Fair Lawn, NJ, USA.
[e] ASA Premix (g 100 g^{-1} premix) : cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250; ferrous sulfate heptahydrate, 4.0; manganous sulfate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; and α cellulose, 81.826.
[f] ASA Premix (g/kg Premix): thiamin HCl, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 IU g^{-1}), 2.40; vitamin D_3 (400,000 IU g^{-1}), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258.
[g] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

IA, USA), sorghum grain (Rangen Inc., Angleton, TX, USA), whole wheat flour (MP Biochemicals Inc., Solon, OH, USA), wheat bran (Siemer Milling Co., Teutopolis, IL, USA), wheat middlings (Alabama Catfish Feed Mill Llc., Uniontown, AL, USA), full fat rice bran (Riceland Foods Inc., Stuttgart, AR, USA), and defatted rice bran (Riceland Foods Inc., Stuttgart, AR, USA). Grains were ground into flour using a laboratory grinder (Glenmills Inc., Clifton, NJ, USA) before preparation of the experimental diets.

Diets were manufactured at Auburn University, Department of Fisheries and Allied Aquaculture, Auburn, AL, USA. They were prepared by mixing pre-ground dry ingredients and menhaden fish oil in a food mixer (Hobart, Troy, OH, USA) for 20 min. Boiling water was then blended into the mixture to attain a consistency appropriate for pelleting. The moist mash from each diet was passed through a 3 mm die in a meat grinder, and the pellets were dried in a forced air drying oven (<45 °C) to a moisture content of less than 10%. Diets were stored at −20 °C, and prior to use each diet was crumbled and sieved to an appropriate size.

Fish were maintained in a semi-closed recirculating system at Claude Peteet Mariculture Center in Gulf Shores, AL, USA. It consisted of 12 circular fiberglass culture tanks containing 600 L of water with a salinity of approximately 20 g L^{-1}, a reservoir tank (900 L) with a biological filter, water pump, and supplemental aeration provided using a central line, a regenerative blower and air diffusers. The system was inside a greenhouse which provided a natural light cycle, approximately 14 h light day^{-1} and 10 h dark day^{-1}. Routine system maintenance such as siphoning of solids and partial water exchanges were performed as needed. During the experimental period water quality parameters including dissolved oxygen (DO), salinity and pH were measured once a day, and temperature was measured twice a day, in the morning and afternoon, using a multi probe meter YSI 556 MPS (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia nitrogen was determined weekly using an ion selective electrode (Orion EA 940, Thermo Electron Corporation, Beverly, MA, USA). Morning and afternoon temperature and salinity were controlled at 27.5 ± 1.82 °C, 30.0 ± 1.08 °C and 20.8 ± 0.95 g L^{-1}, respectively. Dissolved oxygen averaged 6.5 ± 0.72 mg L^{-1}. Total ammonia nitrogen and pH averaged 0.10 ± 0.15 mg NH_4-N L^{-1}, and 7.56 ± 0.20, respectively.

Fifteen fish were collectively weighed (average individual weight of 111.6 g) and stocked into each tank. The first four experimental diets, each with three replicates, were randomly assigned to the twelve experimental tanks in the recirculation system. Feed input was adjusted to provide 4.25% of the body weight per day. The daily ration was provided in two feedings at 0800 h and 1600 h. Fish were fed the diets for one week, then they were anesthetized with dimethylketone alpha methyl quinoline (Hypno, Jungle Lab Cor. Cibolo, TX, USA) to help reduce stress during the manual stripping of the feces, which were pooled into one composite sample per experimental tank. Fish were treated with chloroquine phosphate (Marex, Aquatronics, Oxnard, CA, USA) at 21.1 mg L^{-1} as a preventive measure against protozoan infestations, particularly *Amyloodinium ocellateum*, and were stocked once again into their experimental tanks. After that, fish were fed the reference diet for one week; subsequently they were fed the remaining four experimental diets (each with three replicates randomly assigned to the twelve experimental tanks) for an additional week, at the end of which fish were again manually stripped to collect feces as described previously. Survival was 100% for all experimental treatments.

