Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 271 (2007) 432–438
Aquaculture
www.elsevier.com/locate/aqua-online

Bioimpedance assessment of body composition in cobia *Rachycentron canadum* (L. 1766)

M. Duncan, S.R. Craig, Angela N. Lunger, D.D. Kuhn, G. Salze, E. McLean *

Virginia Tech Aquaculture Center, 1 Plantation Road, Blacksburg, VA 24061-0321, USA

Received 22 February 2007; received in revised form 31 May 2007; accepted 4 June 2007

Abstract

Sixty juvenile cobia (*Rachycentron canadum*; 28.3±0.13 g wet wt) were randomly distributed into each of 12 tanks in a recirculation unit ($n=5$ tank^{-1}). Fish were fed one of two diets (47:8 or 47:20 protein:lipid) at 6–8% body wt d^{-1} for 6 weeks. Each week, the composition of fish ($n=5$) from each dietary treatment was calculated by measuring the impedance (resistance and reactance) of a current (x μA AC and kHz) passed through a live animal. Electrodes were positioned at morphologically discrete points on the dorsal left hand side of the animal. After bioimpedance (BIA) assessment, the identical fish were sacrificed and their body composition determined using traditional, chemical methods. Results generated by chemical analyses were regressed against BIA data. Linear regression analysis was performed utilizing compositional analysis (protein, lipid and ash) as the observed values and BIA assessment for the predicted. Regressions for each body composition parameter produced high correlations in all relationships: resistance (in parallel) and protein (adj. $R^2=0.9569$), resistance (in parallel) and total body water (adj. $R^2=0.9894$), reactance (in parallel) and total body ash (adj. $R^2=0.8547$), reactance (in series) and dry matter (adj. $R^2=0.9272$) and reactance (in series) and fat-free mass (adj. $R^2=0.9916$). The F value tests ($P<0.0001$) revealed significant correlations between the independent and dependent variables for each body composition parameter. Correlations for each regression indicate strong linear relationships between impedance and proximate analysis variables with values of 1:1. This indicates that this BIA methodology can be utilized as an inexpensive, non-lethal, on the farm determination of proximate composition.

Keywords: BIA; Bioimpedance analysis; Non-destructive; Protein; Moisture; Regression

1. Introduction

The emergence of an increasingly sophisticated consumer has highlighted the importance of sustainability, biosecurity, and food safety during aquaculture production. Combined with a greater awareness and higher expectations of product quality by processors, retailers, and consumers, new and complex challenges face the industry in terms of product standardization while maintaining production profitability. For many phases in fish farming, such as harvesting and processing, quality standards do not exist for many species (Rønsholdt and McLean, 1999) and this is particularly true concerning the physico-chemical properties of fish flesh, the composition and nutritional value of which may be influenced by a broad variety of biotic and abiotic factors (Kestin and Warriss, 2001). Conventionally, chemical methods are employed to assess fish body composition.

* Corresponding author. Tel.: +1 540 231 4625; fax: +1 540 231 8865.
E-mail address: emclean@vt.edu (E. McLean).

0044-8486/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2007.06.002

However, comparatively few producers have access to the expensive laboratory facilities and equipment that these techniques demand. Moreover, chemical compositional analyses are destructive, time-consuming and use noxious chemicals. Several other methods have been proposed to forecast body composition of farmed animals. In this respect, Near Infrared Reflectance Analysis (Lee et al., 1992; Rasco et al., 1991), Computerized Tomography (Kolstad et al., 2004; Hancz et al., 2003; Gjerde, 1987) and Magnetic Resonance Imaging (Wille et al., 2004; Nott et al., 1999; Howell et al., 1996) techniques have all been used. However the preceding have no practical (on-farm) application due to the expense of instrumentation and the technical expertise needed during equipment operation and data interpretation.

