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**A factorial approach to defining the dietary  
protein and energy requirements of  
mulloay, *Argyrosomus japonicus*:  
optimising feed formulations and feeding  
strategies.**



**Thesis submitted by  
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**July 2009**

**For the degree of Doctor of Philosophy  
School of Marine & Tropical Biology  
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Townsville  
Australia**

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The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Involving Human* (1999), the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A1102).

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Pirozzi, I., Booth, M.A., Allan, G.L., In press. The interactive effects of dietary protein and energy on feed intake, growth and protein utilization of juvenile mulloway (*Argyrosomus japonicus*). *Aquaculture Nutrition*. doi: 10.1111/j.1365-2095.2007.00641.x

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# Abstract

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The costs associated with feeds and feeding typically constitute the largest proportion of operating expenses in the production of fish in intensive culture. Sub-optimal feeds and inefficient feeding regimes result in direct economic losses through food wastage and sub-optimal growth, deterioration of water quality and increased environmental pressures from excessive waste production. The formulation of cost-effective, nutritionally optimal diets are therefore imperative to maximising profitability and reducing waste output on marine fish farms. Recent interest by industry in New South Wales and South Australia has focused on mulloway culture; however, little information exists on the protein and energy requirements for this species. Prior to the commencement of this research there were no published data on the requirements for digestible protein (DP) and digestible energy (DE) for mulloway and, as a consequence, no specific diet formulations or feeding standards were available.

Mathematical modelling in animal nutrition provides an extremely useful tool in the development of practical feed evaluation systems (i.e. feeding standards and practices) to describe and predict nutrient requirements, body composition and growth of the animal. Factorial bioenergetics is the quantitative study of energy gains, losses and transfers within the whole organism based on thermodynamic principles and has been widely applied to animal nutrition and the development of feed evaluation systems.

The general aim of this thesis was to establish a practical feed evaluation system for mulloway based on the factorial approach. This was achieved by

conducting a series of interrelated studies which determined the requirements for DP and DE for maintenance and growth and described aspects of metabolism relating to the fasting and feeding physiology of this species. The following is a brief overview of these studies.

A comparative study was undertaken to establish the routine metabolic rates (RMR) for similar sized mulloway, a sedentary species, and yellowtail kingfish, a highly active species, acclimated at one of several temperatures ranging from 10 – 35 °C. RMR increased linearly with increasing temperature ( $T$ ) for both species. RMR for mulloway was  $5.78T - 29.0 \text{ mg O}_2 \text{ kg}^{-0.8} \text{ h}^{-1}$  and for yellowtail kingfish was  $12.11T - 39.40 \text{ mg O}_2 \text{ kg}^{-0.8} \text{ h}^{-1}$ . The energetic cost of routine activity can be described as a function of temperature for mulloway as  $1.93T - 9.68 \text{ kJ kg}^{-0.8} \text{ day}^{-1}$  and for yellowtail kingfish as  $4.04T - 13.14 \text{ kJ kg}^{-0.8} \text{ day}^{-1}$ . RMR for mulloway was least thermally dependent at 28.5 °C and for yellowtail kingfish at 22.8°C. The results of this study have direct implications with regard to the appropriate temperatures at which to culture these species.

Specific dynamic action (SDA) is the energy expended on the physiological processes associated with meal digestion and is strongly influenced by the characteristics of the meal and the body weight (BW) and temperature of the organism. The effects of temperature and body weight on the RMR and SDA response in mulloway were assessed at 3 temperatures (14, 20 or 26 °C). RMR and SDA were shown to represent significant energetic costs in the overall energy budget of mulloway. Many of the SDA indices measured in this study were within the ranges of those reported for other temperate marine fish; however, these values are not fixed and are highly dependent on temperature, body size and feed intake. The effect of body size on the mass-specific RMR ( $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) varied significantly depending

on the temperature with a greater relative increase in the mass-specific RMR demonstrated for smaller mullet with increasing temperature. The gross RMR ( $\text{mg O}_2 \text{ fish}^{-1} \text{ h}^{-1}$ ) of mullet can be described as function of temperature as:  $(0.0195T - 0.0454)\text{BW}(\text{g})^{0.8}$  and the mass-specific RMR ( $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) can be described as:  $(21.042T - 74.867)\text{BW}(\text{g})^{-0.2}$ . SDA duration occurred within 41-89 h and was influenced by both temperature and body weight. The average proportion of energy expended over the SDA period (SDA coefficient) ranged from approximately 7 – 13 % of the total DE intake while the proportion of total energy expended on SDA above RMR ranged from approximately 16 to 27 %.

