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project

Improving feed sustainability for marine aquaculture in Vietnam and Australia

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In Australia the barramundi work had a suite of challenges. First there was the need to build a fish-lab at Cleveland in 2009 and then shutdown those facilities in 2010 for 18 months while there was the construction of the new laboratory at Bribie Island. Alas we didn't access the new fish-lab until 2012! This unfortunately delayed much of the work. We also had to bring the implementation of the nutrigenomics work forward into 2010 (rather than the planned 2012) which resulted in a range of issues through to the end of the project. However, we managed to get things mostly on back track due to the hard work at CSIRO from the varied contributions of Michael Anderson, Stuart Arnold, David Blyth, Nicholas Bourne, Giles Campbell, Susan Cheers, Natalie Habilay, Simon Irvin, Ben Maynard, Katherine Morton, Nick Polymeris, Dylan Rylatt, Simon Tabrett and Nick Wade.

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Thanks,
Brett

2. Executive summary

This project brought together a collective of important aquaculture sectors in Vietnam and Australia under the common issue of resolving the problems associated with the reliance on the use of fishery resources as their main feed source. Finfish (barramundi =Asian seabass, grouper and cobia), mud crabs and spiny lobster were the key species studied in this project. This project combined the efforts related to diet development and low-value fish replacement of four previous ACIAR projects under one collective project to ensure maximisation of resource sharing and knowledge transfer among both the Vietnamese and Australian collaborators. The focus of issues in this project extended to socio-economic risk assessment, optimisation of nutrient and energy specifications for key species, broadening of the raw material options for use in feed and enhancing capability development in both Vietnam and Australia.

Through a series of socio-econmic studies this project identified the extent of feed ingredient resource risks and the barriers (perceived and real) to adoption of manufactured feed by marine aquaculture sectors. Using this analysis, the risks (scientific, social, economic and environmental), were identified and it was suggested that an appropriate strategy to address these problems was to improve the design specifications of manufactured feed in Vietnam and improve our understanding on the complexities of use of alternative raw materials in both Vietnam and Australia.

In collaboration with Vietnamese researchers and industry partners, feeds were developed that optimise nutrient and energy delivery to a range of marine aquaculture species. This entailed the definition of protein and energy requirements of key species, which was followed by a series of laboratory and farm-based validation of the findings.

Feed ingredient resource risk in both Vietnam and Australia was minimised by increasing the scope of raw material use. This involved the development of research capacity in Vietnam and a focused research effort in both Vietnam and Australia to define the digestible nutrient and energy values of different raw materials. This also helped with understanding some of the limitations to the use of different raw materials. As an output of this project, a compendium on the digestibility assessment of over 50 ingredients when fed to Asian seabass (*Lates calcarifer*) was developed.

This project improved the capacity for nutritional research in both Vietnam and Australia. In Vietnam, improved capacity for the determination of nutritional requirements using bioenergetic modelling capabilities were developed. Additionally, the development of digestibility assessment in key species has underpinned the capacity for robust assessment of ingredients in Vietnam. Progress on optimising nutritional models to better account for growth prediction and nutrient and raw material utilisation was achieved, as well as the development of nutrigenomics capability that has underpinned significant scientific progress in Australia.

The project included a significant training and extension program. This included hosting of Vietnamese researchers and students in Australia. The project also facilitated several training workshops (nutrition and feed extrusion) and the Regional Aquafeed Forum (RAF), which each year was sponsored by an industry partner. The RAF forums continue beyond the project where they allow technology exchange amongst the project participants and other aquaculture nutrition researchers in the broader Southeast Asian region. The close involvement of the feed manufacturing sector has been critical to the effective adoption of outputs from this project leading to the replacement of direct feeding of low value fish for marine aquaculture in Vietnam, but also major advances in our understanding of nutrition of fish in Australia.

3. Background

There is increasing pressure on feed sustainability throughout the aquaculture industry worldwide. In Vietnam, where low-value fish (termed ”trash-fish”) constitutes the bulk of aquaculture feeds for marine species, there is increasing pressure on sustainability of the fisheries being harvested to provide that feed. In Australia, despite significant adoption of alternative raw materials, fishmeal still constitutes the main ingredient in most aquaculture feeds and there are considerable risks (especially economic supply) to the reliance on this limited, imported resource.

In Vietnam, marine aquaculture is concentrated in Ha Long bay and around Cat Ba island in the north and around central Vietnam (including near Nha Trang) in the south. Projected production of marine fish from coastal areas is 30,000 to 50,000 tonnes (Dr Luu, Director RIA-1 personal communication, 2008). In addition, pond farming barramundi occurs in south Vietnam including around the delta regions. Approximately 22,000 tonnes of mud crabs (Mr Thach, deputy Director of RIA-3, personal communication, 2007) are produced annually from ponds in Vietnam and more than 1,000 tonnes of lobsters are cultured annually.

The Vietnamese aquaculture feed manufacturing industry comprises three sectors. The government agencies (each of the RIAs) have invested in pilot-scale extruders to assist with development of technology for extruded feeds for new species. Secondly, a number of small Vietnamese companies have extruders, some established to make feeds or products for other purposes and see a market opportunity to make aquaculture feeds. Thirdly, large multi-national commercial feed manufacturers (including Ewos, Cargill, Ocialis, Proconco) are interested in diversifying their production of extruded feeds from catfish or other species to include marine fish. All three sectors have a role to play in development of pelleted feeds for the marine aquaculture industries in Vietnam. In total, there are some 36 companies producing aquaculture feeds with production estimated at around 3.2 M tonnes of catfish and shrimp feeds per annum. Although feed production is well established for catfish and shrimp production, there has been reluctance by the aquaculture industry to adopt formulated, manufactured feeds for marine fish and crustaceans. To assist the adoption of manufactured feeds, an improved research and development capacity in Vietnam and closer linkages between the feed, farming and research sectors are required.

In Australia, production of barramundi occurs in sea cages, cages within freshwater ponds and in ponds. Total production was estimated as 2,500 t in 2006/07 (ABARE Fisheries Statistics, 2008). In Australia there are two major feed manufacturers, Ridley Agriproducts and Skretting. Both produce feeds for salmonids and barramundi and Ridley also produce feeds for prawns. In Australia, about 80,000 tonnes of aquaculture feed are produced per annum, worth an estimated $120M. There is an increasing priority to use alternative raw materials to fishmeal in aquaculture diets and this makes it increasingly complex to meet nutritional requirements and to manufacture acceptable quality pellets using available extrusion equipment. These nutritional and feed processing issues require an improved knowledge base to sustain confidence in the use of these raw materials by both feed manufacturers and fish farmers.

In Australia and indeed globally, there is also an increasing level of scrutiny on the environmental sustainability associated with aquaculture. Feed formulation, processing and feed management remain the primary way to manage this issue. Significant advances in feed formulation and management have occurred through the development and adoption of nutritional models by both the feed and farming sectors and further gains will be dependent on refinement of those models.

Communication has been identified as a critical limitation to both industrial adoption of science, but also for technology exchange among researchers not only within Vietnam, but also throughout the broader Southeast Asian region.

In the past ten years there have been several ACIAR funded aquaculture nutrition projects. The present project (FIS/2006/141) proposes to build on these earlier initiatives in an attempt to consolidate the move towards reducing the reliance of the aquaculture industry in Vietnam on the use of low-value fish as a feed. In this regard, the present project will comprise several combined elements of each of five aquaculture species that have formed the basis of earlier projects. These earlier projects include:

- FIS/2002/068 Improving feeds and feeding for small scale aquaculture in Vietnam and Cambodia. Project leaders Brett Glencross, Nguyen Thanh Phuong and Dinh Van Trung (Vietnam) and Chhouk Borin (Cambodia).
- FIS/2000/065 Assessing the potential for low cost formulated diets for mud crab aquaculture in Australia, Indonesia and Vietnam. Project leaders Peter Mather (Aust), Ketut Suwirya (Indonesia) and Nguyen Co Thach (Vietnam).
- FIS/2002/077 Improved hatchery and grow-out technology for marine finfish in the Asia Pacific region. Project leaders Mike Rimmer (Aust), Ketut Sugama, Inneke Rumengan, Usman, Adiasmara Nyoman (Indonesia), Jobert Toledo (Philippines), Mike Phillips (NACA), Le Thanh Luu (Vietnam).
- FIS/2001/058 Sustainable tropical spiny lobster aquaculture in Australia, Vietnam and Indonesia. Project leaders Clive Jones (Aust), H. Safirin (Indonesia), Nguyen Bich Thuy, Pham Thi Du and Nguyen Dinh Mao (Vietnam)

In summary, these projects have investigated nutritional requirements and commenced the evaluation of ingredients with potential for use in formulated diets. During FIS/2002/068, a bioenergetic model for Tra catfish was constructed to help quantify requirements for protein and energy, and digestibility of key ingredients was measured to assist with formulation of new diets. New feeds have performed well and information has been adopted by commercial feed companies. Importantly, the Vietnamese partners have consolidated their role as key technical service providers to the feed manufacturing industry and national leaders in aquaculture nutrition for freshwater species. The approach used in FIS/2002/068 forms the basis for that proposed here.

In addition to this, the Network of Aquaculture Centres in Asia-Pacific (NACA) is also undertaking a FAO-funded project in China, Vietnam, Thailand and Indonesia aiming to facilitate the adoption of manufactured pellets in the grouper aquaculture sectors in these countries. The present project will interact closely with the NACA project to share data, workshops and disseminate results from both projects throughout the region.

In early 2008 the Vietnamese Ministry of Science and Technology (MOST, Government of Vietnam) funded a collaborative project led by Regional Institute of Aquaculture (RIA) -2, but with input from RIA-1 on "*Studies on producing compounded feeds for grow-out phase of Asian seabass (Lates calcarifer), Cobia (Rachycentron canadum) for exporting target".* Many elements of this project cover aspects of the proposed ACIAR project. Collaboration between the two projects is being ensured through the involvement of the MOST project leader and data exchange between the two projects. Extension of the MOST project outcomes will also be facilitated through the ACIAR project extension processes. In addition RIA-2 has requested the involvement of Dr Glencross as a key advisor to the MOST project as part of the collaboration.

Dr Elizabeth Petersen leads a project **(**PLIA/2007/050) on the assessment of policy, institutional and economic constraints to aquaculture research adoption in Vietnam. Dr Glencross and Dr Williams are collaborators with Dr Peterson and it is proposed to learn from elements of that project to improve the research adoption process. In addition, Dr Peterson will be a co-investigator in the present project to provide input in to project design, and to examine key socio-economic issues associated with changes in feed management systems from low-value-fish to the use of manufactured feeds.

4. Objectives

The following objectives will be applied to five key marine aquaculture species (Asian seabass, grouper, cobia, mud crabs and spiny lobster). For some species, like barramundi (Asian seabass), several of the objectives have already been achieved and will only require validation of previous results generated elsewhere. For other species, like cobia, most of the objectives will need to be addressed within this project. The aim of the project is to attempt to bring the basic nutritional technology for all five species to similar level suitable for formulating and manufacturing effective feeds and developing appropriate feeding strategies. However, it is recognised that the ability to achieve all these objectives within the scope, resources and timeframe of this project is ambitious. The proposed specific objectives are to:

1. Determine the soci-economic barriers to adoption of manufactured feed
2. Model the optimal dietary nutrient and energy specifications
3. Measure the digestibility of diets and specific ingredients
4. Benchmark optimal feed specifications and feed management strategies
5. Improve the capacity for feed manufacturing technology
6. Improve the capacity in Vietnam to undertake industry applicable research
7. Explore some of the mechanistic elements of fish models for barramundi

5. Methodology

Vietnam Components (numbers relate to objective number)
1.1 To identify barriers to feed technology uptake by aquaculture sectors
By interviewing fish farmers and feed manufacturers, the extent to which low-value trash-fish is used in the production of marine species in Vietnam was characterised. This was corroborated using Ministry of Agriculture and Rural Development (MARD) data and provided a clear benchmark of current industry practices that was used for subsequent assessment of the impact of the research. Socio-economic evaluations of the impact of the current feeding practices was studied at this point to provide an assessment of the impact of the research and industry changes over the life of the project.

1.2 To identify barriers to feed technology uptake by fish production sectors
Barriers to the adoption of pelleted feeds by the farmers of marine species in Vietnam will be characterised by interviewing the farmers to gain an understanding their perceptions towards using pellets compared with the use of low-value fish. This information will guide the choice of key issues to address at a demonstration level and will be used to design extension strategies that address major concerns.

2. To model the optimal nutrient and energy specifications for each species.
Dietary protein and energy specifications for formulated feeds will be optimised through an improved understanding of energy and protein demands. This knowledge will be fast-tracked using factorial modelling technologies. The outcome of this work will allow better formulation of feeds to match animal nutrient requirements and sustain maximal animal growth with the most efficient resource utilisation. Development of these nutritional models also allows for calculation of optimal feed rations based on energy demands.

3. To define the protein and energy digestibility of a suite of locally available and/or key raw materials
Methods and/or experience will be established to enable the assessment of the digestible nutrient and energy values of a range of raw materials for Asian seabass, cobia and grouper, mud crabs and lobster. The raw materials to be evaluated will be those based on an assessment of key local and imported raw materials that are likely to underpin future feed development requirements. These studies will also be used to examine the influence of different raw materials on feed palatability for each species.