Alternatives to costly and intricate medical imaging technologies and time-consuming and hazardous chemical analyses are clearly needed if aquaculturists are to standardize their product line(s) based upon consumer and or processor demands. Substitute technologies however, must enable rapid on-site evaluation of the impact of feeding strategies, dietary treatments, selective breeding and otherwise, on carcass characteristics. Such technologies must be inexpensive, safe, reproducible and preferably non-lethal to the fish. With these provisions in mind, Rønsholdt et al. (2000) evaluated trout carcass composition using computer-assisted image analysis and flatbed scanning techniques. However, these studies involved manual image processing that may have induced analytical bias which prompted Wille et al. (2004) to remove this partiality by automating image processing of stained materials. While the latter two methods proved successful in especially determining body lipid content of fish flesh, each were destructive techniques. Clearly, non-lethal methods of compositional analyses would be preferred since financial loss would not be incurred by the farmer.

Electrical impedance-based methods have been employed since the 1920s to evaluate meat lean and fat content (Callow, 1936) and in fish, the Total Body Electrical Conductivity or TOBEC system has been used to determine body composition of various fish species (Bai et al., 1994; Brown et al., 1993). Although a useful tool, TOBEC equipment is expensive and clumsy and is only able to accept samples of relatively small size. An alternative method for evaluating lean or lipid content of animals is Bioelectrical Impedance Analysis (BIA). BIA measures sample resistance to the flow of an electrical current and the method has been used successfully to examine carcass yield and composition in various species including sheep, skunk, pigs and cattle (Hwang et al., 2005; Berg et al., 1996; Johns et al., 1992; Swantek et al., 1992). Importantly, the equipment for BIA is hand held, relatively rugged and inexpensive (by a factor of ~20 versus TOBEC). Moreover, because compositional data can be acquired non-lethally, BIA can be used to follow changes in composition throughout a growing season. However, few studies have evaluated the application of BIA to fish (Cox and Hartman, 2005; Bosworth and Wolters, 2001) and none have examined its potential as a method for determining composition in farmed marine fish. Accordingly, we examined the utility of BIA as a method to analyze body composition in growing juvenile cobia by comparing results against traditional chemical analyses.

2. Materials and methods

2.1. Experimental system

All studies were undertaken using a 3400 L recirculating life support system, which comprised 24× 110 L glass aquaria, of which 12 were dedicated to this study, serviced with a 750 L KMT-based (Kaldnes Miljøteknologi, Tønsberg, Norway) fluidized bed biofilter for nitrification, a bead filter (Aquaculture Technologies Inc., Metaire, LA, USA) for solids removal, a protein skimmer (R&B Aquatic Distribution, Waring, TX, USA), and a 40 W UV sterilizer (Emperor Aquatics, Pottstown, PA, USA) for sterilization. The fluidized bed and aquaria were oxygenated using diffusion air lines connected to a 1 HP Sweetwater remote drive regenerative blower (Aquatic Ecosystems, Apopka, FL, USA). Each aquarium received a water flow of 4 L min^{-1}.

Table 1
Composition of diets: g/100 g on a dry matter basis

	Low lipid diet (47% crude protein) (5.6% lipid)	High lipid diet (47% crude protein) (20% lipid)
Herring meal [a]	63.9	63.9
Dextrin [b]	4.8	7.8
Lipid (Menhaden oil) [c]	0	14.4
Mineral mix [d]	4.0	4.0
Vitamin mix [e]	3.0	3.0
Carbohydrate cellulose (CMC) [b]	1.0	1.0
Cellufil [b]	23.3	5.9

[a] International Proteins Corporation, Minneapolis, MN, USA.
[b] US Biochemical Corporation, Cleveland, Ohio, USA.
[c] Omega oils, Reedville, VA, USA.
[d] ICN Corporation, Costa Mesa, CA, USA.
[e] See Moon and Gatlin (1991).

Fig. 1. Photograph illustrating positioning of BIA probes during data acquisition from cobia.

Water temperature (27.0±0.3 °C) and pH (8.09±0.06) were monitored using a Hanna Instrument 9024 pH meter (Aquatic Ecosystems, Apopka, FL, USA). Dissolved oxygen (5.92±0.09 mg L^{-1}) and total ammonia nitrogen (0.37±0.04 mg L^{-1}) were measured using an YSI 85 Series dissolved oxygen meter (YSI Inc., Yellow Springs, OH) and by spectrophotometric analysis (Hach Inc., Loveland, CO, USA), respectively. Nitrite (0.31±0.06 mg L^{-1}) and nitrate (71±11 mg L^{-1}) were quantified by spectrophotometric analysis. Salinity was maintained at 17.0±0.5 g L^{-1} using Crystal Sea synthetic sea salt (Marine Enterprises international, Baltimore, MD, USA). A 12 h photophase-scotophase, with a 30 min dusk/dawn period was maintained using phosphorescent tubes positioned 1.8 m above the system.