The utilization of DP and DE is dependant on the composition of the diet and the efficiency with which tissue deposition (growth) occurs. A detailed understanding of the relationships between nutrient intake, tissue deposition and body composition is necessary to accurately determine feed requirements. The effects of body weight, temperature and feed intake level on the utilization of DP and DE and the requirements for maintenance in mullet were investigated. Utilization efficiencies for growth based on linear regression for DP (0.58) and DE (0.60) were found to be independent of fish size, temperature and feed intake level. The partial utilization efficiencies of DE for protein ( $k_p$ ) and lipid ( $k_l$ ) deposition, estimated using a factorial multiple regression approach, were 0.49 and 0.75 respectively. Maintenance requirements estimated using linear regression were independent of temperature for DP ( $0.47\text{g DP kg}^{-0.7} \text{ day}^{-1}$ ) while maintenance requirements for DE increased with increasing temperature ( $44.2$  or  $49.6 \text{ kJ DE kg}^{-0.8} \text{ day}^{-1}$  at  $20$  or  $26$  °C respectively).

The interactive effects of DP and DE on the feed intake, growth and body composition of mullet were investigated using the dose-response method to identify the optimal DP content and DP:DE ratio for the growth of mullet. This

was achieved by feeding mulloay diets containing one of four different DP levels (250 - 550 g kg<sup>-1</sup>) at two DE levels (16 or 21 MJ kg<sup>-1</sup>). The results indicated that feed intake was not governed solely by energy demands but was also dependant on the DP content of the diet. Protein utilization did not improve with diets containing decreasing protein and increasing lipid content indicating that mulloay have a limited capacity to spare dietary protein. Optimal DP content was found to be 444-491 g kg<sup>-1</sup> depending on the DE content of the diet and the size of mulloay and is within the range reported for other sciaenid species. The use of formulated diets with 28.6 g DP MJ DE<sup>-1</sup> will achieve optimal growth and protein deposition for 70 – 275g mulloay.

The final study consolidated the results of the previous experiments to establish a feed evaluation system for mulloay using a factorial approach based on the requirements for DP and DE. Assessments of the growth potential of mulloay and the allometric relationships between body size and protein and energy metabolism and protein and energy whole body composition were combined with data previously established on the utilization efficiencies and maintenance requirements for DP and DE. Factorial modeling of the data allowed estimations of the decreasing requirement of the ratio of DP:DE for mulloay with increasing body size through grow-out production up to 2 kg. Estimations using the factorial method were found to be close to those estimated independently using the dose-response method. From this information theoretical diet formulations and feeding regimes were iteratively derived to match the predicted shifting requirements for DP and DE dependant on body size and the grow-out stage of mulloay.

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## List of Abbreviations

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ABT	Arrhenius breakpoint temperature		
BW	Body weight	$MO_{2sda-d}$	SDA duration
BOD	Biochemical oxygen demand	$MO_{2sda-p}$	Peak post-prandial $MO_2$
DE	Digestible energy	$MO_{2sda-pd}$	Time to peak post-
$DE_m$	Maintenance digestible energy		prandial $MO_2$
DO	Dissolved oxygen	NFE	Nitrogen free extract
DP	Digestible protein	NSW	New South Wales
DP:DE	Ratio of g DP MJ DE <sup>-1</sup>	OT	Oxygen transfer
ERE	Energy retention efficiency	OTR	Oxygen transfer rate
FCR	Food conversion ratio	PD	Protein deposition
FE	Feeding efficiency	PE	Protein energy
GE	Gross energy	PRE	Protein retention efficiency
GMBW	Geometric mean body weight	PSFI	Port Stephens Fisheries Institute
IBW	Initial body weight	QLD	Queensland
$k_l$	Partial energy efficiency for lipid	RE	Retained energy
$k_p$	Partial energy efficiency for protein	RFI	Relative feed intake
LD	Lipid deposition	RMR	Routine metabolic rate
LDO <sup>TM</sup>	Luminescent dissolved oxygen	SA	South Australia
LE	Lipid energy	SDA	Specific dynamic action
MBW	Metabolic body weight	$SDA_E$	SDA energy expenditure
$MO_2$	Oxygen consumption	SMR	Standard metabolic rate
$MO_{2rnr-g}$	Gross RMR	$T$	Temperature
$MO_{2rnr-s}$	Mass-specific RMR	TE	Total energy
$MO_{2scope}$	Post-prandial metabolic scope		
$MO_{2sda}$	Cumulative $MO_2$ above RMR over the SDA period		