4. To benchmark modelled feed specifications and management strategies against independent data.
In close collaboration with commercial feed manufacturers, feeds will be formulated based on technology gained during the project. Initially these new feeds will be assessed in controlled laboratory trials to observe and understand the behaviour of each species in order to optimise the management of the feeding process. Following confirmation of the efficacy of new feeds in the laboratory, on-farm trials will be undertaken at R&D farms and focus on extension towards large and medium scale farmers maximise project impact and to further validate the technology. The positive outcomes obtained from the R&D farm commercial scale trials with key fish producers and feed companies was coordinated and demonstrated the technology under commercial situations. Socio-economic evaluations of the impact of the change in feeding practices were studied at this point.

It is worth noting that each of these key parts in the feed technology development process need to occur for each species. However, feed development for some species is more technically advanced than for others, and as such there is capacity to expedite certain elements of this work for some species. A technology status matrix for each of the five key marine aquaculture species being studied is presented in Table 1.

5. To improve the capacity for feed manufacturing technology
Training courses on extrusion technology were prepared and delivered in Vietnam with the aim of improving the technical capacity of the Vietnamese feed industry. Using a combination of lectures and practicals participants were trained in the principles of extrusion to produce floating and sinking feeds and to also develop skills for moist-pellet production.

6. To improve the capacity in Vietnam to undertake industrially applicable nutrition research
The local Vietnamese researchers were assisted in the design, structure and implementation of experiments to address each of the research issues presented. Through the experience gained in this process, their capacity to undertake independent research was improved, and encompassed elements of scientific writing and presentation at conferences. A key part of improving the capacity for nutrition research in Vietnam was to consolidate the expertise in the country and improve linkages between different research groups. The Regional Aquafeed Forums (RAF) were proposed as an annual event to facilitate this.

Australian Components
1. To identify barriers to feed technology uptake by aquaculture sectors
By interviewing fish farmers (Marine Produce Australia, Humpty Doo, Good Fortune Bay among others) and feed manufacturers (Ridleys and Skretting) the issues affecting fishmeal replacement technology in Australia to produce diets for marine species were characterised. This also provided a guide to the key perceived issues of each sector in furthering ingredient sustainability for feed production and allow them indirect input into guiding the research being undertaken to address those issues.

3. To define the protein and energy digestibility of suite of locally available and/or key raw materials
Over several years and using refined techniques, a range of raw materials were evaluated for their nutrient and energy digestibilities, following a survey of key feed manufacturers to ensure that the raw materials assessed were those that are likely to underpin future feed development requirements. These studies also examined the influence of different raw materials on feed palatability for barramundi. NIRS calibrations of feeds to estimate diet protein and energy digestibility were developed. A focus was made on the effects of plant-derived raw materials on the digestive physiology of fish, with a view to improving our understanding of the effects of carbohydrate complexity on the nutrition of carnivorous fish species.

7. To explore mechanistic elements of fish nutritional modelling systems
Australian nutritional models for barramundi are primarily based on factorial methods that combine a series of empirically derived mathematical equations that describe various growth and nutritional relationships. One of their principal flaws is that they do not describe actual discrete biochemical processes nor attribute the energy value of either diets or growth into actual compositional parameters. In this study, mechanistic models were developed that better represent discrete biochemical processes and nutritional values of macronutrients. Based on actual processes and nutrients rather than simple mathematical relationships of energy flow, these models underpinned the development of a more refined interpretation of nutritional requirements and total feed delivery management. Additionally, a suite of molecular (nutrigenomic) tools were developed, and these were applied to better understand the role that different nutrients had in providing nutritional energy to barramundi by exploring key metabolic pathways involved in energy metabolism.

Table 1 Analysis of data/knowledge gaps for each of the five key marine aquaculture species in Vietnam. Where data is known and available, the source is referenced.

Species	Asian seabass	Grouper	Cobia	Spiny Lobster	Mud Crab
1.1. Production characterization	RQD	RQD	RQD	RQD	RQD
1.2. Farmer Surveys	RQD	RQD	RQD	RQD	RQD
1.3. Socio-Economic Analysis	RQD	RQD	RQD	RQD	RQD
2.1. Maintenance demands	Glencross 2008	RQD	RQD	N/A	N/A
2.2. Protein and Energy Utilization	Glencross 2008	RQD	RQD	N/A	N/A
2.3. Growth Model	Glencross 2008	RQD	RQD	RQD	RQD
2.4. Factorial model	Glencross 2008	RQD	RQD	RQD	RQD
3.1. Digestibility Methods	McMeniman, 1998	Usman et al 2007	Zhou et al 2004	Irvin & Tabrett 2005	Richardson 2008
3.2. Ingredient Digestibilities*	RQD	RQD	RQD	RQD	RQD
4.1. Protein + Energy	Williams et al 2003	Tuan & Willams 2007	Chou et al 2001	Smith et al 2003	Richardson 2008
4.2. Feeding Rations	Glencross 2008	RQD	RQD	RQD	RQD
5. Feed Processing Capacity	RQD	RQD	RQD	RQD	RQD
6.1. Feeding Behavior	Williams & Barlow 1999	Tacon et al 1991	RQD	Williams, 2007	Richardson 2008
6.2. Commercial Feed Trials	RQD	RQD	RQD	RQD	RQD
7. Mechanistic model	RQD	N/A	N/A	N/A	N/A

RQD: Required. N/A: Not applicable or premature. *Ingredient digestibilities are required for all species on an ongoing basis as variability in raw material quality is extensive and only through long-term analysis of this variability will methods to manage this become apparent

6. Achievements against activities and outputs/milestones

Objective 1: Determine the barriers to the adoption of manufactured feed …

no.	activity	outputs/ milestones	completion date	comments
1.1	Construction of survey form	Survey form complete	March 2010	Completed
1.2	Initiation of survey	Data collection commenced	May 2010	Completed
1.3	Completion of survey	12-months of data collected	May 2011	Completed.

Objective 2: Model the optimal dietary nutrient and energy specifications …

no.	activity	outputs/ milestones	completion date	comments
2.1	Collection of growth data from farms and laboratory experiments (V)	Data on weights and temperatures collected at regular intervals on each species	May 2011	Completed
2.2	Collection of animal composition variation with size (V)	Samples of each species at varying sizes collected and analysed	May 2011	Partially completed. Data for some species requires refinement.
2.3	Determination of maintenance energy and protein requirements (V)	Data on energy and protein loss from animals of varying sizes after starvation	June 2011	Partially completed. Data for some species requires refinement.
2.4	Determination of partial utilisation efficiencies of energy and protein (V)	Data on energy and protein gain achieved with varying levels of energy and protein intake	Dec 2012	Partially completed. Data for some species requires refinement.

2.5	Construction of a factorial model for each species (V/A)	A functional MS Excel based model		(V) Factorial models prepared and in some cases published for Barramundi and Cobia. Data for Grouper, Lobster and Crab models incomplete. (A) Factorial model for Barramundi is now fully functional. Model for Cobia is now also operational.

V = Vietnam, A = Australia

Objective 3: Measure the digestibility of diets and specific ingredients …

no.	activity	outputs/ milestones	completion date	comments
3.1	Identifying key ingredients for evaluation	A range of ingredient samples and associated data	(V) May 2011 (A) May 2010	Completed. Completed.
3.2	Digestibility of a selection of key ingredients determined and methods validated	Digestible protein and energy data from a suite of ingredients	(V) Dec 2011 (A) May 2010	Completed. Completed and published.
3.3	Effect of dietary fibre complexity on digestibility examined	Digestible nutrient and energy data from a suite of diets in response to varying compositional complexity	(A) Dec 2012	Completed and published.
3.4	Use of NIRS to measure digestibility examined	Validation of a conceptual basis for improving quality control of feeds	(A) Dec 2014	In Australia development of a NIRS calibration for diet protein and energy digestibility was developed and published.

V = Vietnam, A = Australia

Objective 4: Benchmark optimal feed specifications and feed management strategies …

no.	activity	outputs/ milestones	completion date	comments
4.1	Iteratively designed diets determined and formulated	A series of formulations to test	Completed August 2010	Iterative diets for barramundi designed based on latest modelling technology
4.2	Laboratory Growth trials instigated	Diets made and experiment initiated	(A) Completed in 2013 (V) Not implemented	Completed
4.3	Laboratory Growth trials completed	Experiment completed	(A) Completed in Dec 2013 (V) Not implemented	In Australia a small model validation trial has confirmed aspects of model validity and explored key assumptions. Was skipped and the Vietnemese went direct to the farm trial.
4.4	Farm Growth trials instigated	Diets made and experiment initiated	(V) Dec 2014 (A) Trials progressed in 2010.	Completed Trials completed but given that they were not funded by ACIAR are held commercial-in-confidence. Independent benchmarking trials to validate project outcomes was also undertaken.

V = Vietnam, A = Australia

Objective 5: Improve the capacity for feed manufacturing technology …

no.	activity	outputs/ milestones	completion date	comments
5.1	Identify manufacturing limitations (V)	Documented priority issues pertaining to feed manufacturing	November 2009	Visit undertaken to several feed mills in Vietnam in August 2009 and again November 2009 to identify key issues and constraints
5.2	Plan feed manufacturing courses (V)	Content, trainers, trainees and venues identified.	May 2010	Two training courses delivered.
5.3	Undertake feed manufacturing courses (V)	Courses completed	July 2011	1st training course completed 28th - 30th June 2010 in Hanoi. 2nd course completed 27th - 29th June 2011 in CaiBe.

V = Vietnam, A = Australia

Objective 6: Improve the capacity in Vietnam to undertake industrially applicable research.…

no.	activity	outputs/ milestones	completion date	comments
6.1	Annual planning workshops	That plans for each successive 12-months are agreed on and documented	Completed	1st Project Planning Meeting held in NhaTrang in August 2009. 2nd - Hanoi in July 2010. 3rd - HoChiMinh in June 2011. 4th - NhaTrang in April 2012. 5th - NhaTrang in June 2013. 6th – Cairns in May 2014
6.2	Annual Regional Aquaculture Nutrition Forums	Proceedings from each forum produced	Still ongoing (2017)	1st Regional Aquafeed Forum held in NhaTrang in August 2009. 2nd - Hanoi in July 2010. 3rd - HoChiMinh in July 2011. 4th - NhaTrang in April 2012. 5th - VungTau in December 2013. 6th - NhaTrang in September 2014.
6.3	Two Extrusion Workshops	Two completed training courses	July 2011	1st Aquafeed Extrusion Masterclass held in Hanoi in June 2010. 2nd Aquafeed Extrusion Masterclass held in CaiBe in June 2011.

6.4	Two Nutrition Shortcourses	Two completed training courses	June 2014	Only one Nutrition Masterclass Shortcourse was held (March 2014).
6.5	Australian based training	Six Vietnamese scientists gaining experience in Australia	June 2014	Eight Vietnamese scientists visited Australia as part of this project.

V = Vietnam, A = Australia

Objective 7: Explore the mechanistic elements of fish nutritional modelling systems

no.	activity	outputs/ milestones	completion date	comments
7.1	Develop a mechanistic model of fish physiology and molecular processes	A process diagram and associated MS Excel model has been mapped	(A) Aug 2010	A visit by Dr Glencross to the University of Guelph in Canada supported by the Australian Academy of Sciences was used to expand an existing factorial model to a more advanced mechanistic one.
7.2	Define effects of temperature x photoperiod on barramundi growth	Growth data defining animal response to test parameters	(A) Dec 2012	Experiment was completed. Not written up due to staff/legal complications.
7.5	To examine the effects of dietary protein and energy density on feed intake	Feed intake data defining animal response to test parameters	(A) Dec 2010 (A) Jun 2012	Experiment undertaken Sept - Nov 2010. Follow up experiment undertaken Jan - Feb 2012. Completed and published.
7.6	To examine the effect of dietary nutrient deletion/ addition on feed intake	Feed intake data defining animal response to test parameters	(A) Dec 2010	Included with experiment 7.5 and experiment undertaken Sept - Nov 2010. Experiments now completed and published.

V = Vietnam, A = Australia

Table 2 Reanalysis of data/knowledge gaps for each of the five marine aquaculture species in Vietnam. Where data is known and available, the source is referenced. Where published within this project the first author is linked to relevant Results and Discussion section below.

Species	Asian seabass	Grouper	Cobia	Spiny Lobster	Mud Crab
1.1. Production characterization	Petersen et al (6.2)	Petersen et al (6.1)	Petersen et al (6.3)	Petersen & Tuan (6.5)	Petersen et al (6.4)
1.2. Farmer Surveys	Petersen et al (6.2)	Petersen et al (6.1)	Petersen et al (6.3)	Petersen & Tuan (6.5)	Petersen et al (6.4)
1.3. Socio-Economic Analysis	Petersen et al (6.2)	Petersen et al (6.1)	Petersen et al (6.3)	Petersen & Tuan (6.5)	Petersen et al (6.4)
2.1. Maintenance demands	Glencross 2008	RQD	Tien et al (6.7)	RQD	RQD
2.2. Protein and Energy Utilization	Glencross 2008	RQD	Tien et al (6.7)	RQD	RQD
2.3. Growth Model	Glencross 2008	RQD	Tien et al (6.7)	RQD	RQD
2.4. Factorial model	Glencross 2008	RQD	Tien et al (6.7)	RQD	RQD
3.1. Digestibility Methods	McMeniman, 1999 Blyth et al (6.14)	Usman et al 2007	Zhou et al 2004	Irvin & Tabrett 2005	Richardson 2008
3.2. Ingredient Digestibilities	Binh et al (6.10) Glencross et al (6.17)	Tien et al (6.9)	Tien et al (6.9)	Tuan et al (not submitted)	Phuong et al (6.11)
4.1. Protein + Energy	Williams et al 2003	Tuan & Willams 2007	Chou et al 2001	Smith et al 2003	Richardson 2008
4.2. Feeding Rations	Glencross 2008	RQD	Tien et al (6.7)	RQD	RQD
5. Feed Processing Capacity	Aquafeed Extrusion Masterclasses held in Hanoi in June 2010 and in CaiBe in June 2011				
6.1. Feeding Behavior	Williams & Barlow 1999	Tacon et al 1991	RQD	Williams, 2007	Richardson 2008
6.2. Commercial Feed Trials	Binh et al (6.12)	RQD	RQD	RQD	Phuong et al (6.13)
7. Mechanistic model	Glencross et al (6.19; 6.20) Wade et al (6.21)	RQD	RQD	RQD	RQD

RQD: Required. N/A: Not applicable or premature.