Gross energy of experimental diets, feces, and feed ingredients was analyzed with a Semimicro-bomb calorimeter (Model 1425, Parr Instrument Co., Moline, IL, USA). Chromic oxide concentrations were determined by the method of McGinnis and Kasting (1964) in which, after a colorimetric reaction, absorbance was read on a spectrophotometer (Spectronic Genesys 5, Milton Roy Co., Rochester, NY, USA) at 540 nm. All sample analyses were conducted in triplicate. The ADC of energy and dry matter of each diet, and the ADC of energy of the feed ingredients were determined using standard formulas (Cho et al., 1982). The ingredient ADC of energy was adjusted according to Forster (1999).

Proximate composition of the plant feedstuffs including moisture, protein, fat, fiber, and ash, were determined by the New Jersey Feed Laboratory Inc. in Trenton, NJ, USA, and the total starch content was determined at the Poultry Science Laboratory of Auburn University, Auburn, AL, USA, in accordance with the Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland) modification of AOAC (2002) Official Method 996.11, for determination of the total starch content of samples containing resistant starch, but no D-glucose and/or maltodextrins (KOH format) (Table 2).

The ADC of energy and dry matter of diets, and the ADC of energy of ingredients were subjected to analysis of variance and Duncan's multiple range test for comparison of means (P<0.05) using the SAS software package (SAS Institute Inc., 1999–2000).

3. Results

Gross energy of experimental diets, ADC of dry matter and energy of each diet, and the ADC of energy of the evaluated feed ingredients for Florida pompano are presented in Table 3. ADC of the dry matter of the diets ranged from a low value of 24.4% for the diet with wheat middlings to a high value of 63.3% for the diet containing whole wheat flour. The reference diet had an ADC of dry matter of 69.1%. The ADC of energy of the diets also showed a wide variation depending on the feedstuff included, ranging from 58.6% for the wheat middlings' diet, to 74.1% for that including whole wheat flour, and that of the reference diet was 81.6%. The ADC of energy determined for the feed ingredients were lower than those of their corresponding experimental diets. The two lowest ADC of energy were observed for wheat middlings and defatted rice bran, with values of 8.2 and 12.6%, respectively. They were followed by sorghum grain and full fat rice bran, with 21.6 and 24.9%, respectively, then by corn grain and wheat bran with almost identical values, 44.8 and 44.9%, respectively. Finally, the highest ADC of energy was observed for whole wheat flour, 55.4%.

Table 2
Proximate composition, starch content, and gross energy of the evaluated vegetable feed ingredients.

	Corn grain	Sorghum grain	Whole wheat flour	Wheat bran	Wheat middlings	Full fat rice bran	Defatted rice bran
Moisture (%)	12.9	11.6	11.5	13.3	12.6	9.6	9.0
Crude protein (%)	8.2	10.3	15.8	15.5	14.6	14.1	17.7
Crude fat (%)	3.6	3.2	1.6	2.5	3.4	19.5	3.4
Crude fiber (%)	2.3	2.4	2.8	11.1	10.2	7.3	8.4
Ash (%)	1.1	1.3	1.5	6.1	4.2	12.9	13.8
Starch (%)	66.9	65.1	57.9	16.0	17.3	21.5	22.1
Gross energy (cal/g)	4151.2	4795.5	4403.6	4391.4	4327.8	4926.6	3731.0

4. Discussion

The requirement for digestible energy in fish feeds ranges from 2.33 to 4.10 kcal g^{-1} diet, depending on species and the amount of dietary protein included (NRC, 1993). Typically a significant component of the dietary energy should be provided by lipid and carbohydrate to spare the protein. Digestible energy values of some of the vegetable feed ingredients investigated in this study have been reported for various species. McGoogan and Reigh (1996) reported the ADC of energy of sorghum, corn, wheat middlings and rice bran in red drum (*Sciaenops ocellatus*) close to 52.7, 56.0, 33.7 and 12.0%, respectively. Compared to the observed ADC in this study (Table 3), particularly wheat middlings and sorghum grain, appear to be less efficiently digested by Florida pompano, although the full fat rice bran evaluated in this study showed an ADC of energy of 24.9%, more than double the amount reported in the previous study for rice bran. Sullivan and Reigh (1995) had previously reported the ADC of energy of rice bran of 47.0% for hybrid striped bass (*Morone saxatilis* ♀ × *M. chrysops* ♂). They also reported values of 54.5, 60.7 and 40.7% for wheat flour, wheat middlings and corn. Gaylord and Gatlin (1996) reported an ADC of energy of 61.6% for wheat grain in a study with red drum, comparable to the ADC of energy of whole wheat flour of 55.4% for Florida pompano observed in this study. Gothreaux (2008) reported an ADC of energy of 41.4% for corn in Florida pompano, very similar to the 44.8% reported in the present study. Information on the ADC of energy of some of the investigated feedstuffs for other marine finfish species is relatively scarce.