2.2. Animals and husbandry

Sixty juvenile cobia (*Rachycentron canadum*; mean individual weight, 28.3±0.13 g) were randomly distributed into each of 12 tanks ($n=5$ $tank^{-1}$). Fish were hand-fed experimental diets (6–8% body wt. d^{-1}; Table 1) two times per day, at 09.00 and 16.00 h. The ration was divided equally between the two feedings and cobia were fed to apparent satiation without overfeeding. Tanks were group weighed weekly to adjust feeding rates and to monitor growth performance. Six tanks were fed the high lipid diet and the remaining six tanks the low lipid diet. At the end of each week, fish from both treatments were randomly sampled for analysis.

2.3. Experimental diets

Two different diets were employed in attempts to modify lipid content of experimental fish. Ingredients of the two experimental diets were mixed in a Patterson-Kelley twin shell® Batch V-mixer (Patterson-Kelley Co. Inc., East Stroudsburg, PA, USA) for 20 min, transferred to a D300 Floor Mixer (Hobart Co., Troy, OH, USA), and appropriate levels of menhaden oil added as the lipid source. Distilled water (20–40% of feed weight) was added to the dietary mixture prior to pressure pelleting, using an appropriate die to provide pellets of suitable size for the fish. After air-drying, feed was frozen at −20 °C until needed. To determine dry matter, duplicate samples from each feed were heated at 135 °C for 2 h in a gravity oven (Blue M Electric, Blue Island, IL, USA), and differences calculated by subtraction. Prior to feeding, small quantities of diet were thawed and refrigerated.

2.4. Principles of BIA

Body composition estimates using BIA were calculated by measuring the resistance and reactance of a current (800 μA AC and 50 kHz) passed through an animal's tissue and then regressing these readings with proximate composition measures. Resistance is proportional to the voltage of an applied current as it passes through a substance. Reactance is the opposition to alternating current by a capacitor (the cell membranes). Both resistance (R) and reactance (X_c) are measured using a bioelectrical impedance analyzer. The electrical conductance of an organism is determined by its water compartments and solutes within those areas. Typically, electrical conductivity is greater in lean tissue than lipids due to higher water (i.e. less lipid) and electrolyte content. Cell membranes are comprised of a non-conductive lipid bilayer packed between two conductive protein layers. The cell membrane acts as a capacitor causing the signal for the voltage to be out of phase. At high frequencies, the storage is insignificant and the current passes straight through the membrane without a voltage shift (Liedtke, 1997). BIA uses low voltages and high frequencies which allows the current to pass through extra cellular fluids but not through the cell membranes.

2.5. Analytical procedures

At the end of each sampling event, five fish from each dietary treatment were euthanized by an overdose of clove oil (Sigma-Aldrich, St. Louis, MO, USA), blot

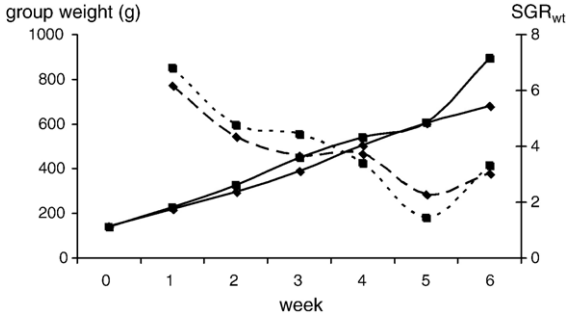

Fig. 2. Group weight gain (—) and weight specific growth rates (———) of cobia fed on high (■) and low (◆) lipid diets.