7. Key results and discussion

7.1 - Bioeconomics of Grouper, Serranidae Epinephelinae, Culture in Vietnam

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Reviews in Fisheries 21, 49-57.

The results of a survey of 37 small-scale grouper farmers in Vietnam are presented in this section (approximately 5% of the industry by production). The data from the survey are used to inform two bioeconomic models; one each for the northern and central regions of Vietnam. From the bioeconomic analysis using these models it is inferred that the profitability of grouper farming in Vietnam is variable and depends on the region and grow-out method used. Net revenue was found to be very high in northern cage systems compared with central cage and pond systems. This is despite the northern systems completing one crop every 2 years compared with annual crops in the centre. Profitability was significantly higher in the north compared with the centre as harvest biomass is significantly higher. In turn, this higher harvest biomass is due to significantly higher numbers of fish stocked and a larger individual fish biomass at harvest. Central cage systems have many benefits over the other systems, including a relatively low price of seed, relatively high average stocking density and high survival rate. However, the average aquaculture area is very low compared with northern cage and central pond systems. Hence, they rely on extremely high output prices relatively to input prices to make their systems profitable.

The profitability of northern cage and central pond systems is low to moderate compared with other high-valued species in Vietnam. For example, the profitability of farming lobster is approximately 870 million VND/year and mud crab was 41 - 136 million VND/year. The profitability of these systems is moderate to high compared with that of lower-value species such as cobia (25 - 185 million VND/year) and Asian seabass (11 - 39 million VND/year).

Costs for all systems are dominated by feed, and to a lesser extent seed and labour. Northern farming systems have a higher level of profitability and return on costs than central farms. The revenue per unit costis 1.84 in the north compared with 1.03 for central cage and 1.19 for central pond systems. In other words, northern farmers earn significantly more per dong per unit of investment than central farmers. This return is high for northern farmers and low for central farmers compared with other medium and high-valued farmed species in Vietnam. For example, revenue per unit cost for other high-valued species in Vietnam are 2.05 for lobster and 1.92 – 3.55 for mud crab. The revenue per unit cost for other medium-valued species are 1.2 for cobia and 1.04 – 1.23 for Asian seabass depending on the region (Petersen et al. 2011a-d). These values are heavily depending on the season and region. Most northern farmers and central pond farmers do not have alternative sources of income. Most central pond farmer also produce black tiger shrimp and have orchards.

Grouper feed is exclusively low-value finfish. Most farmers in both regions perceived that grouper are not adaptable to manufactured diets such as pellets, and although they did not perceived pellets to be more costly than other diets (except for central pond farmers), they did not perceive that pellets would lead to faster growth rates. Most farmers did not perceive that pelleted diets are readily available to them, and most farmers indicated they would not use them even if they were available.

There are many advantages to the use of pellets including faster growth rates (feed to biomass conversion ratios (FCRs) are generally less than 2, compared with ratios of 10 and higher for low-value fish diets), fewer parasites and diseases, fewer environmental problems, and more stable water quality. Conversely, problems with the current low-value finfish diets include a short storage life, rapid decline of nutritional quality if stored for too long, unstable supply (depending on the season), relatively low growth rates (compared with pelleted diets), localised pollution and water quality degradation, and transmission of parasites and diseases. The larger-scale cooperatives exclusively use pelleted feeds.

If pellets are to be widely adopted by grouper farmers, negative perceptions regarding the poor adaptability of grouper to pellets, relatively slow growth rates compared with low-value finfish and lack of availability of pellets need to be overcome. There is preliminary evidence that while pelleted diets are more expensive then low-value fish, their growth rates are generally significantly higher and justify the extra expense.

The future potential for grouper culture is good, with strong and potentially growing demand in Asia. Grouper aquaculture is a viable alternative livelihood for Vietnamese fishers, particularly in the north. This is also the case for central fishers if they could increase their aquaculture area and growout production time. Further research into the cost-effectiveness of pelleted diets for grouper grow-out farming would provide useful information to both northern and central farmers. More extensive bioeonomic analysis of grouper farming in Vietnam is also a potential area for further research, including sensitivity analysis of key model parameters to investigate the robustness of profitability given seasonal and market variability.

7.2 - Bioeconomics of Asian seabass, *Lates calcarifer*, culture in Vietnam

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The results of a survey of 12 small-scale Asian seabass farmers in Vietnam are presented in this paper. The data from the survey are used to inform two bioeconomic models; one each for the central and southern regions of Vietnam. From the bioeconomic analysis using these models it is inferred that Asian seabass farming in Vietnam is profitable. This profitability is low compared with other aquaculture species in Vietnam such as cobia, grouper, lobster and mud crab. However, this is highly dependent on region and seasonal conditions.

Although this study focuses on small-scale farming operations, culture practices and level of intensity of Asian seabass farming does not differ significantly across regions of Vietnam, despite the fact that the central farmers surveyed produced Asian seabass using ponds, whereas the southern farmers were using cage systems. The similarities across regions included the price and size of seed, the length of the growout season, the survival rate of stocked fingerlings, quantity and cost of feed, the size of the harvested Asian seabass, the price received for the harvested fish, the number of household members working on the operation, and the amount of credit borrowed.

Given the different systems used to produce the Asian seabass (ponds in the centre and cages in the south), there were differences across regions in stocking densities, the number of fish stocked, survival rate, total harvest biomass and equipment requirements. The central pond systems had a significantly lower stocking density but larger total number of fish stocked. Due to the large total number of fish stocked, the total seed cost was significantly higher in the centre compared with the south. However, the total harvest biomass was also greater. Overall, the greater biomass does not offset the larger costs in the centre compared with the south, and the overall profitability is higher in the south compared with the centre. As farms in both regions produce one crop per year, this profitability per crop is equivalent to annual profitability. The return on costs is also higher in the south compared with the centre. In other words, central farmers spend more money and produce more Asian seabass, but the money earned per dong of investment is lower than southern farmers. Most farmers in both regions do not have other significant income generating activities.

Overall, the return to investment of Asian seabass farming is similar to that of other medium-valued farmed species in Vietnam; for example, cobia (BCR ~ 1.20). However, the return to investment is low compared with high-valued farmed species; for example, grouper (BCR ~ 1.06 – 1.84), mud crab (BCR ~ 1.92 – 3.55) and lobster (BCR ~ 2.05).

The two dominant cost sources of Asian seabass farming are feed and labour. All other costs sources contribute less than 10% to total costs. Asian seabass diets are dominated by low-value finfish. Pellets were not used by any respondents in the central region, but were used by approximately 60% of respondents for the nursery phase and then 25% of respondents for the rest of the grow-out period. Low-value finfish is preferred by central farmers due to perceived faster growth rates, and by southern farms due to perceived faster growth rates and the lower cost. Pellets were used by southern farmers due to their ease of availability and storage, but were generally not used for most of the grow-out cycle as there is a perception that pellets are only suitable for fingerlings. Central farmers perceive that Asian seabass are not easily adaptable to manufactured diets, that they are more expensive than current diets but they do lead to faster growth rates. Half the farmers indicated that if pellets were available, they would not be interested in using them for these reasons. Southern farmers did not know whether Asian seabass are adaptable to pellets and many did not know whether they lead to faster growth rates compared with low-value finfish. They did indicate that pellets were generally available to them. Still, only half the farmers indicated they would use pellets if they were available for ease of management and less labour requirements for feeding.

Given the quantities of feed used, individual fish size at harvest and grow-out period of farmers in this analysis, the feed conversion ratio of low-value finfish was calculated to be 6.2 for the central pond farmers and 4.0 for the southern cage farmers (on a wet-weight basis). Approximately 25% of respondents in the south used pellets throughout the production cycle, and the calculated feed conversion ratio for this diet was 1.7. This is evidence that pellets do lead to faster growth rates. There are many advantages to the use of pellets including faster growth rates (feed to biomass conversion ratios (FCRs) are generally less than 2, compared with ratios of 4 and higher for low-value fish diets), fewer parasites and diseases, fewer environmental problems, and more stable water quality. Conversely, problems with the current low-value finfish diets include a short storage life, rapid decline of nutritional quality if stored for too long, unstable supply (depending on the season), relatively low growth rates (compared with pelleted diets), localised pollution and water quality degradation, and transmission of parasites and diseases. The larger-scale cooperatives exclusively use pelleted feeds.

If pellets are to be widely adopted by Asian seabass farmers, negative perceptions regarding the poor adaptability of Asian seabass to pellets, relatively slow growth rates compared with low-value finfish and lack of availability of pellets need to be overcome. There is preliminary evidence that while pelleted diets are more expensive then low-value fish, their growth rates are generally significantly higher and justify the extra expense.

Asian seabass aquaculture is a viable alternative livelihood for Vietnamese fishers, particularly in the south. This is also the case for central fishers if they could increase stocking density, survival rate and/or feeding costs. Further research into the cost-effectiveness of pelleted diets for Asian seabass grow-out farming would provide useful information to both central and southern farmers. More extensive bioeonomic analysis of Asian seabass farming in Vietnam is also a potential area for further research, including sensitivity analysis of key model parameters to investigate the robustness of profitability given seasonal and market variability.

7.3 - Bioeconomics of cobia, Rachycentron canadum, culture in Vietnam

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Aquaculture Economics and Management 18, 28-44.

Cobia farming in Vietnam is profitable, with net revenue being approximately 2,000 and 7,900 USD/year in the northern and southern regions, respectively. This profitability is comparable with high-valued aquaculture species in Vietnam such as grouper (approximately 400 – 9,000 USD/year) and mud crab (2,000 – 7,000 USD/year), although lower than the profitability of lobster (50,000 USD/year). Profitability of cobia is high compared with Asian seabass (barramundi) (600 – 2,000USD/year) which has a similar price to cobia. The variation in profitability quoted from these cited sources is due to regional and seasonal differences.

Culture practices and level of intensity of small-scale cobia farming differs significantly across Vietnam. The intensity level of inputs to cobia farming is significantly higher in southern Vietnam than northern Vietnam. Initial stocking density, total number of fish stocked, number and size of cages, and quantity of feed used are all higher in the south than the north. The number of fish initially stocked is almost double that of northern farms and the total water volume of all cages combined at the end of the grow-out cycle is 8.5 times higher in the south than the north. Feeding rates on a per fish basis start lower in the south than the north, but increase at a faster rate so that they use higher feeding rates per fish by the end of the growout cycle.

This higher intensification in the south leads to significantly higher total costs. It also leads to significantly higher productivity and profitability. Fingerlings are more expensive in the south, but are also purchased at a larger size which contributes, in part, to the higher survival rate in the south. Better quality seed, higher feeding rates and lower stocking densities in the latter phases of grow-out, lead to a higher harvest size in the south. Higher individual fish weight, higher stocking biomass and higher survival rates lead to significantly higher harvest biomass in the south (12,400kg/crop) than the north (2,700kg/crop). The harvest price of cobia is lower in the south than the north, but the higher harvest biomass ensures that net revenue from the operations is significantly higher in the south (8,000 USD/crop), than the north (2,000 USD/crop).

The average length of the grow-out period is shorter in the south (10 months) than in the north (19 months), such that southern crops get one crop each year, whereas northern farmers get one crop every two years. Hence, net revenue per year is half the net revenue per crop for northern farmers.

Despite the higher level of intensity, productivity and profitability in the south than the north, the overall value for money is equivalent across regions. The benefit cost ratio for both regions is 1.2. Depending on the region, overall, the value for money of cobia farming is similar to that of other low to medium-valued mariculture alternatives in Vietnam, such as Asian seabass (BCR ~ 1.0 - 1.2). However, the value for money is low compared with high-valued farmed species, such as grouper (BCR ~ 1.1 – 1.8), mud crab (BCR ~ 1.9 – 3.6) and lobster (BCR ~ 2.1).

The dominant cost source for cobia farming is feed. Cobia diets are dominated by low-value fish. Pellets are used by a minority of southern farmers during the nursery phase only. There are many advantages to the use of pellets including faster growth, fewer parasites and diseases, fewer environmental problems, and more stable water quality. There is preliminary evidence that while pelleted diets are more expensive then low-value fish, their growth rates are generally significantly higher and justify the extra expense. If pellets are to be widely adopted by cobia farmers, negative perceptions regarding the poor adaptability of cobia to pellets, relatively slow growth rates compared with low-value fish, and lack of availability of pellets, need to be overcome.

Further research into the cost-effectiveness of pelleted diets for cobia would provide useful information for both northern and southern farmers. More extensive bioeonomic analysis of cobia farming in Vietnam is also a potential area for further research, including sensitivity analysis of key model parameters to investigate the robustness of profitability given seasonal and market variability.