An understanding of the milling constituents derived from the wheat kernel is essential for determining the feeding value of wheat flour milling by-products. The Association of American Feed Control Officials (AAFCO, 1996) describes wheat bran as the coarse outer covering of the wheat kernel as separated from cleaned and scoured wheat in the usual process of commercial milling. Wheat flour consists principally of wheat flour together with fine particles of wheat bran, wheat germ, and the offal from the "tail of the mill", and must not contain more than 1.5% crude fiber. Wheat middlings consist of fine particles of wheat bran, wheat shorts, wheat germ, wheat flour, and some of the offal from the "tail of the mill", and must contain no more than 9.5% crude fiber.

Aside from differences in maximum crude fiber restrictions, total starch content for the analyzed wheat by-products varied greatly in this study, 57.9% for whole wheat flour, compared to 17.3 and 16.0% for wheat middlings and wheat bran, respectively (Table 2), with values for the ADC of energy of 55.4, 8.2 and 44.9% (Table 3), respectively. In terms of digestible energy, the difference between wheat middlings and wheat bran could be attributed to their milling constituents, resulting in superior feeding value of wheat bran for Florida pompano in this study, in spite of having a comparable starch content (Fig. 1). According to Hertrampf and Piedad-Pascual (2000), wheat middlings are less fibrous than wheat bran (7.8 vs. 12.3%), contain more wheat protein (16.9 vs. 15.6%) and have a higher feed value. However, inconsistencies during the wheat milling process and the proportions that ultimately result in wheat by-products' final nutrient composition, result in difficulties encountered when ascertaining their nutritional value for most species, which may explain the differences observed in this study and others. Moreover, the chemical and nutrient composition of wheat itself is affected by the variety, precipitation, temperature, irrigation, texture and composition of the soil, and the use of fertilizers (Hertrampf and Piedad-Pascual, 2000).

Digestibility of energy-yielding nutrients to a particular species is believed to depend on the nature of the feed ingredient ingested. An important consideration derived from the analyzed feed ingredients is that, even if the total starch content is high, *e.g.* in sorghum or corn grains, their ADC of energy may be relatively low as demonstrated for this species in this study (Fig. 1). Having a high starch content does not guarantee that a plant feedstuff will serve as an adequate energy source for fish. The availability of energy from starch is influenced by a number of factors, including the source or type of starch as well as the processing. For example, the low digestibility of sorghum grain in this study is probably due to this starch requiring higher temperatures for gelatinization which is required to enhance the digestion of carbohydrates by disrupting the starch granules. Hence, under different processing conditions the digestible energy could be improved.

Other factors may have affected the ADC. It has been suggested that some supplementation levels of chromic oxide, the inert marker usually employed for digestibility determinations, may cause significant differences in food digestibility (Shiau and Liang, 1995; Tacon

Table 3
Analyzed dietary gross energy, apparent digestibility coefficient (ADC) of dietary dry matter and energy, and ADC of energy of the evaluated vegetable feed ingredients for Florida pompano.

Experimental diet	Gross energy of diet (cal/g)	ADC of dry matter of diet (%)	ADC of energy of diet (%)	ADC of energy of ingredient (%)
Diet $1_{\text{Reference}}$	4813.8	$69.1^a \pm 2.45$	$81.6^a \pm 0.64$	–
Diet $2_{\text{Corn, grain}}$	4704.9	$58.1^b \pm 3.66$	$71.4^c \pm 1.70$	$44.8^b \pm 6.14$
Diet $3_{\text{Sorghum, grain}}$	4788.5	$45.4^d \pm 4.03$	$63.9^d \pm 1.03$	$21.6^c \pm 3.52$
Diet $4_{\text{Whole wheat flour}}$	4739.7	$63.3^b \pm 1.81$	$74.1^b \pm 1.21$	$55.4^a \pm 4.25$
Diet $5_{\text{Wheat bran}}$	4876.8	$51.5^c \pm 3.47$	$70.1^c \pm 2.23$	$44.9^b \pm 7.10$
Diet $6_{\text{Wheat middlings}}$	4881.9	$24.4^e \pm 5.45$	$58.6^e \pm 0.71$	$8.2^d \pm 2.25$
Diet $7_{\text{Full fat rice bran}}$	4805.4	$42.2^d \pm 2.71$	$64.7^d \pm 0.85$	$24.9^c \pm 2.85$
Diet $8_{\text{Defatted rice bran}}$	4611.2	$44.6^d \pm 0.62$	$63.8^d \pm 1.36$	$12.6^d \pm 5.28$

Values represent means ± SD (n = 3). Values in the same column with different superscripts are significantly different ($P<0.05$).