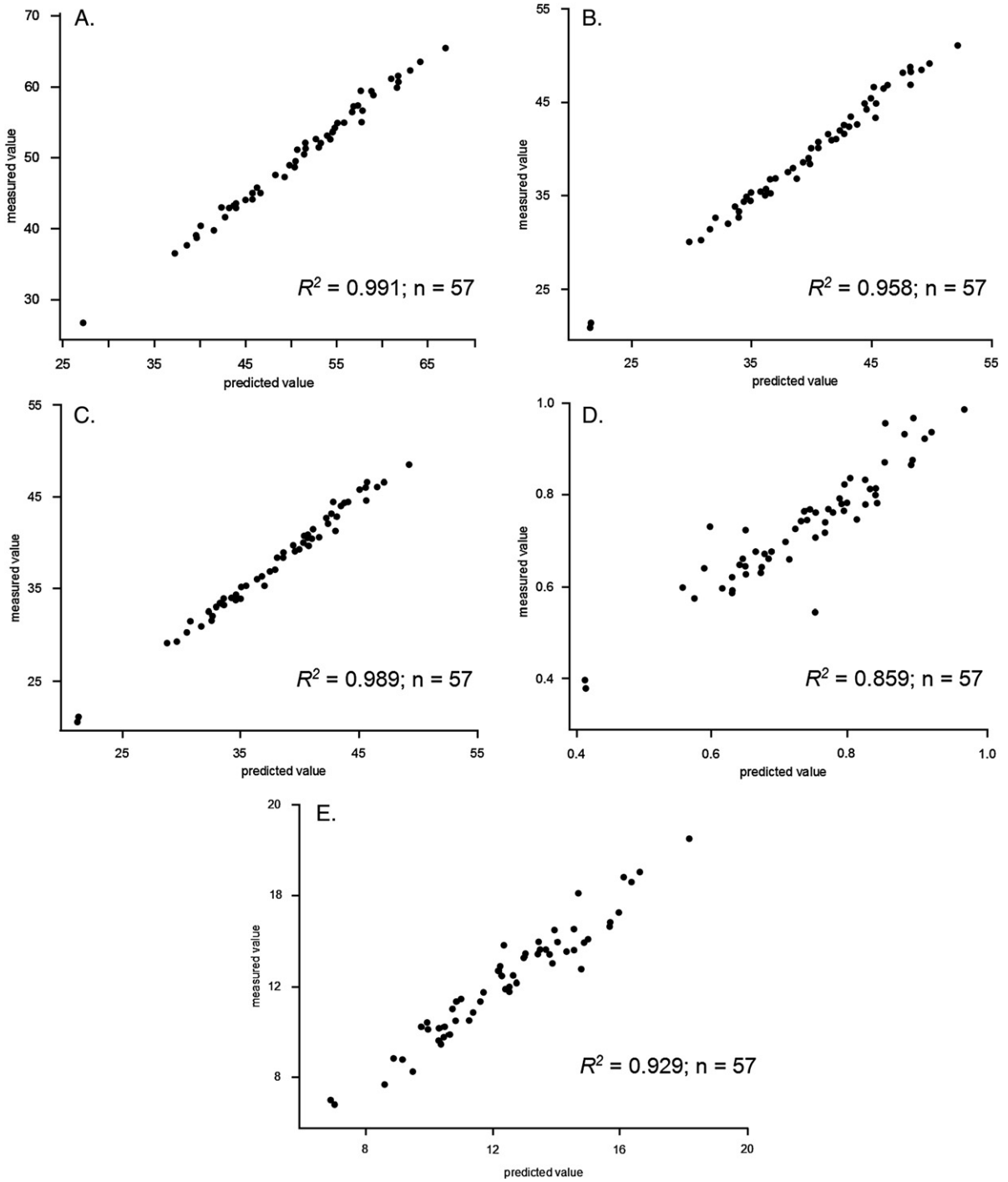

Fig. 3. Predicted and measured parameters using BIA and direct chemical analyses of 57 cobia for: A. Total fat free mass and predicted fat-free mass; B. Relationship between total body protein and predicted protein levels; C. Relationship between measured body moisture and predicted values; D. Measured body ash versus predicted ash content and, E. Measured and predicted dry matter.

dried, and placed on a nonconductive board. In addition to individual weights and total lengths, resistance and reactance values were immediately determined using a Quantum II Tetrapolar Bioelectric Impedance Analyzer (RJL Systems, Detroit, MI, USA) with stainless steel 28 gauge 12 mm needles (Grass Telefactor, West Warwick,