7.4 - Bioeconomics of mud crab, *Scylla paramamosain, c*ulture in Vietnam

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Reviews in Aquaculture 5, 1-9.

The results of a survey of 80 small-scale mud crab farmers in Vietnam are presented in this paper. The data from the survey are used to inform two bioeconomic models; one each for the central and southern regions of Vietnam. From the bioeconomic analysis using these models it is inferred that mud crab farming in Vietnam is profitable. Profitability was significantly higher in the centre compared with the south as almost three times the biomass is harvested in the centre compared with the south (in turn, due to significantly higher survival rates) and almost double the harvest price received for this harvested biomass. Profitability in the central region is high, and that in the south is comparable, to other aquaculture species in Vietnam. For example, the profitability of farming other high-value species such as lobster and grouper are generally higher than mud crab. However, the profitability of mud crab farming is higher than that of lower-value species such as cobia and Asian seabass, depending on region and seasonal conditions. Moreover, return on investment is extremely high for central farmers and moderately high for southern farmers compared with other medium and high-valued farmed species in Vietnam.

Results provided in this paper suggest that the input intensity level of mud crab farming does not differ significantly across regions of Vietnam, despite the greater experience of farmers southern farmers compared with central farmers. The crabs are almost exclusively fed on low-value finfish. A minority of central farmers also use pellets and shellfish. Feeding rates are higher in the centre compared with the south. However, assuming a feed conversion ratio (ratio of food eaten to weight gain) of 20, the biomass gain due to feeding is only 5% in both regions.

The biological, economic and environmental advantages to the use of pellets instead of low-value fish diets are well documented. If pellets are to be widely adopted by mud crab farmers, negative perceptions regarding the poor adaptability of mud crab to pellets (northern farmers only), relatively slow growth rates compared with low-value finfish and lack of availability of pellets need to be overcome. There is preliminary evidence that while pelleted diets are more expensive than low-value fish, growth rates using pellets are generally significantly higher and justify the extra expense.

Further research into the cost-effectiveness of pelleted diets for mud crab grow-out farming would provide useful information to both central and southern farmers. More extensive bioeonomic analysis of mud crab farming in Vietnam is also a potential area for further research, including sensitivity analysis of key model parameters to investigate the robustness of profitability given seasonal and market variability. Manufactured diets are available in some of these countries (e.g. China and the Philippines) but adoption is poor and there is significant scope to improve formulations and to reduce their costs. As farming techniques and farmer perceptions differ across countries, there is significant scope to extend this research to these countries.

7.5 - Bioeconomics of lobster, *Panulirus ornatus*, culture in Vietnam

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Culture-based grow-out of tropical spiny lobster is a nascent and unique industry which faces a number of challenges (such as reducing dependency on wild stocks for peurulus and food, the management and treatment of disease and low-value fish feeding issues) and opportunities (such as unmet export demand and therefore high export prices). Of particular interest to this study is the imminent development of pelleted diets for lobster that will potentially reduce the industry's dependence on by-catch for feed (with external benefits to wild-stocks of fish and reduced downstream environmental impacts of current feeding regimes). The aim of this paper is to conduct bioeconomic analysis of lobster grow-out farming in Vietnam, to determine farmer perceptions regarding the potential use of pelleted diets.

Results of the bieconomic analysis suggest that the net revenue for lobster grow-out operations is approximately 869 million VND/crop, 44,800USD/year, with a crop cycle averaging 18 months (although stocking is staggered and one crop is harvested each year). This profitability is very high compared with other aquaculture enterprises in Vietnam. For example, the profitability of farming other high-value species such as grouper is approximately 8 - 171 million VND/year and mud crab is 41 - 136 million VND/year (Petersen et al. 2011a,b). The profitability of lobster farming is also very high compared with that of lower-value species such as cobia (25 - 185 million VND/year) and Asian seabass (11 - 39 million VND/year).

Investment in the enterprise is also high compared with other enterprises in the region. For example, total costs for lobster farming is 828 million VND/year (43,000USD/year) compared with 44 – 877 million VND/year for other species such as Asian seabass, cobia, grouper and mud crab (Petersen et al. 2011a–d). Moreover, disease has the potential to devastate lobster crops and there is little information available to lobster farmers about disease prevention and management. Hence, the lobster enterprise is a high-risk high-return industry.

The return to investment to grow-out lobster farming is high. The benefit cost ratio is 2.05, which is moderate to high compared with Asian seabass (1.04 – 1.23), cobia (1.20), grouper (1.06 – 1.84) and mud crab (1.92 - 3.55).

The harvest price of market sized lobster (approximately 1kg) has increased by 30% from last year to 1.3 million VND/kg, making it a very high-valued seafood species. This increase in harvest price has been partially offset by a 270% increase in seed price from last year to 250,000VND/kg. These differences in market conditions have been the main drivers of a 120% increase in net revenue from last year, and an in 42% increase in return on investment (BCR).

The dominant cost sources for these farms are seed (61% of costs) and feed (24%). All other costs contribute 5% or less each. This includes labour which generally consists totally of household labour of approximately 3 person-years per crop. In this bioeconomic analysis, household labour is costed at half the cost of hired labour rates with the assumption that the household worker could find alternative employment but at reduced income if they were not working on the lobster operation.

A sensitivity analysis of key model parameters show that model results remain robust to significant changes in key model parameters with the exception of the cost of seed and harvest price (where low seed costs and high harvest price significantly increase the BCR). The enterprise remains profitable with significant changes in all parameters.

Pellets were not used by any of the surveyed households. However, there are many advantages to the use of pellets including faster growth rates (feed to biomass conversion ratios (FCRs) are generally less than 2, compared with ratios of 10 and higher for low-value fish diets), fewer parasites and diseases, fewer environmental problems, and more stable water quality. Conversely, problems with the current low-value finfish diets include a short storage life, rapid decline of nutritional quality if stored for too long, unstable supply (depending on the season), relatively low growth rates (compared with pelleted diets), localised pollution and water quality degradation, and transmission of parasites and diseases. The larger-scale cooperatives exclusively use pelleted feeds.

The households surveyed in this study perceive that lobsters do not easily adapt to manufactured diets (pellets), and they do not know whether pellets would be more or less expensive than current diets, or whether they would lead to faster or slower growth rates. Ninety percent of surveyed households indicated they would not use pellets if they were available to them (it is expected that pelleted diets formulated specifically to lobster will be available in Vietnam within 3 years). Significant effort will be needed to negate some farmer perceptions when introducing formulated diets to the industry. There is preliminary evidence that while pelleted diets are more expensive then low-value fish, their growth rates are generally significantly higher and justify the extra expense.

Further research into the cost-effectiveness of pelleted diets for lobster grow-out farming would provide useful information. More extensive bioeonomic analysis of lobster farming in Vietnam is also a potential area for further research, including sensitivity analysis of key model parameters to investigate the robustness of profitability given seasonal and market variability.

7.6 - Bioeconomic analysis of improved diets for marine aquaculture in Vietnam

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Vietnam is experiencing significant growth in the value and volume of production and exports of aquaculture products. This growth is increasing pressure from the global community to increase the sustainability of the aquaculture industry, including reducing the reliance on low-value fish for feed. Manufactured diets have significantly less economic, risk management and environmental problems.

Pelleted diets are generally more expensive than low-value finfish diets, but have significantly lower FCRs. The aim of this paper is to study the trade-off between increased costs and increased feed conversion ratios of these diets to understand the implications for profitability and return to investment with the potential adoption of manufactured diets for farmers of each of these species. Seven bioeconomic models were developed for four species (cobia, Asian seabass, lobster and grouper) across several regions of Vietnam based on a 47-question survey of 130 small to medium-scale mariculture growout farmers.

The results of the analysis show the change in return to investment (measured through the change in benefit cost ratio) for small-scale mariculture farmers from a change in feed regime to manufactured diets. It should be noted that the price and feed conversion ratio of manufactured diets specifically formulated for the target species is plastic, depending on local and global conditions associated with diet specifications, formulation, cost of feed ingredients, seasonal and economic conditions (including exchange rates) and management practices. Hence, the results of this paper are presented for a range of feed prices and feed conversion ratios.

It is estimated that adoption of pelleted diets by grouper farmers in the central region is expected to lead to increased returns to investment (approximately 35%). Although current low-value fish diets are significantly cheaper (approximately 8,000VND/kg) compared with proposed manufactured diets (approximately 40,000VND/kg), they are also significantly less efficient (with a fish feed conversion ratio of approximately 12) compared with that of manufactured diets (approximately 1.5). Hence, manufactured diets are expected to produce economic benefits for these farmers. In fact, given current economic conditions, manufactured diets with feed conversion ratios of approximately 1.5 are likely to lead to economic benefits, so long as the diets are cheaper than approximately 65,000VND/kg.

Given current economic conditions, adoption of pelleted diets by lobster farmers is expected to lead to equivalent returns to investment. However, the adoption of pelleted diets by cobia farmers, grouper farmers in the north and seabass farmers is expected to decrease a farmer’s return to investment. It appears that the current diets are relatively cheap and efficient. The improvements in feed conversion ratios are not expected to outweigh the additional costs of these diets, such that they may not produce economic benefits for these farmers.

If the manufactured diets formulated for each of these species are a functional feed (providing additional benefits to the fish other than those nutritional, such as disease resistance), then the benefits to these fish are expected to be significantly higher. For example, if these feeds lead to a reduction in mortality of approximately 20%, then farmers of all these target species in all regions would be economically better off adopting these functional diets, than continuing with their current low-value fish diets. The equivalent feed price (the feed price that matches current profitability given changes in the feed conversion ratio) is raised by an average of 10,000VND/kg for cobia, grouper in the centre and seabass. It is raised by 27,000VND/kg for lobster and 35,000VND/kg for grouper in the north.

The sensitivity analysis shows that the equivalent feed price is generally very sensitive to changes in management, seasonal and economic conditions (such as stocking density, mortality and harvest price). Hence, the price at which a pelleted diet becomes cost-effective for a farmer is very fluid. Given this, functional feeds are significantly more likely to be cost-effective and therefore adopted by small-scale mariculture farmers in Vietnam than standard manufactured feeds, and potentially pose a smaller investment risk for feed manufacturers.

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

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Factorial models have proven to be useful in defining both protein and energy demands and total feed ration management for a range of fish species. This study reports on the development of a model for a new carnivorous fish species and as such adds to the volume of data on such fish species.

There are actually only a few studies examining growth by cobia in the literature. Growth rates in that study were consistently 94% (at 27.8°C) and 82% (at 25.5°C) of those reported in the present model at water temperature of 27.8°C*.* A common issue with the comparison of the growth model against much of the published literature is that most of the published literature appears to be with very small fish and often under limiting conditions of feed quality situations and with fish growing much slower than that encountered from our farm-based data collection. Such vagaries in the growth rates throughout the literature highlight the need to develop a benchmark standard against which laboratory studies should be compared.

The metabolic weight exponent for protein metabolism in cobia is 0.697. This is similar to the generic protein exponent for most fish species is 0.70. The efficiency of protein use by cobia, based on the regression of the protein gain against the digestible protein intake, was linear over the protein intake range examined and had a coefficient of 0.456. This coefficient value for the partial efficiency of protein gain for this species is also similar to that observed for most other fish species – barramundi: 0.48, gilthead seabream: 0.53, rainbow trout: 0.40 - 0.47, yellowtail kingfish: 0.51. Although in most other studies this relationship between protein gain and protein intake has usually been observed to be curvilinear, in the present study this response was linear over the feed intake ranges studied. Though it has been argued that such linear responses are indicative of underfeeding as even the curvilinear responses reported are close to linear at the lower levels of feed intake.

A notable feature of this study was the higher maintenance protein requirements (DP_{maint}) observed of this species. Based on the point of zero net protein gain a DP_{maint} intake of 0.99 g/ $kg^{0.70}$/d was calculated (Figure 4). This is about 50% higher than the value of 0.66 g /$kg^{0.70}$/d determined for *D. labrax* and double the 0.45 g /$kg^{0.70}$/d determined for barramundi. However, it is only about half that reported for yellowtail kingfish (1.70 g /$kg^{0.70}$/d), another highly active pelagic carnivorous species.

The relationship between this specie's energy metabolism and its body weight also conform to the allometric equation: $a \bullet BW(kg)^b$ as is the case for virtually every other fish species studied. Similarly, the exponent value of body weight ($BW^{exponent\ value}$) for energy metabolism in cobia was observed to be 0.822 which is like the result determined using indirect calorimetry with this species (0.809). It is also like other fish species including barramundi (0.80), grouper (0.79), gilthead seabream (0.82), European seabass (0.80), Pangasius catfish (0.84) and tilapia (0.85).

The maintenance energy requirements ($DE_{maint} =$ 74.3 kJ/$kg^{0.80}$/d), as defined by the point of zero net energy gain, in this study was substantially higher from that seen for other species like rainbow trout

(40.1 kJ / $kg^{0.80}$ /d), barramundi (42.6 kJ / $kg^{0.80}$ /d) and mulloway (26.3 kJ / $kg^{0.80}$ /d). However, the DE_{maint} was like the 87.4 kJ/$kg^{0.80}$/d reported for another pelagic carnivorous fish species the yellowtail kingfish (*Seriola lalandi*). This observation poses a question whether it is this active pelagic nature of these animals that results in such a higher or some other feature like the partial endothermy observed in some Scombrid species.

