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# Development of a nutritional model to define the energy and protein requirements of cobia, *Rachycentron canadum*

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ABSTRACT

This study assessed the protein and energy requirements of Cobia (*Rachycentron canadum*) using a bio-energetic factorial approach. Using a series of inter-related studies, several parameters were defined to enable the construction of a bio-energetic factorial model for this species. The studies included two controlled laboratory experiments and also extensive field-data collection from commercial and research farms in Vietnam. The devised model includes parameters for both maintenance and protein demands; the effect of fish live-weight on maintenance protein ($LW^{0.697}$), lipid ($LW^{0.972}$), and energy demands ($LW^{0.815}$); the efficiencies of protein, lipid and energy utilisation at various protein, lipid and energy intake levels; and the variability in whole body composition with varying live-weight. The protein utilisation efficiencies (0.456 · [protein intake] − 0.445), lipid utilisation efficiencies (1.292 · [lipid intake] − 1.120) and energy utilisation efficiencies (0.651 · [energy intake] − 48.41) were similar to other carnivorous fish species. However, the maintenance requirements for both energy (74.3 kJ/kgBW$^{0.8}$/d$^{-}$ at 28 °C) and protein (0.99 g/kgBW$^{0.7}$/d at 27.9 °C) were about double to other species. Using this modelling approach it was possible to iteratively derive optimal dietary protein and energy specifications for this species.



## 1. Introduction

Cobia (*Rachycentron canadum*) is the only species in the family Rachycentridae. The species is distributed worldwide in warm marine waters; except for the central and eastern Pacific. The species is generally regarded as a fast growing, tropical pelagic animal. In offshore net cage systems, cobia can grow from 0.5 kg fingerling to 6.0 to 8.0 kg marketable size within 6 to 8 months with a feed conversion ratio of 1.5 (Liao et al., 2004) or 6 kg after 1 year at 28 °C (Benetti et al., 2010). Due to their high quality white flesh, cobia is suitable for sashimi or fillet production (Chou et al., 2001). The global aquaculture production of Cobia has increasing rapidly from 2002, reaching to 41,774 MT in 2012 (FAO, 2014). The three main producers of cobia in 2012 were China, Taiwan and Vietnam, where annual production was approximately 38,014 metric tons (MT), 1384 MT and 2000 MT, respectively (FAO, 2014). While Cobia cultured in offshore net cage systems is generally reared using formulated feeds (Liao et al., 2004), most cobia production in traditional inshore sea cages is still based on trash fish (Petersen et al., 2015). Currently, the limited supply of trash fish as the main feed source for cobia grow-out has become a major constraint for cobia culture in Viet Nam and other countries.

Cobia culture has been rapidly gaining in popularity since the early 1990s, but formulated feed development for aquaculture of this species is still lagging behind compared with other fish species such as salmon or barramundi (Zhou et al., 2007; Xiao et al., 2009; Liu et al., 2010). Despite, many studies have been undertaken to identify a range of nutritional requirements of this species, the energy and protein requirements are still undefined and pelleted feed are still not well established (Fraser and Davies, 2009; Salze et al., 2010). Earlier studies have suggested that the optimum dietary protein and lipid levels in juvenile cobia were 45% and 5–15% dry weight, respectively (Chou et al., 2001; Craig et al., 2006). Maximum growth and the best feed conversion ratios have been recorded at 27–29 °C in juvenile cobia with an optimum feed ration level determined at 9% initial body weight per day for fish of 10–200 g live-weight (Sun et al., 2006; Webb et al., 2009; Sun and Chen, 2014).

The requirements for protein and energy for most aquaculture species have traditionally been determined using empirical dose-response studies (Mercer, 1982). More recently, the use of bio-energetic factorial modelling has proven to be a useful alternative method in estimating these requirements (Shearer, 1995; Glencross, 2008; Trung et al., 2011). The benefits of bio-energetic factorial modelling are that it

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provides a method for estimating nutritional requirements independent of animal size and it results in a series of nutrient specifications that are indexed against energy demand and as such it underpins the potential for a wide range of diet specifications to be developed subject to different formulation strategies (Lupatsch et al., 2003; Booth et al., 2010; Glencross et al., 2011). Additionally, this modelling approach also has an advantage over an empirical approach in that it can also be used to define the optimal feed rations as well as specifications. This has further merits in that total nutrient and energy budgets, including losses through wastage and excretion, and also raw material demands can be determined and strategies examined by which to improve fish production (Glencross, 2010).

This paper describes a series of studies designed to determine the energy and protein requirements of cobia (*R. canadum*). Using farm-collected data, samples and experiments from both Vietnam and Australia, and a series of studies undertaken to determine key parameters of the model. These parameters include; the estimation of growth potential of fish with varying size, changes in body protein and energy composition with fish size; determination of the energy and protein requirements for maintenance, determination of the protein and energy digestibility of a reference diet, and determination of the partial efficiencies of both protein and energy utilisation. From this series of studies the results are then integrated to present an iterative approach to the determination of the protein and energy requirements for this species over a range of fish sizes.