RI, USA). The analyzer has two sets of subdermal needles, one signal electrode and one detecting electrode, spaced 1.0 cm apart. Two readings were taken using the bioimpedance analyzer: one dorsal and one ventral (Fig. 1). For dorsal readings, one set of electrodes was placed at the posterior apex of the operculum and the second set was positioned along the posterior region of the caudal peduncle. Ventral readings were taken with one set of electrodes directly below the pectoral fin while the second set of needles was placed at the anterior end of the anal fin (Fig. 1). Electrode needles penetrated just below the skin. The distance between the two sets of electrodes was measured and then resistance and reactance readings were taken. These readings were used to determine resistance in both series and parallel, reactance in series and parallel, and capacitance. Samples of the muscle were collected for proximate analyses, including crude protein, total lipid, dry matter, and ash (AOAC, 1994). All proximate compositional measurements were performed in duplicate.

2.6. Statistical analyses

All data were subjected to analysis of variance procedure utilizing SAS v9.1 (SAS, Cary, NC, USA). Regression analysis for BIA was also completed using SAS v9.1. Regression models included whole weight, resistance in series and parallel, reactance in series and parallel, and capacitance. Backwards stepwise selection methods were used to determine the best fit model based on the adjusted R^2.

3. Results

Average group weight gain and weight specific growth rates of experimental animals are presented in Fig. 2. No differences were observed between dietary treatments in weight gain for the first 5 weeks of the trial although at week 6, fish fed the high lipid diet were heavier ($P<0.05$) than cobia offered the low lipid diet. Strong correlations were found between independent variables calculated from BIA values and proximate analysis values. Regressions for each body composition parameter (Fig. 3) illustrated the following relationships: resistance (in parallel) and protein (adj. $R^2=0.9569$), resistance (in parallel) and total body water (adj. $R^2=0.9894$), reactance (in parallel) and total body ash (adj. $R^2=0.8547$), reactance (in series) and dry matter (adj. $R^2=0.9272$) and reactance (in series) and fat-free mass (adj. $R^2=0.9916$). The F value tests ($P<0.0001$) revealed a significant correlation between the independent and dependent variables for each body composition parameter presented (Fig. 3). Correlations for each regression indicate strong linear relationships between impedance and proximate analysis variables with values of 1:1. For lipid content however, poor correlation between measured and predicted values were observed ($R^2=0.3337$).

4. Discussion

The major objective of the present study was to determine whether BIA represented a useful tool to accurately and non-destructively assess body composition of farmed fish. When compared against traditional chemical methods of compositional analysis, the utility of BIA as a method for non-lethal assessment of marine fish composition, and especially for body moisture, protein, and fat-free mass was extremely high, as evidenced by correlations with adjusted R^2 values greater than 0.95 for protein, fat-free mass and moisture. The lower correlations obtained for dry matter, ash and lipid levels, while disappointing, does not preclude use of BIA as a tool to estimate fish body lipid, especially given the reproducibility and resolving power of BIA body protein and moisture datasets. There are several reasons that explain the poorer performance of BIA regarding lipid quantification. Most likely, the lower correlation for lipid reflected the small overall size of the rapidly-growing experimental fish. Earlier studies both within our lab and elsewhere illustrate that cobia, relative to other species, and at the life cycle stages studied, have a tendency towards extremely lean body mass, usually less than 4% on a dry weight basis (Craig et al., 2007; Lunger et al., 2006, in press; Chou et al., 2004) and this fact, when combined with the method employed for lipid analysis (Folch et al., 1957), might explain the apparent reduction in precision of the BIA method. Other studies have had to resort to employing unifying morphometric analyses with resistance and reactance values to more accurately determine body moisture content for example, in gray seals, grizzly and polar bears (e.g., Bowen et al., 1999; Farley and Robbins, 1994). It may be that similar methodologies might be required to more accurately determine lipid levels in fast-growing young fish but this requires further research.

The selection of cobia as the experimental species for this study was primarily based on its rapid growth providing the means to assess changes in body composition of individuals of varying size, over a comparatively short timeframe. Previous experience with cobia indicated that manipulation of dietary protein:energy ratios, while influencing hepatic, and to a lesser extent, fillet lipid levels, was without effect upon overall fish performance (Craig et al., 2007). The specific growth

observed during the present trial for the size of growing animal were generally similar to that reported previously for this species (Webb et al., in press; Resley et al., 2006; Zhou et al., 2006; Chou et al., 2001, 2004). As the animal ages however, greater lipid accumulation occurs (Shiau et al., 2001) and a higher level of correlation between traditional chemical methods of lipid analysis and BIA would be anticipated.