The partial efficiency of energy use is determined as the slope of the regression of the energy intake against energy retention, on a metabolic body weight basis. In the present study for cobia species, the response of full energy intake range was recorded to be linear. This contrasts with the curvilinear response observed with other species, but is consistent with the linear response reported in other studies.

In the present study, the partial efficiency of energy gain was observed to be 0.651. This value is consistent with other carnivorous fish species e.g. Gilthead Seabream (0.65), white grouper, *Epinephelus aeneus* (0.69), barramundi (0.68), rainbow trout (0.62), yellowtail kingfish (0.65) and mulloway (0.60).

Key dietary parameters of energy and protein specifications can be derived iteratively from this model for fish at any phase of its production cycle. This iterative approach was also used to define the energy and protein requirements for cobia from 100g to 2000g at each of three dietary energy densities. Based on a combination of the somatic and non-somatic (maintenance) energy demands a simplistic energy budget was created that dictates how much energy the fish needs to consume to achieve a prescribed growth potential. The amount of feed (g/fish) rationed to the animal then being this energy demand divided by the digestible energy density of that feed.

Similarly, the needs for protein for both somatic and non-somatic demands can also be defined using this approach which defines the appropriate DP:DE ratio. Using the empirically derived equations from studies 1 to 5 the requirements for protein and energy at a range of fish sizes was determined. Based on a combination of the predicted growth, the protein and energetic cost of that weight gain, the efficiencies associated with those gains and the maintenance requirements, the total daily requirements for both protein and energy at a range of fish sizes were calculated. From this both the daily energy and protein intake requirement were defined. This has subsequently allowed us to iteratively specify a series of hypothetical diets of varying energy density (12 MJ/kg, 16 MJ/kg and or 20 MJ/kg).

In applying this iterative approach, it is assumed that the fish will eat to an energetic demand and as such the energy content of each diet will define total feed consumption. This total feed consumption also influences the amount of dietary protein required to satisfy the daily protein demand.

Using this iterative approach, the present study shows that there are several strategies that can be employed to define the theoretically optimal diet energy and protein specifications and that these change with fish size, consistent with what has been reported in numerous other similar studies. When the diet energy density and/or fish size varies the present model demonstrates that there is a need to vary the dietary protein supply for this species. This model also demonstrates how the choice of diet energy density has an effect on the biological feed conversion ratio (FCR). When a lower FCR is achieved with a higher energy density simply due to the energetic demands being satisfied by fewer grams of feed. Importantly though, this lower feed ration combined with the same daily protein requirement also means that the protein concentration required in that diet for it to satisfy the daily protein demands has to increase for it to be effective. Similar to other species, it was noted that the most dramatic changes in the protein demand (based on the required protein :

energy ratio) of cobia occur over the first 500 g of its growth, where the optimal DP:DE changes from 36 g/MJ at 50 g to 24 g/MJ at 500 g.

For cobia, the optimal DP:DE ratios at 100 g and 1000 g were 32 and 22 g/MJ, respectively and by comparison barramundi optimal DP:DE ratios at 100 g and 1000 g were 30.2 and 19.9 g/MJ. This contrasts those determined for yellowtail kingfish which had optimal DP:DE ratios at 100 g and 1000 g of 39 and 27 g/MJ, respectively. For each of the sizes of cobia, examined in the present study that the optimal DP:DE ratios were marginally higher than those of barramundi, but substantially lower than those of yellowtail kingfish.

This study used a factorial method for determining the protein and energy requirements for cobia. This study adds to the volume of literature using this method to estimate these requirements for a range of fish species. Comparison of the data derived from this study with that obtained for other species indicates a high degree of homology of most energetic parameters. The primary difference, in comparison to the many other models developed for most other carnivorous fish species, is that this species has a marginally higher demand for protein, but most notably its maintenance requirements for protein and energy are substantially higher than other studied species. The only exception to this being the comparison with another pelagic marine fish, the yellowtail kingfish, which also has similarly high maintenance demands.

This study represents a series of estimations based on a series of inter-related studies and their derived parameters. As such the estimations deduced from this modelling exercise are only as robust as the weakest data estimates. It would be prudent to take the outputs from this model and independently validate them and also test some of the assumptions used to increase the robustness of this model.

7.8 - Inter-laboratory Benchmarking Exercise

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During the course of any nutrition research a significant number of chemical and biochemical analyses will generally be carried out on feeds, feed ingredients and tissues of the species being studied. The results of these analyses can be used to formulate feeds, calculate the digestibility of feeds and their ingredients and to determine nutrient utilisation efficiencies. The results of the research would be of little value if the results of the analyses were unreliable, inaccurate or too variable. In this project five research institutions have been collaborating with the collection of many different types of samples and then independently analysing them. The data is being shared among the collaborators and integrated to answer the questions under investigation in the project. It was recognised that it was essential to ensure that the results of the chemical analyses from each of the collaborating institutions was as accurate and as precise as necessary to answer the questions under investigation.

"Benchmarking" is defined as establishing a standard or point of reference against which other things may be compared or assessed. The purpose of the inter-laboratory benchmarking exercise was to establish the accuracy with which a series of selected samples were analysed by each of the participating institutions (or their preferred outsourced laboratory) and to identify problem areas and then provide guidance on how the analytical techniques could be improved to meet the project requirements.

Objectives

- Ensure that the level of accuracy and precision of analytical data collected and used by the project was appropriate for the purpose for which it was intended.
- Support the development of internationally acceptable standards of nutritional analysis at participating laboratories.
- Give confidence to the participating laboratories that their nutritional analyses are accurate and reliable

Results from Laboratory 6 were generally more variable and inconsistent with the results of the other four laboratories. As this was an outsourced laboratory, the collaborating institute elected to use a different laboratory for all further chemical analyses.

In the determination of moisture in the samples, the accepted method is to dry the sample at 105°C for 16 hours. Laboratory 4 and 6 dried the samples by freeze drying them. This process resulted in an underestimate of the moisture content of between 1 and 3% (Table 1).

The determination of ash content of the samples was very consistent except for the results from Laboratory 5 which were consistently greater than the results from the other laboratories. The reason for this was identified as a problem with the temperature control of the muffle furnace used in ashing the samples.

The results of the crude protein analyses were very consistent, with the exception of the results from Laboratory 6, which were considered to be outliers. When the data from Laboratory 6 was omitted from the data set, the coefficient of variation was less than 0.5% for all but one sample, which was 0.54%.

The determination of the lipid content of the samples was the most inconsistent of all the analyses. This was mainly due to the fact that some of the laboratories determined total lipid, using a chloroform: methanol extraction method, while two of the laboratories determined crude fat using a soxhlet extraction with petroleum ether or hexane as the solvent. One laboratory determined total fat, which uses a combination of mild acid hydrolysis followed by a soxhlet extraction. Soxhlet extraction on its own will always result in a lower estimate of lipid content of a sample as it does not extract a significant proportion of the polar lipids in the sample. However, the soxhlet extraction method is widely used within the feeds industry, it is easy to use as semi-automated equipment is readily available and as a consequence it is relatively quick and inexpensive method. However, for nutritional research and the purposes of this project, the crude fat estimate is not recommended. A project workshop discussion was held to explain the limitations of the soxhlet extraction method and the reasons that total lipid or total fat were the preferred methods of analysis.
Further discussions were held about the analysis of the most widely used digestibility marker, chromic oxide and the requirements for the determination of Gross Energy. Due to the inherent difficulties of the chromic oxide analysis method and the very dangerous combination of concentrated acids that are used, the project participants all decided on sending their chromic oxide samples to the Larael laboratory for analysis using ICP-MS. As none of the laboratories were confident about analysis of Gross Energy, given the small amounts of available material from some samples, all elected to send their samples to Larael for analysis. It is appropriate to note that the results from Lareal were among the most consistent of all the laboratories participating in this benchmarking exercise.

Conclusions
As a result of the benchmarking exercise the following achievements were made:
- Obtained accurate estimates of proximate composition of the five ingredients
- Identified problems at individual laboratories and have taken steps to resolve them
- Identified which laboratories could provide reliable analyses
- Demonstrated the importance of determining total lipid or total fat rather than crude fat

7.9 - A comparison of the diet and ingredient digestibility between cobia (*Rachycentron canadum)* and orange spotted grouper (*Epinephelus cooides)*

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Cobia showed generally a high capacity for diet digestibility. Most of diets also given digestibility value over 0.85 when fed cobia accept for diet dry matter digestibility. The apparent digestibility coefficients for crude protein, crude lipid, and energy of both feed and ingredients were lowest in the brewery yeast when fed to both species. The apparent digestibility coefficients of diet and ingredient in the MBM were also lower compared to that of FMK, CGM and SBM when fed to cobia. Similarly, the apparent digestibility coefficients of diet and ingredient in the BLM were also lower compared to that of the FMK, CGM and SBM when fed to grouper. Both cobia and grouper digested well the fish meal, corn gluten meal and defatted soybean meal. Grouper showed that apparent digestibility is not as high as cobia in all parameter examined.

Observation of lower feed intake and feed palatability in cobia and grouper fed the BRY diet or grouper fed the BLM diet was recorded during feeding trial period. Therefore, the inclusion level about 30% of brewery yeast in cobia and grouper diets or blood meal in grouper diet should be regarded with some precaution.

Therefore, the present study aimed to examine the potential for using digestibility data from one species as an estimate for that obtained by another. Given the costs and difficulty of undertaking digestibility studies with some species this could offer a significant advancement in terms of being able to consolidate resources through the use of a species that is easier to work with (cobia) to provide data relevant for the formulation of another species (grouper).

Diet digestibility differences between species

In this study it was observed that there was a high degree of correlation between the cobia and grouper for all diet digestibility parameters examined. Regression equation coefficients ranged from 0.786 to 3.192 and were strongest (R^2 = 0.961) for diet energy and weakest (R^2 = 0.900) for diet lipid digestibility. Estimated for cobia based on the diet dry matter (0.862), protein (0.929), lipid (0.946) and energy (0.928) digestibility of the fish meal (FMK) fed to grouper given apparent digestibility coefficients of 0.825 for dry matter (cf. actual of 0.823), 0.906 for protein (cf. actual of 0.915), 0.929 for lipid (cf. actual of 0.931) and 0.859 for energy (cf. actual of 0.882). These findings clearly show that there is a close relationship between the digestibility responses of the two species when fed the same diets. This observation is in consistent with findings of other competitive studies between rainbow trout and Atlantic salmon when fed the same diet or between barramundi and rainbow trout.

A better diet digestibility of the most parameters examined in cobia suggested a close correlation to their habits. Cobia is more active in both taking formulated feed and actively swimming most of the time, while grouper feeding very slowly to formulated feed and sitting at the tank's bottom most of the time. It's not too difficult for cobia use pellet feed but it's difficult for grouper changed favourite feed is trash fish. These characters might led to relatively lower capability of grouper to digest formulated feed compared to that of the cobia.

Ingredient digestibility differences between species

There was close correlation in values of the most important parameters examined (crude protein, crude lipid and energy) between the two species, supporting that the ingredient digestibility data is homologous between the two species, except the weak correlation in ingredient dry matter digestibility coefficient. For example, an analysis of ingredient dry matter (0.774), protein (0.909), lipid (0.914) and energy (0.869) digestibility of cobia for soybean meal diet would have given digestibility coefficients of 0.652 (cf. actual of 0.662) for dry matter, 0.854 (cf. actual of 0.832) for protein, 0.889 (cf. actual of 0.906) for lipid and 0.744 (cf. actual of 0.700) for energy. These findings clearly show that there is a close relationship between the digestibility responses of the two species when fed the same ingredients. This observation is in consistent with findings of other competitive studies between rainbow trout and Atlantic salmon when fed the same diet or between barramundi and rainbow trout.

Cobia and grouper show similar digestibility ability to the ingredients examined. Cobia and grouper showed poorer digestibility to brewery yeast but better digestibility on the fishmeal, soybean meal and the corn gluten meal. This suggested that those ingredients can be incorporated in formulated of both species. These observations were in consistent with findings in grouper juveniles. Another study about apparent digestibility of juvenile (about10g) cobia *Rachycentron canadum*. Apparent dry matter, protein, lipid and energy digestibility for soybean meal ranged 68.3-70.5, 90.9-92.8, 92.4-95.4, 86.9-90.6 respectively.

The ingredient digestibility of parameters examined (dried matter, crude protein, crude lipid and gross energy) were significantly higher when fed to cobia compared to that of grouper. This observation was supporting the findings digestibility of the tested diets in this study. The liner correlation equations, showing strong relationship between diet and ingredient digestibility between cobia and grouper, that could be a useful tool to assess the digestible value of both diets and ingredients. Since this study could only examine the most common feed ingredients, it is necessary to test the digestibility in a wider range of feed ingredient to calibrate the correlations of this study.

The findings of this study confirm that there are strong correlations in both diet and ingredient digestibilities between cobia and grouper. However, the cross-application of diet lipid digestibility need further work. This has important implications in that it supports that the use of data generated from one species will most likely be valid if applied to the other species (though this concept should be validated with each additional species prior to application).

7.10 - Apparent digestibility of common ingredients in Vietnam for feed development for Asian seabass (*Lates calcarifer)*

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This study aimed to characterise a range of ingredients of priority in Vietnam to the feed sector. Additionally, the study also determined the digestible protein and energy (among others) of these resources on the basis that we hypothesised that there would be substantial variability in the digestibility parameters of the different ingredients and that knowledge of this would be able to be used to guide future feed formulation.