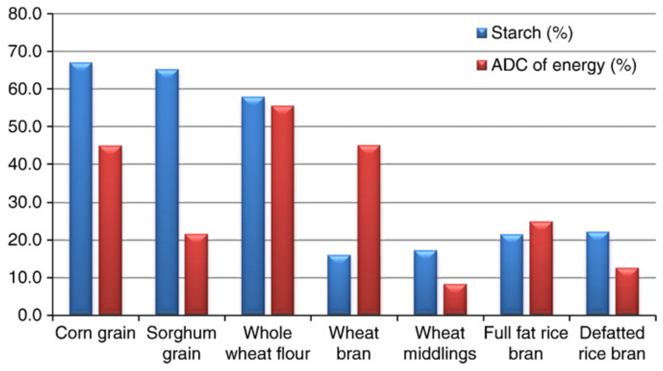

Fig. 1. Total starch content (%) and ADC of energy (%) of the vegetable feed ingredients.

and Rodrigues, 1984). However, Fernandez et al. (1999) found no differences in the ADC of the organic fraction of the food, such as carbon and nitrogen, caused by dietary chromic oxide in gilthead sea bream (*Sparus aurata)*, but found ADC differences for mineral components, such as calcium and phosphorus. The fecal collection method also influences the acquisition of reliable data on the ADC of ingredients; therefore, a standardized methodology that avoids any possible leaching of nutrients is necessary (Kaushik, 2000), such as the manual stripping of the feces performed in this study.

In the case of cereal grains, relatively low values for the ADC of dry matter are usually reported. McGoogan and Reigh (1996) reported the ADC of dry matter of 58.1, 67.7, and 35.6% for corn, sorghum, and wheat middlings, respectively, in red drum, while Sullivan and Reigh (1995) reported 49.7 and 31.1% for wheat flour and rice bran in hybrid striped bass. The ADCs of dry matter of diets in this study are comparable and ranged from 24.4% to 63.3%.

Differences between species in values reported for the ADC of dry matter and energy for a particular feed ingredient are evident, even if they share similar feeding habits. Available data agree in that energy from vegetable feed ingredients with relatively high levels of carbohydrate is not as readily available to some carnivorous fish species (Gaylord and Gatlin, 1996). Major differences in the anatomy of the digestive tract, and the enzyme complement and activity may explain these differences in fish. The predominant starch digestive enzymes are α-amylase, disaccharidase, and α-glucosidase, which hydrolyze α-glycoside bonds of starch to yield glucose (Stone, 2003). It is difficult to make direct comparison of digestive enzyme activity between species because methodologies vary considerably, but in general, the enzyme complement and their activity level appear to be closely related to the trophic level, with low activities usually measured in the intestinal tract of carnivorous fish (Krogdahl et al., 2005).

All things considered, plant carbohydrates are the least expensive source of dietary energy and their inclusion in aquafeeds is necessary for proper shaping and pellet stability. Knowing the contribution of common vegetable feed ingredients with relatively high levels of carbohydrate to the digestible energy content of production diets is critical to achieve cost-effective formulations that may lead to a reduction in the overall feeding cost of Florida pompano production, providing useful information to improve feed formulations for this species.

Acknowledgements

The authors would like to express their appreciation for the assistance provided by reviewers, and staff at Auburn University Claude Peteet Marine Laboratory (Gulf Shores, AL, USA), particularly the authors would like to thank Melanie A. Rhodes for her valuable technical assistance. Funding for this research was provided in part by the American Soybean Association Soy in aquaculture program, the National Oceanic and Atmospheric Administration (NAO60AR4170191), the NOAA Marine Aquaculture Program (NAO80AT4170840), and the Consejo Nacional de Ciencia y Tecnologia (CONACYT), Mexico. The mention of trademarks or proprietary products does not constitute an endorsement of the product by Auburn University and does not imply its approval to the exclusion of other products that may also be suitable.

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