The gaining prominence of global cobia aquaculture (Liao and Leaño, 2007) and its high growth rates may ultimately present the aquaculture industry with a species suited to and competitive in process markets previously dominated by traditional fisheries catch as exemplified by preformed fish sticks, nuggets and cakes, surimi, pates and spiced and marinated foods. Optimal selection of fish for each product line however, will rely on the availability of an inexpensive and reproducible method to assess body composition. Final quality of processed fish flesh is contingent on the quality of raw material and, depending on product destination, specific flesh characteristics might be stipulated by processors. Thus, for the sushi and sashimi trade, high lipid levels in the belly-flap region are desirable. Fillet lipid content is important during smoking (Robb et al., 2002; Rasmussen et al., 2000) while protein and water content impacts frozen storage quality and potentially, sensorial qualities of thawed fish (Lunger et al., in press; Ayala et al., 2005; Careche et al., 1999).

The plastic nature of fish body composition, rather than being a hindrance to producers presents a number of potential advantages. We have suggested previously that specifically formulated diets might be used to produce "designer fish" — that is, fish that express optimum flesh characteristics for specific processing needs (Powell, 2003; Rasmussen et al., 2000; González et al., 2006), or production requirements, as exemplified by the organic sector (Craig and McLean, 2005). It has been demonstrated that finishing diets represent an effective means for influencing and establishing specific flesh quality criteria at harvest (Robin et al., 2003; Rasmussen et al., 2000) and for body fat this is of particular importance because lipids impact appearance, flavor, tenderness and juiciness, as well as processability and flavoring in final products (Wille et al., 2002;Beltran and Moral, 1991). More importantly, at least from a human health perspective, body fat is the delivery vehicle for the beneficial n-3 fatty acids found in marine species. By having a non-destructive method for tracking changes in body composition producers would be able to tailor their produce for specific processes while authenticating the quality of their products. An ability to verify the composition of aquacultured fish would also improve product branding opportunities. Because BIA is relatively inexpensive, the equipment portable and robust and results comparatively easy to generate and understand, the method clearly offers high potential for on-farm application. Future studies must examine the effect of temperature upon BIA measurements and species-specific responses. Because the BIA probes puncture the skin and muscle of the animal, fish must be provided with adequate recovery times before re-assessment since punctures lower tissue resistance and could potentially impact BIA results. Long-term effects of single BIA readings on muscle quality (e.g., spotting) must also be examined. Future studies must be undertaken to evaluate the developed model using independent data sets as well as to test the method with fattier fish such as Atlantic salmon.

Acknowledgements

The authors would like to thank the Acorn Alcinda Foundation, Inc. for providing funds for the purchase of the BIA equipment.

References

AOAC, 1994. Official Methods of Analysis, 15th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

Ayala, M.D., López Albors, O., Blanco, A., García Alcázar, A., Abellán, E., Ramírez Zarzosa, G., Gil, F., 2005. Structural and ultrastructural changes on muscle tissue of sea bass, *Dicentrarchus labrax* L., after cooking and freezing. Aquaculture 250, 215–231.

Bai, S.C., Nemantipour, G.R., Perfra, R.P., Jaramillo, F., Murphy, B.R., Gatlin III, D.M., 1994. Total body electric conductivity for nondestructive measurement of body composition of red drum. Prog. Fish-Cult. 56, 232–236.

Beltran, A., Moral, A., 1991. The effects of fat contents and storage temperature on the storage life of smoked sardine fillets (*Sardina pilchardus* W.), prepared from frozen sardine. Food Chem. 42, 347–356.

Berg, E.P., Neary, M.K., Forrest, J.C., Thomas, D.L., Kaufmann, R.G., 1996. Assessment of lamb carcass composition from live animal measurement of bioelectrical impedance or ultrasonic tissue depths. J. Anim. Sci. 74, 2672–2678.