High levels of digestible protein were found in all the ingredients in this study. The protein digestibility of Asian seabass for the local fishmeal is similar to that for tuna meal (92%) and only slightly higher when compared with the value obtained for Danish fishmeal (87.9%). The previous studies showed that there was significantly different in utilisation of various kinds of fishmeal in the diets. For example, protein digestibility of juvenile Asian seabass for Thailand fishmeal was 92.8%.

The apparent digestibility of protein in poultry by-product meal in the present study was much higher than that reported in the literature of poultry by-product meal was ranged from 64.4–77.7%. In addition, poultry by-product meal is also an excellent ingredient for Asian seabass in term of high digestibility of dry matter and energy. The AD of dry matter and energy were quitely high (89.7 and 85.5%, respectively).

For plant-based ingredients, extracted soybean meal and whole soybean meal were also options for use in the diets for Asian seabass. Protein of extracted soybean meal was highly digested (93%) but there was low digestibility of dry mater (72%). It is suggested that the high levels of non-starch polysaccharides in the extracted soybean meal reduced the digestibility of dry matter and energy. In contrast, the apparent digestibility of dry matter and lipid for whole soybean meal, in this study, were high when compared among the plant-based ingredients within the present study. These digestibility parameters were found to be 83% for dry matter and 94% for lipid. The AD values for soybean meal were also found to be high for many species including seabass (*Lates calcarifer)*, red drum (*Sciaenops oulatus)*, channel catfish (*Ictalurus punctatus)*. Cassava meal is characterised by having a very low level of protein and a high level of starch. This ingredient had low digestibility of dry matter, consistent with poor digestion of the starch (43%) by this species. That report demonstrated that Asian seabass have a limited ability to digest starch and that there was an effect of inclusion level on the ability of the animal to digest this nutrient. However, this product has potential to be used in fish feed because it is a key ingredient in the formulation to enable the appropriate balance the nutrients in the feed and to manipulate the floating characteristics of the feed.

7.11 - Assessment of the nutritional value of feed ingredients for feed development for Mud crab, *Scylla serrata*

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High digestibility coefficient for all diet digestibility parameters examined was observed in this study, the results were similar other studies. Diets digestibility for dry matter, protein, energy were high, similar with result studies using keys ingredient in diets for mud crab that were same with this current study. For examples, there were high on ADC (apparent digestibility coefficient) for dry matter (ranged from 90.9% to 89.2%), protein (ranged from 95.5% to 94.2%) when using Peruvian fish meal, acetes meal, rice bran, defatted soybean meal into diets for mud crabs also reported that high ADC for dry matter (ranged from 75.8% to 85.7%), protein (from 93.2% to 84.7%), energy (from 83.8% to 92.2%) when using rice bran, cassava meal, defatted soybean meal into diets for mud crab *S. serrata*.

This study examined the digestible protein and energy content of a suite of ingredients of commercial interest to the feed sector and also a suite of ingredients that we will use in future strategic research trials. This finding showed that mud crabs can digest diets which keys ingredient were used (fish meal, soybean meal, rice bran, cassava, acetes meal). These were also considered in other studies. Relatively high ingredient ADC values were obtained when using diets containing soybean meal or fish meal. Similar studies have demonstrated that ADC of soybean meal for dry matter, protein and energy were 95.7%, 97.1%, 97.9% respectively and fish meal were 85.7%, 95.0%, 91.5% respectively if using each ingredient into diet for juvenile mud crab, *S. serrata*. reported that ADC were relatively high in the soybean meal and fish meal incorporate into diets formulated for same species with values for dry matter was 80.4% and 85.4%; protein was 91.7% and 88.3%; energy was 89.1% and 87.8%. These were also similar results with the findings of current study.

The ADC of cassava meal for dry matter, protein, energy were very poor and significant poorer than other ingredients. Similar findings have reported that relatively poor digestibility of cassava meal in diets formulated for mud crab *Scylla paramamosain* with values 54.8% (dry matter), 70.3% (protein), 67.8% (energy), though all these values were higher than our study result, but it also showed that mud crab was not digest protein and energy from cassava meal as much as the other key ingredients. More further, in this study, the ADC of rice bran was lower than the finding of others who observed that ADC for dry matter, protein, energy was 77.3%, 93.8%, 90.2%. The reason may be the differently initial trial sizes of mud crabs. Acetes meal is a key ingredient had been used into formulated diet for mud crabs which generally showed high ADC and values of protein and energy ADC were 79.5% and 88.9% in the study. High diet ADC (94.9% for protein ADC, 88.3% for dry matter ADC) were also found by others, where *acetes* sp. had been used into experiment diet for mud crab. Alternatively, shrimp meal can be used to replace acetes meal in mud crab formulated feeds as it is also highly digestible by juvenile mud crab *Scylla serrata*, 91.9% for protein and 84.4% for energy. This study has demonstrated that mud crab can digest protein and energy from a wide range of feed ingredients. These data will be useful in enabling the formulation of a range of nutritionally-efficient, cost-effective feeds for this species.

7.12 - Determinig the growth performance of two different feed types in Asian seabass, *Lates calcarifer*

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Objective: The objective of this study is to compare growth performance, feed conversion ratio and survival in Asian seabass fed different feed types on earthen pond productivity and the economic returns in staged increment of pond inputs in the Asian seabass culture under practical condition. The results of this trial can be used as a base to assess the quality of ACIAR feed in reality of fish farming.

Experimental design

Two different formulated feeds including AF and UF were used to compare growth performance in seabass juveniles in earthen ponds during 6-month period. The AF (ACIAR feed) was formulated and produced from research results of ACIAR project, while the UF is a commercial feed for seabass made by Uni President Company that are being popularly used in Southern Viet Nam. There were three replicates of each diet for the study.

Table 7.12.1. Composition of ACIAR feeds

Formula	**ACIAR feed 1**	**ACIAR feed 2**	**ACIAR feed 3**
Fish size	10-300 g/fish	300-500 g/fish	500-1000 g/fish
Pellet size	2, 4 mm	4, 6 mm	6, 8, 10 mm
Nutrients on DM basic:			
Protein	55	50	45
Lipid	18	20	22
Gross energy	22	21	21
Digestible energy	20	19	19
LOA	3	3	3
LNA	1	1	1
EPA	0.5-0.7	0.5-0.7	0.5-0.7
DHA	0.5-0.7	0.5-0.7	0.5-0.7
Total phosphorus	>1.0	>1.0	>1.0
Calcium	>2.0	>2.0	>2.0

Diet preparation

The ACIAR feeds were produced basically on three diet formulas according to three growth periods of fish (10 – 300 g/fish; 300 – 500 g/fish; and 500 – 1000 g/fish). These formulas were different in terms of protein, lipid and gross energy (Table 1). The pellets were also produced in five different sizes with diameter of 2, 4, 6, 8 and 10 mm in order to suit fish mouth sizes. All AF feeds will be made using a feed extruder pilot line of RIA 2 that being located at Bac Lieu experimental station for aquaculture (BLESA). The AF feed will contain moisture content of around 11%.

Pond and fish preparation

Six 500 m^2 earthen ponds were used for this experiment. These ponds were filled with seawater, water depth was remained at least 1.4 m during the experiment. All ponds were aerated by paddle wheels and maintained dissolved oxygen level of above 4.0 ppm. Water was changed regularly to suitably keep water quality for fish to growth in terms of pH from 7.0 – 9.0. salinity from 5 – 35 ppt, and total alkalinity from 100 to 130 ppm. The Asian seabass juveniles with similar sizes of around 10 g/fish were used for the experiment. The fish was bought from seabass hatchery in Vung Tau, and transferred to BLESA.

Feed management
During the experimental period, the fish were manually fed twice daily (7:00 AM and 4:00 PM) to apparent satiation, as determined by the loss of feeding activity. The diet was adjusted following the growth rate of fish culture. All feed is to be kept in the cold room (16-18 ºC) except when feeding or weighing events.

Data analysis

Survival rate was calculated as
Survival rate = $100 \times \frac{\text{Total number of fish harvest}}{\text{Total nuber of initial fish stocking}}$

Weight gain (AWG, g) was determined using the following equation:
AWG (g) = $\text{Final Weight} - \text{Initial Weight}$

Specific growth rate (SGR, (g/fish/day) was determined using the equation:
SGR (g/fish/day) = $\frac{\text{Wf} - \text{Wi}}{\text{T2} - \text{T1}}$

Where*:* W_f and W_i are final and initial weights, respectively; T is the period of measurement in days.
Feed conversion ratio (FCR) was determined using the equation:

FCR = $100 \times \frac{\text{F}}{\text{Final Weight} - \text{Initial Weight}}$
Where: *F* is the weight of feed supplied to fish during the study period.

Table 7.12.2 Number of stocked and harvested fish, initial and final average weight, culture period and total initial and final weight of the Asian seabass fed two extruded feeds

Parameters	**ACIAR feed**			**UP feed**			**Mean**	
	AF1	AF2	AF3	UF1	UF2	UF3	**AF**	**UP**
Number of fish stocking	1,000.0	650.0	650.0	1.000.0	650.0	650.0	**766.7**	**766.7**
Initial average weight (g)	108.5	91.4	74.5	112.4	89.6	74.1	**91.5**	**92.0**
Total initial weight (kg)	108.5	59.4	48.4	112.4	58.2	48.2	**72.1**	**72.9**
Period (days)	223.0	233.0	233.0	223.0	223.0	233.0	**229.7**	**226.3**
Number of harvested fish	661.0	623.0	602.0	712.0	550.0	335.0	**628.7**	**532.3**
Final average weight (g)	1,178.2	1,111.6	1,036.0	1,127.5	1,232.5	1,339.4	**1,108.6**	**1,233.2**
Total final weight (kg)	778.8	692.5	623.7	802.8	677.9	448.7	**698.3**	**643.1**

Table 7.12.3 Survival rate, yield, weight gain, specific growth rate and daily growth rate of Asian seabass fed two extruded feeds

Parameters	**AF feed**			**UP feed**			**Mean**	
	AF1	AF2	AF3	UF1	UF2	UF3	**AF**	**UP**
Survival rate (%)	66.1	95.8	92.6	71.2	84.6	51.5	84.9	69.1
Yield (kg/ha)	15,576.0	13,850.0	12,474.0	16,056.0	13,558.0	8,974.0	13,966.7	12,862.7
Weight gain (g)	1,069.7	1,020.2	961.6	1,015.1	1,142.9	1,265.3	1,017.1	1,141.1
SGR (%)	1.1	1.1	1.1	1.0	1.2	1.2	1.1	1.2
DGR (g/fish/day)	4.8	4.4	4.1	4.6	5.1	5.4	4.4	5.0

Table 7.12.4 Total feed fed, feed conversion ratio, price, total feed cost and feed cost per kg of fish increase for Asian seabass fed two extruded feeds

Parameters	**AF feed**			**UP feed**			**Mean**	
	AF1	AF2	AF3	UF1	UF2	UF3	**AF**	**UP**
Total Feed	1,209.3	1,015.3	974.5	1,217.1	1,080.6	790.7	1,066.4	1,029.5
FCR	1.8	1.6	1.7	1.8	1.7	2.0	1.7	1.8
Feed price (VND)	28,500.0	28,500.0	28,500.0	28,500.0	28,500.0	28,500.0	28,500.0	28,500.0
Total feed cost (VND/pond)	34,465,050	28,936,050	27,773,250	34,687,350	30,797,100	22,534,950	30,391,450	29,339,800
Feed cost (VND/kg of fish)	51,419.9	45,706.1	48,276.4	50,242.4	49,700.0	56,265.2	48,467.5	52,069.2

Table 7.12.5 Variable cost, total cost, fish price, income, profit and total profit of two extruded feeds for Asian seabass

Parameters	AF feed			UP feed			Mean	
	AF1	AF2	AF3	UF1	UF2	UF3	**AF**	**UP**
Variable cost (VND)	22.396.166,7	19.061.166,7	19.061.166,7	22.396.166,7	18.896.166,7	19.061.166,7	**20.172.833,3**	**20.117.833,3**
Total cost (VND)	56.861.216,7	47.997.216,7	46.834.416,7	57.083.516,7	49.693.266,7	41.596.116,7	**50.564.283,3**	**49.457.633,3**
Fish Price (VND/kg)	78.000,0	78.000,0	78.000,0	78.000,0	78.000,0	78.000,0	**78.000,0**	**78.000,0**
Income (VND/0.05ha)	60.746.400,0	54.015.000,0	48.648.600,0	62.618.400,0	52.876.200,0	34.998.600,0	**54.470.000,0**	**50.164.400,0**
Profit (VND/0.05 ha)	3.885.183,3	6.017.783,3	1.814.183,3	5.534.883,3	3.182.933,3	(6.597.516,7)	**3.905.716,7**	**706.766,7**
Profit (VND/ha)	77.703.666,7	120.355.666,7	36.283.666,7	110.697.666,7	63.658.666,7	(131.950.333,3)	**78.114.333,3**	**14.135.333,3**

7.13 - A comparison of the completed diets with different digestible protein and energy densities for juvenile mud crab, *Scylla paramamosain*

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After three moulting periods, crabs fed the diets with lower DP:DE density (MLD) showed lower growth rates compared with crab fed higher DP:DE density (MHD). However, the intermolt period was similar at the two formulated diets. These data suggested that thehigher DP:DE (higher DP and DE) density could increase growth of crab under these experimental conditions. The high crude protein levels in the current study (50.3% and 57.5%) were similar to previous determined optimum dietary protein requirement values.