**Table 1**
Reference diet formulation (% as used) and composition (% dry basis unless otherwise indicated).

| Ingredients | (%) |
|---|---|
| Brown fish meal | 65.5 |
| Wheat flour | 14.5 |
| Wheat gluten | 10.0 |
| Fish oil | 9.4 |
| Mineral and vitamin premix[a] | 0.5 |
| Marker (yttrium oxide) | 0.1 |
| Composition | |
| Dry matter (% as fed) | 95.8 |
| Crude protein | 48.9 |
| Digestible protein | 40.6 |
| Total lipid | 13.8 |
| Digestible lipid | 13.0 |
| Crude ash | 11.0 |
| Carbohydrate[b] | 21.1 |
| Gross energy (kJ $g^{-1}$) | 22.2 |
| Digestible Energy (kJ $g^{-1}$) | 19.7 |

[a] Vitamin and mineral premix includes (IU $kg^{-1}$ or g $kg^{-1}$ of premix): Vitamin A, 1.3 MIU; Vitamin D3, 0.5 MIU; Vitamin E, 0.17 MIU; Vitamin K,3, 3.4 g; Vitamin B1, 6.7 g; Vitamin B2, 5.8 g; Vitamin B6, 6.7 g; Vitamin B12, 0.003 g; Folic acid, 0.8 g; D-Calpan, 20 g; Niacin, 11.7 g; Biotin, 0.17 g; Vitamin C, 33 g; Inositol, 45 g; Iron, 8.3 g; Zinc, 16.7 g; Copper, 8.3 g; Manganese, 3.0 g; Cobalt, 0.67 g; Iodine, 0.17 g; Selenium, 0.07 g.
[b] Calculated only.

## 2. Methods

### 2.1. Study 1 – endogenous losses of protein, lipid and energy

This experiment was conducted at the Cat Ba National Broodstock Center of Marine Aquaculture of the Research Institute for Aquaculture - 1 (RIA-1), in Vietnam. Twelve 1000 L tanks were each stocked with ten cobia (*R. canadum*). Fish sizes within each tank were in one of four general size classes (100 g, 200 g, 500 g and 1000 g $fish^{-1}$), with three replicates being used for each size class. Additional fish ($n = 5$ for each size class) of similar approximate weights to those four size classes were euthanized at the beginning of the study to determine the dry matter, ash, protein, lipid and energy composition of the fish at the beginning of the study. The experimental tanks were supplied with aeration, flow-through marine water (salinity 32PSU) at 28.4 ± 1.58 °C. The transferred fish were kept in the tanks for 21 days, without feeding. After this period the fish were re-weighed and all fish from each tank were used as a replicate to determine weight, energy, lipid and protein loss. Following weighing five of the fish from each size class were euthanized, pooled and assessed for composition change in dry matter, ash, lipid, protein and energy concentrations.

### 2.2. Study 2 – energy and protein digestibility

This study was conducted at the Cleveland Laboratory of the CSIRO Aquaculture Program in Australia. A single basal diet was formulated to provide protein and lipid at 489 g/kg and 138 g/kg diet at a gross energy level of 22.2 MJ/kg (estimated digestible protein and energy of 406 g/kg and 19.7 MJ/kg, respectively) (Table 1). The dry ingredients were first blended in a series of batches using a 60 L upright Hobart mixer (HL600, Hobart, Pinkenba, QLD, Australia), to produce a single batch of basal mash which was extruded using a laboratory-scale, twin-screw extruder with intermeshing, co-rotating screws (MPF19:25, Baker Perkins, Peterborough, United Kingdom). The resultant pellets produced through a 3 mm Ø die were cut into 4 to 5 mm lengths using a four-bladed variable speed cutter and collected and dried at 60 °C for 12 h in a fan-forced drying oven. The remaining oil allocation was vacuum infused post-drying according to the methods reported by Diu et al. (2015).

Three 100 L tanks of flow through seawater (27.9 ± 0.32 °C) were each stocked with 10 juvenile (~200 g) fish. The transferred fish were allowed to acclimate to the tanks and were fed the reference diet for 23 days before faecal collection was initiated. Faeces were collected using stripping techniques similar to that used for barramundi (Blyth et al., 2014).

Diet and faecal samples were analysed for dry matter, yttrium, protein, total lipid, gross energy and ash content (AOAC, 2005). Differences in the concentrations of the protein, lipid, energy and yttrium in the feed and faeces on a dry matter basis in each treatment were calculated to determine the apparent digestibility ($AD_{diet}$) of each nutritional parameter. Those digestibilities examined were based on the following equation:

$$ADC = \left(1 - \frac{Y_{feed} \times \text{Parameter}_{faeces}}{Y_{faeces} \times \text{Parameter}_{feed}}\right)$$

where $Y_{diet}$ and $Y_{faeces}$ represent the yttrium content of the diet and faeces respectively, and $\text{Parameter}_{diet}$ and $\text{Parameter}_{faeces}$ represent the nutritional parameter of concern (protein, lipid or energy) content of the diet and faeces respectively.

### 2.3. Study 3 – energy and protein utilisation efficiency

This study was conducted at the Cleveland Laboratory of the CSIRO Aquaculture Program in Australia. Twenty four 100 L tanks were each stocked with 10 cobia juveniles ( mean weight 136.2 ± 0.71 g). A series of six feed ration treatments were assigned in quadruplicate to the array. The same diet as used in the digestibility study was used in this study (Table 1). Each ration level was determined based on satiety, 80%, 60%, 40%, 20% of satiety and starved. The sub-satietal levels were estimated based on feed intake measured in the three days preceding the initiation of the experiment when fish were being acclimated to the tanks. Water temperature was maintained at 27.9 ± 0.32 °C for the duration of the study. The trial was run for 23 days to minimize the time that fish were unfed before a result could be obtained. The apparent satiety ration level was determined based on the loss of feeding activity after the fish being offered food on three or more independent feeding episodes within a one-hour period. Any uneaten food was collected by siphoning and accounted for. After 23 days the weight gain was assessed by weighing all fish within each tank to determine tank mean weight gain. At this point three fish from each tank were also euthanized and whole fish samples were collected for the analysis of dry matter, protein, lipid and energy content.