Bosworth, B.G., Wolters, W.R., 2001. Evaluation of bioelectric impedance to predict carcass yield, carcass composition, and fillet composition in farm-raised catfish. J. World Aquac. Soc. 32, 72–78.

Bowen, W.D., Beck, C.A., Iverson, S.J., 1999. Bioelectrical impedance analysis as a means of estimating total body water in gray seals. Can. J. Zool. 77, 418–422.

Brown, M.L., Gatlin III, D.M., Murphy, B.R., 1993. Non-destructive measurement of sunshine bass, *Morone chrysops* (Rafinesque) × *Morone saxatilis* (Walbaum), body composition using electrical conductivity. Aquac. Fish. Manage. 24, 585–592.

Callow, E.H., 1936. The electrical resistance of muscular tissue and its relation to curing. Special Report 75. Department of Scientific and Industrial Research Food Investigation Board, London, pp. 75–81.

Careche, M., Herrero, A.M., Rodriguez-Casado, A., Del Mazo, M.L., Carmona, P., 1999. Structural changes of hake (*Merluccius merluccius* L.) fillets: effects of freezing and frozen storage. J. Agric. Food Chem. 47, 952–959.

Chou, R.L., Su, M.S., Chen, H.Y., 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 193, 81–89.

Chou, R.L., Her, B.Y., Su, M.S., Hwang, G., Wu, Y.H., Chen, H.Y., 2004. Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. Aquaculture 229, 325–333.

Cox, M.K., Hartman, K.J., 2005. Nonlethal estimation of proximate composition in fish. Can. J. Fish. Aquat. Sci. 62, 269–275.

Craig, S.R., McLean, E., 2005. The organic aquaculture movement: a role for NuPro as an alternative protein source. In: Jacques, K., Lyons, P. (Eds.), Nutritional Biotechnology in the Food and Feed Industry: Create, Innovate, Elevate. Nottingham University Press, UK, pp. 285–293.

Craig, S.R., Schwarz, M.H., McLean, E., 2007. Juvenile cobia (*Rachycentron canadum*) can utilize a wide range of protein and lipid levels without impacts on production characteristics. Aquaculture 261, 384–391.

Farley, S.D., Robbins, C.T., 1994. Development of two methods to estimate body composition of bears. Can. J. Zool. 72, 220–226.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Gjerde, B., 1987. Predicting carcass composition of rainbow trout by computerized tomography. J. Anim. Breed. Genet. 104, 121–136.

González, S., Flick, G.J., O'Keefe, S.F., Duncan, S.E., McLean, E., Craig, S.R., 2006. Chemical, physical and sensorial differences in farmed southern flounder (*Paralichthys lethostigma*) fed commercial or crab meal-supplemented diets. J. Aquat. Food Prod. Technol. 15, 69–79.

Hancz, C., Romvári, R., Szabó, A., Molnár, T., Magyary, I., Horn, P., 2003. Measurement of total body composition changes of common carp by computer tomography. Aquac. Res. 34, 1–7.

Howell, N., Shavila, Y., Grootveld, M., Williams, S., 1996. High-resolution NMR and Magnetic Resonance Imaging (MRI) studies on fresh and frozen cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). J. Sci. Food Agric. 72, 49–56.

Hwang, Y.T., Larivière, S., Messier, F., 2005. Evaluating body condition of striped skunks using non-invasive morphometric indices and bioelectrical impedance analysis. Wildl. Soc. Bull. 33, 195–203.

Johns, J.V., Brackelsberg, P.O., Marchello, M.J., 1992. Bioimpedance measurement of lean and fat tissue in the beef carcass. J. Anim. Sci. 70 (Supplement 1), 45.

Kestin, S.C., Warriss, P.D., 2001. Farmed fish quality. Fishing News Books. Oxford, UK.

Kolstad, K., Vegusdal, A., Baeverfjord, G., Einen, O., 2004. Quantification of fat deposits and fat distribution in Atlantic halibut (*Hippoglossus hippoglossus* L.) using computerised X-ray tomography (CT). Aquaculture 229, 255–264.

Lee, M.H., Cavinato, A.G., Mayes, D.M., Rasco, B.A., 1992. Noninvasive short-wavelength near-infrared spectroscopic method to estimate the crude lipid content in the muscle of intact rainbow trout. J. Agric. Food Chem. 40, 2176–2181.