There is a subtle difference in growth between the current study and othe reported studies. With increasing dietary protein from 45% to 55% no further increase in final weight was observed while there was significant difference in growth rate of MLD and MHD (50.33% and 57.51%). The difference in final weight at the two formulated diets in the current study therefore may be driven by the high DP:DE density ratio in the diet. It was found that the digestible protein specification level for mud crab*, S.serrata*, was 53 % at the digestible energy value of 18 MJ/kg. The high density in DP:DE also promoted growth in carnivorous fish such as Asian seabass and Gilthead seabream.

The high protein diets (MHD) which were similar to trash fish in terms of their capacity to promote growth in a laboratory-based culture system has the potential to replace trash fish in mud crab aquaculture. There was no significant difference in the intermolt period between the two formulated diets containing 47% and 54 % DP. These results are consistent ith other lieterature that reports that crabs fed with diets containing crude protein in the range 32-48 % did not have significantly the intermolt period difference. However, there was a significant decrease of the intermolt period values in crabs fed trash fish.

The FCR value of 1.73-2.08 was obtained with MHD and MLD in the present study were lower than those in mud crab, *S. serrata* studied by others, who found no significant difference in FCR ranging from 3.37 to 4.21 in the mud crab *S. serrata* fed protein levels ranging from 32 % to 48 %. The similar FCR value of 1.7 was reported for juvenile mud crabs, *S. serrata,* when fed them with a diet containing the DP of 46 % at 18 MJ/kg. In conclusion, the completed diet for mud crab, *S. paramamosain,* should be formulated with 540 g kg^{-1}DP diet and 19 MJ/kg to maximize weight gain and minimize feed conversion ratio.

7.14 - A study of the effects of faecal collection method and acclimation time on the digestibility of diets and ingredients when fed to juvenile barramundi (*Lates calcarifer*)

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The key foci of this study were methodological, in that the study sought to define the effects of faecal collection method and also acclimation time to diets, on the digestibility values determined in barramundi. Although studies have been performed comparing the determination of whole diet digestibilities based on faeces collected using either settlement or stripping techniques in salmonids, this is the first study to compare the influence of these faecal collection methods with barramundi. Additionally, the study also examines the variation in digestibility over time to establish what is the best acclimation time to diets prior to faecal collection. Similar such data from other species could not be found.

Faecal collection method influences

There has been much debate on the positives and negatives associated with either faecal collection method used in digestibility studies. However, it is widely acknowledged that the two faecal collection methods do result in different diet digestibility value determinations. These differences imply that there are compositional differences in the faeces collected which immediately have connotations on the use of each faecal collection method. Despite being more laborious and costly to collect, the data produced from faeces collected using the stripping method was more conservative than the data produced from faeces collected using the settlement method. This factor alone means that when provided with the option to use either data set the rational decision is to use the data from the stripping method because of this conservatism.

It was noted previously that the greatest differences between the nutrient digestibility assessments from the two faecal collection methods were those ingredients with higher levels of carbohydrates. A similar result was also observed in the present study with a greater number of significant differences in the digestibility of the Starch diet than either the Basal or MKM diets. It is likely that this is due to high levels of carbohydrates in the faeces decreasing faecal integrity and as such increases the dissolution of the faecal matter collected using settlement techniques.

Temporal variation in digestibility values

One of the key elements of this study was to determine the time period over which the fish should be fed a diet before faecal collection is initiated. Unfortunately there was little literature with which to compare our data in this part of the study. Therefore, in assessing this question the key parameter was considered to be the level of variability (as noted by the magnitude of the standard error) in the data collected and also how the data at any time point compares to that data obtained at the longest acclimation time point. This was based on the assumption that by this time point the fish would have acclimated to the diet. The different digestibility parameters (dry matter, protein, energy) were also subtly different in how they responded over time with respect to the variability and also how they fared compared to the digestibility values from day 12 of the study. Fish fed the MKM diet took the longest to acclimate to it and there was a higher level of data variance within the dry matter digestibilities determined from that diet even up to day 10. However, the protein and energy digestibility parameters for that diet showed little variance and were relatively consistent from day four onwards.

An important observation in this study though is the level of variability seen of the data from the Basal diet. As indicated in the methods, the fish were fed this diet for one week before any faecal collection commenced, yet on day one of faecal collection a decline in dry matter digestibility was observed relative to the longer-term mean. In fact, throughout the two week study period there was an inconsistency in the digestibility values determined for dry matter from this diet (and the other two) which perhaps indicates that some variation in digestibility might be a natural feature independent of acclimation time.

Conclusions

The two faecal collection methods used in this study are the two main methods used by fish nutritionists worldwide and this study provides a good estimate of how well each method compares when used with barramundi. The faecal stripping collection method is the more conservative of the two assessments used in this study and therefore is the one we recommend for use with this species.

When assessing the variability in digestibility over time, it was observed that in the first three days after a new diet is introduced, that the digestibility data obtained using the faecal settlement methods, was particularly variable. After this time this variability diminished and values became more uniform. We therefore recommend at least four days acclimation to new diets for barramundi before any faeces are collected for digestibility studies.

7.15 - A study of the discrete and interactive effects of different polysaccharides on the digestibility of diets fed to barramundi, *Lates calcarifer*

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It has been demonstrated that different polysaccharide types cause varying effects on nutrient and energy digestibilities when included in fish diets. It is now understood that the presence of these complex carbohydrates has a significant bearing on the variability in many of the nutritional responses by fish to diets. While the discrete effects of different polysaccharides have been studied, little information is available on the potential interactive effects among the different polysaccharides classes. As raw materials contain different polysaccharide types and are typically added in a combination rather than added in isolation to aquaculture feeds, it is important to consider the implications of the addition of several different types of these polysaccharides to the nutritional value of feeds. It has been suggested that it is the interactive effect of these different polysaccharides that contributes to the variable effects seen in the digestibility values of many raw materials.

Polysaccharide class discrete effects

Each of the dietary non-starch-polysaccharide classes had a clear effect on the digestibility of dry matter content of diets fed to barramundi. This is a clear contrast to the effect seen with the pregelatinsed wheat starch, which had little effect on dry matter digestibilities. This difference clearly shows that although this species of fish can digest pregelatinised starch that it has almost no ability to digest any of the non-starch-polysaccharide (NSP) classes tested in this study.
With the majority NSP classes the decline in dry matter digestibility is directly related to the level of inclusion on of each NSP sample. This effect was seen directly with cellulose and to a lesser degree with lignin. These observations are similar to those observed earlier where a significant decline in organic matter digestibility of diets with different levels of cellulose inclusion when fed to rainbow trout. Similar such effects have been reported by others, who also report a significant decline in organic matter digestibility of diets with high cellulose inclusion when fed to rainbow trout. The same effects with cellulose on dry matter and energy digestibilities and also the insoluble NSP content of lupin kernel meals. These observations combined with the present one's support that in most cases the NSP are simply acting as non-nutritive filler and have limited greater interactive effect on diet dry matter digestibility.

However, in the present study, the single or blended addition of pectin or lignin resulted in reductions in digestibility at levels which exceed their inclusion level and are indicative of an interactive anti-nutritional factor. Pectin is a soluble fibre, commonly used as a gelling agent in products for human consumption. It has been suggested that NSP's in soluble form are most detrimental to fish, due to their ability to disrupt digestive function by increasing the viscosity of intestinal contents. When pectin is blended with any of the fibre types it is possible this interaction is occurring and leading to reduced diet digestibility. A similar interaction was observed with the cellulose + lignin blend diet.

A multivariate approach assessed over seventy lupin meals with rainbow trout. It was shown in that study that lignin content negatively affected nitrogen digestibility and that in combination lupin protein level and lignin content was the strongest predictor of protein digestibility. This result is was supported by a subsequent study with rainbow trout in which lignin inclusion (as lignosulphate) in particular was observed to have a significant effect on diet protein digestibilities. That study assessed three inclusion levels 25, 50 and 100g/kg and interestingly, the effect on protein digestibility ceased to be linear after the 50 g/kg inclusion level. It was suggested that this could be due to a saturation effect of the lignosulphate on whatever loci it is disrupting in the digestion process. These findings are consistent with observations from other studies on the lignin fibre class, with lignosulphanate also found to have significant effects on diet digestibility even at very low inclusion levels.

The different dietary NSP types had clear effects on the digestibility of the energy content of diets fed to barramundi. The response of diet energy digestibility to inclusion of the different NSP's, eitherincluded singly or blended, is consistent with the fact that these fish are obtaining nominal energetic value from the presence of NSP's in the diet. This is also consistent with recent findings that show a limited energetic value from the inclusion of NSP's in diets for trout. Interestingly, when lignin (100g/kg) was pair combined with any of the fibre classes it resulted in an exacerbated reduction in diet energy digestibility indicative of a negatively synergistic interaction. However, this was not observed in the diet which contained a 50g/kg inclusion of each of the fibre classes, suggesting that providing the inclusion of lignin is $\leq$ 50g/kg it is unlikely to significantly disrupt energy digestibility in barramundi.

Conclusions

This study clearly demonstrates that there are both discrete and interactive effects of the different polysaccharides on the nutrient and energy digestibilities of diets fed to barramundi. While theis directly manipulative approach of the present study provides some clear indications on which NSP from different feed grains might affect their own digestibility, it would be useful to follow up this work with an assessment of a broad suite of feed grains and cross reference these observations using a multivariate analysis approach, similar to that done with lupin meals, but instead the proposed study should examine a cross section of the different feed grains available. Being able to corroborate the results from the present study with that from a multivariate analysis of actual feed grains will help consolidate the hypothesis that it is the NSP complexity in these raw materials that is the a key cause of nutritional value variability. Secondly further research to identify why both pectin and lignin have the effects that the do would be useful to understand the mode of action of these NSP on the digestion of diets by fish.

7.16 - An assessment of cereal grains and other starch sources in diets for barramundi (*Lates calcarifer*) – Implications for nutritional and functional qualities of extruded feeds

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There is a large volume of studies examining the digestible value of plant derived raw materials when fed to a variety of fish species. Most of these studies have been based on the assessment of the digestible value of protein and energy from those raw materials that are generally included in diets for their protein value. However, plant derived raw materials also contribute a substantial amount of carbohydrates. These carbohydrates can be loosely classed as starch or non-starch polysaccharides (NSP). One class of raw materials, the cereals, are usually included in formulations for their starch content, not their protein content, for diet processing reasons. Despite this functional need, these raw materials still contribute significant nutritional value. Therefore the present study aimed to examine the variability in the nutritional value of a range of cereal grains and other grains which have a large proportion of starch. As such this study provides a good overview of the effect that different starch polysaccharide compounds have on the digestible value of these raw materials to barramundi and also the technical qualities of extruded diets in which they are included.

Effects of different starch sources on pellet technical qualities

This study showed that there was significant variability in the functional effects achieved on the extruded pellets through the use of different cereal sources. Principally these cereals are included in extruded fish feeds for their starch contribution which acts as the primary binding mechanism to give the pellet is structural integrity.

However there was substantial variability in the structural integrity (compressional strength) observed with the different cereals used. For example, those pellets produced with the inclusion of Faba beans and some varieties of barley had substantially higher compressional strengths than others like triticale and oats, which were quite soft by comparison. In other studies the use of RVA has been effective as a quality assessment tool in predicting some performance parameters of extrusion. In earlier studies, the final viscosity was shown to ne related to compressional strength of pellets. This it is thought because that earlier work and its findings were more related to the NSP content of the ingredients/diets being examined and not the starch properties. However, in the present study no such relationship was observed. The use of RVA was effective however in predicting the pellet expansion and bulk density of products.

There was also substantial variability in the bulk density of the different pellets. It has been shown in earlier work that below a bulk density of 530 g/L pellets will float. Therefore the variability seen among the different cereal types could also be sued as a way of regulating pellet bulk density. For example among the barley varieties the bulk density ranged from 483g g/L to 583 g/L and therefore simple choice of barley variety would have the effect of producing either a floating or sinking pellet. A close relationship was also noted between expansion and bulk density and clearly this can be controlled not only through cereal choice, but also overall inclusion level of starch in the diet.

In addition to the observed functional/physical qualities observed through the addition of different cereal/starch sources to extruded diets it is also well known that extrusion processing improves the energy digestibility and value of feeds for fish by the changes to the chemical and physical structure of the starch molecules within the pellets.

Diet and ingredient digestibility effects

The findings in this study show that there can be significant levels of variability in the nutritive value of different cereals grains as assessed based on the digestible nutrient and energy values. In cereals this variability in nutritive value is largely driven by the variability seen in the digestible value of the starch content of the different grains.

Although the relationship between total diet starch content and diet starch digestibility was not a significant one, a clear trend was evident in that barramundi appear to not have a strong ability to digest high starch levels well. However, studies with other high-trophic order carnivores, like cobia (*Rachycentron canadum*) do show that with increasing starch content there is a decreasing level of starch digestion. Other reports on starch digestibility with barramundi have indicated that gelatinised starch was digested poorly, as was pea starch. It was also reviewed that starch content of the diet had a significant effect on starch digestibility, and those observations are consistent with those seen in the present study.