Liao, I.C., Leaño, E.M. (Editors), 2007. Cobia Aquaculture: Research, Development and Commercial Production. Asian Fisheries Society, Manila, Philippines, World Aquaculture Society, Louisiana, USA, The Fisheries Society of Taiwan, Keelung Taiwan, and National Taiwan Ocean University, Keelung, Taiwan.

Liedtke, R.J., 1997. Principles of bioelectrical impedance analysis. RJL Systems Inc., Clinton, MI. 10 pp.

Lunger, A.N., McLean, E., Craig, S.R., 2006. Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. Aquaculture 257, 393–399.

Lunger, A.N., McLean, E., Craig, S.R., in press. The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters in juvenile cobia (*Rachycentron canadum*). Aquaculture 264, 342–352.

Moon, H.Y., Gatlin III, D.M., 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95, 97–106.

Nott, K.P., Evans, S.D., Hall, L.D., 1999. Quantitative magnetic resonance imaging of fresh and frozen-thawed trout. Mag. Res. Imag. 17, 445–455.

Powell, K., 2003. Eat your veg. Nature 426, 378–379.

Rasco, B.A., Miller, C.E., King, T.L., 1991. Utilization of NIR spectroscopy to estimate the proximate composition of trout muscle with minimal sample pretreatment. J. Agric. Food Chem. 39, 67–72.

Rasmussen, R.S., Ostenfeld, T.H., Rønsholdt, B., McLean, E., 2000. Manipulation of end-product quality in rainbow trout with finishing diets. Aquac. Nutr. 6, 17–23.

Resley Jr., M.J., Webb, K.A., Holt, G.J., 2006. Growth and survival of juvenile cobia, *Rachycentron canadum* at different salinities in a recirculating aquaculture system. Aquaculture 253, 398–407.

Robb, D.H.F., Kestin, S.C., Warriss, P.D., Nute, G.R., 2002. Muscle lipid content determines the eating quality of smoked and cooked Atlantic salmon. Aquaculture 205, 345–358.

Robin, J.H., Regost, C., Arzel, J., Kaushik, S.J., 2003. Fatty acid profile of fish following a change in dietary fatty acid source: a model of fatty acid composition with a dilution hypothesis. Aquaculture 225, 283–293.

Rønsholdt, B., McLean, E., 1999. Quality characteristics of rainbow trout as perceived by the Danish trout processing industry. Aquac. Int. 7, 117–127.

Rønsholdt, B., Nielsen, H., Færgemand, J., McLean, E., 2000. Evaluation of image analysis as a method for examining carcass composition of rainbow trout. Ribarstvo 58, 3–11.

Shiau, C.Y., Linn, R.T., Li, Y.L., Chiou, T.K., 2001. Characterization of chemical composition of cage cultured cobia (*Rachycentron canadum*). 6th Asian Fisheries Forum, 25-30 Nov 2001, Kaohsiung, Taiwan, Book of Abstracts, p. 338.

Swantek, P.M., Crenshaw, J.D., Marchello, M.J., Lukaski, H.C., 1992. Bioelectric impedance: a nondestructive method to determine fat-free mass of live market swine pork carcasses. J. Anim. Sci. 70, 169.

Webb, K.A. Jr., Hitzfelder, G.M., Faulk, C.K., Holt, G.J., in press. Growth of juvenile cobia, *Rachycentron canadum*, at three different densities in a recirculating aquaculture system. Aquaculture 264, 223–227.

Wille, K., McLean, E., Goddard, J.S., Byatt, J.C., 2002. Dietary lipid level and growth hormone alter growth and body conformation of juvenile blue tilapia *Oreochromis aureus*. Aquaculture 209, 221–234.

Wille, K., Jain, R., McLean, E., Goddard, J.S., Kaplan, E.J., Leven, H., 2004. Flatbed scanning as a novel approach for examining lipid distribution in fish: comparison with MRI and traditional chemical methods. Int. J. ReCirc Aquac. 5, 1–17.

Zhou, Q.C., Wu, Z.H., Tan, B.P., Chi, S.Y., Yang, Q.H., 2006. Optimal dietary methionine requirement for juvenile cobia (*Rachycentron canadum*). Aquaculture 258, 551–557.