When examined on a raw material specific basis the relationship between not only total starch content, and more specifically the type of starch within that grain (% of grain) had a highly significant effect on the digestible value of the starch within those raw materials. Notably amylopectin, the branched-chain starch molecule was clearly shown to have a strong negative relationship with starch digestibility in barramundi. It might be expected then that the reciprocal of this, the amylose content, would have a positive effect on starch digestibility, however no significant relationship ($P>0.05$, $R2=0.002$) was observed. It could be reasoned that the variability around the relationships between the different starch classes, the starch digestibility and the diet/ingredient digestibility is probably affected by the different levels and types of the non-starch polysaccharides within each diet/ingredient. As such we suggest that there is some merit in seeking to define the relationships between NSP type and inclusion level, and interactions on not only starch digestibility, but indeed protein, lipid and energy digestibility of diets/ingredients as well. Certainly, other recent studies have demonstrated that some NSP classes have clear effects on protein digestibility within some ingredients. We also suggest that the high level of variance seen in the ingredient protein digestibilities and also that many of those values were calculated as exceeding 100%, is probably further indicative of these interactions. This interaction effect is an important feature to note as it implies that there is a potential loss of additivity in the digestibility assumptions being used in the formulation of diets.

Digesta form effects

Substantial variability was also seen in the consistency in digesta (faeces) obtained from the fish during the stripping process. The inclusion of different carbohydrate types in a fishes diet has also been related to the consistency of the digesta collected. In the present study substantial variability was observed between the different grains. If a category 4 (firm) faecal pellet is considered ideal then corn was the best source of starch in terms of faecal consistency. However if compared to wheat, the industry standard cereal grain used, then there were a range of other cereal grains that were equally as appropriate in terms of the faecal consistency – these included, oats, sorghum and the low starch variety of barley-871.

While digesta consistency may be considered a useful parameter for faecal structural integrity, which is supposedly an issue for recirculation aquaculture systems, it provides little nutritional significant meaning. However, it may provide some indication of bacterial proliferation or diversity issues and potential related gut pathologies. As such, future such studies may consider examining the allochontous bacterial populations to establish if this is such a case with this species.

Conclusions and recommendations

The findings of this study demonstrate that there is substantial variability in the nutritional and technical value of different starch sources when used in feeds for barramundi. These findings show that there is considerable potential for starch sources other than wheat for use in diets for this species. The results also show that there are key functional relationships between amylopectin starch content and the nutritive value of the starch in a grain source included in a diet for barramundi.

While it was hypothesised that it was the variety of carbohydrates present as in the different grains that contributed to the variability in nutritional and physical properties of these grains this study really only details the impacts of starch in this regard. Therefore, the roles of the complex non-starch polysaccharides on the physical and nutritional properties of the diet and by inference the raw materials, remains to be explored.

7.17 - A compendium of raw material digestibilities for Barramundi, *Lates calcarifer*

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This study examined the nutritional value of a series of alternative raw materials to the use of both fishmeal and fish oil in diets for juvenile barramundi (*Lates calcarifer*). The focus of this assessment was the examination of the digestible nutrient and energy value of each of these raw materials so as to provide data suitable for the formulation of diets on a digestible nutrient and energy basis. Additional to this a literature survey was conducted to compile this new data, with other digestibility data available for this species, into a single compendium.

Plant raw material digestibility

A wide range of plant derived raw materials was evaluated in the present digestibility study. Among those examined was a series of soybean products, including solvent extracted soybean meals, soy protein concentrates (SPC) and soy protein isolate (SPI) and several other feed grain varieties. Soy products have generally been favourably used in diets for barramundi without many issues, despite a lack of digestibility data. Of the different feed grain varieties studied in the present work the protein digestibility was generally high at around 90%, though the dry matter and energy digestibilities were typically lower, reflecting the lower concentration of protein found in these raw materials (the exception being the highly processed products of SPC and SPI). Previously examined the digestibility of solvent-extracted soybean meal in diets fed to barramundi and observed protein digestibilities of 85% and 103% respectively. While it is often difficult to compare digestibility values across studies, without some form of reference, it can be seen that the range in protein digestibility values for such a common raw material can be quite expansive (65% to 103%) across all the combined studies. These differences could be due to a range of factors including the soybean genotype, growing environment, processing and not withstanding also the experimental methodologies used.

However, substantial differences were also seen between the two SPC raw materials evaluated in the present studies and although there were subtle differences in the basal diets between the two experiments, otherwise experimental methodologies were kept uniform. Despite these consistencies in methodologies the protein digestibilities of the two SPC raw materials varied from 49% to 95% and the energy digestibilities observed for each raw material were consistent with there being such a substantial difference in protein digestibility between the two. Interesting was the observation of the protein digestibility of SPC-1 in terms of both nitrogen and sum of amino acids, both used as proxies for determining protein, in that they were quite divergent. This observation perhaps suggests that there might have been a significant level of non-protein nitrogen associated with SPC-1 that was not absorbed by the animal. This observation also raises the question as to the more appropriate way to assess protein digestibility, from nitrogen or sum of amino acid data. While in other studies there has been a good regression between these two parameters in the present study this divergence casts some doubt on the validity of either method. Logic suggests that the use of sum of amino acids provides a more valid assessment as it is less likely that there are non-protein amino acids in the raw materials than non-protein nitrogen sources.

There were also no other published reports on the digestible value of SPCs when fed to barramundi, but other data on protein concentrates from lupins were found. Each of the lupin protein concentrates studied also had high protein and energy digestibilities. However protein concentrates from other grain species such as canola/rapeseed, field peas or faba beans remains to be explored. Certainly studies on understanding the influence of different carbohydrate classes and non-starch polysaccharides on the digestibility of diets by this species provides a clear mechanism for understanding why some substantial differences are observed among some of the plant protein raw materials.

Among the other plant protein raw materials examined in the present study a consistently high level of protein digestibility (90% to 95%) was observed. The exception to this was the Camelina meal which produced both protein and energy digestibility values of 36%. There was no other data in the literature on digestibility values of Camelina meal when fed to barramundi in which to compare, however some data was found on growth responses from work with Atlantic salmon. That work found little impact from the inclusion of 100 g kg^{-1} of camelina meal, although the diet did contain high fishmeal levels (~318 g kg^{-1}). There were however several studies on the digestibility of lupin and canola meals. The literature values found for lupin kernel meal protein digestibilities varied among the different lupins species evaluated, but ranged from 81% to 109%. For the *Lupinus angustifolius* species evaluated in the present study this range was substantially smaller (86% to 98%). Notably in some studies where the same sample of lupin kernel meal was used across studies a highly conserved range of protein digestibility values were observed. Values of 96% to 97% were seen for the *L. angustifolius* cv. Myallie variety and 86% to 90% were seen for the *L. angustifolius* cv. Coromup variety. This observations suggests that the between study variation is perhaps smaller than the between variety variation.

Animal raw material digestibility

A range of animal derived raw materials were also evaluated in the present digestibility study. Among those examined was a series of blood meal products and poultry offal meals. Each of the three blood meals examined had protein digestibilities that were observed to be similar to or better than that of the poultry offal meal or fish (tuna by-product) meal. Notably two of the blood meals had protein digestibilities above 100%, with one clearly lower at 83%. Reasons for why this difference existed among the blood meals are not clear as no information was provided from the supplier on the basis of the sample origin variability, other than it is suspected they were from different rendering plants. Clearly to follow this up further a more direct approach to rendering plants needs to be undertaken rather than obtaining samples from a feed producer. Other studies examining rendered mammalian meals with both barramundi and other species have also identified substantial variability in digestible values for these raw materials.

Amino acid digestibilities of the poultry offal meal and tuna offal meal were quite similar, except for one or two amino acids. Those amino acids that were quite different between these two raw materials included cysteine and serine. Overall the sum of amino acid digestibilities were also quite different at 77% and 87% and these contrasted those of the nitrogen digestibilities 87% and 71% respectively for the same two samples. As with the plant protein raw materials there is a range of reasons why this difference may exist, however this cannot be reasonably explored based on the assessment of two raw material samples and clearly further work on this issue is warranted.

Lipid raw material digestibility

Each of the three lipid raw materials examined in the present study had lipid and/or energy digestibilities that were observed to be similar amongst each other with no clear better or inferior product. Although there have been a few studies examining lipid raw materials in barramundi, no data was found determining the digestibility of any lipid resources in this species. It would be useful to not only follow this work up with assessment of additional lipid raw materials, but also to assess the discrete digestibilities of individual fatty acids from within the different lipid raw materials.

Conclusions

The findings from this compendium provide a useful resource to enable nutritionists to formulate diets for barramundi on a digestible nutrient and energy basis. To further reduce feed risk, additional raw materials need evaluation and dissemination of this data remains one of the highest priorities to provide enhanced flexibility for formulation options for use in barramundi feeds (Glencross et al., 2007). In addition to assessing the digestibility of additional raw materials, it was clear from this study that there is considerable variability in the nutritional value of raw materials, not only between types, but even within types. Therefore to follow from this work further effort needs to be spent on defining those factors that affect the nutritional value within classes of raw materials. This can most notably be achieved by defining their digestible value relative to their chemical composition.

7.18 - Using Near Infrared Reflectance Spectroscopy (NIRS) to predict the digestible protein and digestible energy values of diets when fed to barramundi, *Lates calcarifer*.

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Because variability exists in all raw materials, it is important that strategies are devised to manage this variability, and are used to minimise the impacts of this variability when such raw materials are included in compound diets. If not managed there is a risk of diets not achieving their required specifications and then those diets failing to sustain the growth potential of the animals to which they are fed. The extent of these implications was tested in a study examining the use of lupin meals with varying degrees of digestibility and demonstrated that digestible variability within in a single ingredient can have a significant impact on the performance of fish. Traditionally this management of variability was achieved by the bulk pooling of raw materials to obtain a more homogenous representation of each. However, this can be difficult to achieve on a temporal basis and undermines the potential greater value that can be obtained from higher-quality raw materials through their blending with lower quality ones. An option to optimise the utilisation of this inherent variability and capitalise on it has been to evaluate the composition and qualities of batches of raw materials and then adapt formulation practices based on this data. While the use of technologies like NIRS to assist the process have been routine for some time in terms of screening the crude chemical compositional parameters, its use for screening digestible/utilisable parameters of raw materials has been less common.

In addition to management of the inputs to the formulation process, another option in product quality control is the review of compound diet specification criteria following production. In most modern aquaculture fed mills it is now routine practice to use NIRS to evaluate crude composition specifications of products like; moisture, protein and lipid. However, there are no reports on the use of this technology being used to examine the diet digestible nutrient and energy specifications for aquaculture species. While such an approach doesn't allow the production process to be as reactive as in the case of screening raw material inputs, it does provide a quality control for the output products prior to despatch to the users.

Data variance

Over the present series of five independent experiments a substantial range in the diet composition and digestibility parameters were observed. Among the compositional parameters, the most variable was the carbohydrate content which had a coefficient of variation (CV) of 35.8%. This variability was driven by the inclusion of diets in the study which were based largely on only proteinaceous raw materials to those diets which had high inclusion levels of cereal grains and purified non-starch polysaccharides included. The least variable compositional parameter in the study was that of the dry matter which had a CV of only 2.3%. Protein content of the diets ranged from 396 g kg^{-1} DM to 664 g kg^{-1} DM with a CV of 10.7%, which contrasted that of the energy content of the diets which ranged from only 19.9 MJ kg^{-1} DM to 23.1 MJ kg^{-1} DM with a CV of just 3.6%. This lower variability in the gross energy content reflects the similarity in the energy density achieved with the interchange of protein and carbohydrates. However, this interchange had a more substantive impact on the digestibility of the diets.

The most variable digestibility parameter was that of the dry matter diet digestibilities which had a CV of 19.7%. The least variable digestibility parameter was that of lipid digestibility, which had a CV of 5.5%. Diet protein digestibilities ranged from 44.3% to 95.4% and had a CV of 15.0%. Diet energy digestibilities ranged from 45.6% to 85.5% and had a CV of 12.5%. These data are generally consistent with other such data published on diet digestibilities in barramundi.

The digestible nutrient and energy parameters are those derived from a combination of the compositional and digestibility ones, therefore they are likely to compound the variability of each. The variability in each digestible parameter was compounded by variability in both diet composition and diet digestibilities combining to exacerbate the range of values observed in the present study. Diet digestible protein was the more variable of the two parameters a coefficient of variation of 19.5%, with a range in digestible protein levels of 228 to 587 g kg^{-1} on a dry basis. The diet digestible energy levels had a coefficient of variation of 13.4%, with a range in ingredient digestible energy of 9.5 to 18.9 MJ kg^{-1} on a dry basis.

NIRS calibration statistics

Although in theory calibrations could be generated for digestibility values, it was deemed more appropriate to focus the present study on the development of calibrations against the digestible characteristics as these represent a more tangible assessment of the nutritional value of the diets. Calibrations were successfully developed for both the digestible protein and digestible energy parameters in this study. Among the digestible protein and digestible energy calibrations the number of factors used to derive the calibration varied from 10 (digestible protein) to 7 (digestible energy). The calibration R^2 values ranged from 0.864 for digestible protein to 0.852 for digestible energy. The cross validation R^2 values were closely aligned with the calibration R^2 values, albeit typically a little weaker. The standard errors of cross validation (SECV) ranged from 0.8643 for digestible energy to 0.0382 for digestible protein.

The digestible protein and digestible energy calibrations defined within the present paper appear to be quite unique within the scientific literature. Not only are the present calibrations the only such ones found for compound diet digestible value parameters in fish, they also appear to be relatively unique within broader monogastric research. As with other studies recently published in aquaculture, much of the monogastic NIRS calibration work has focussed on the assessment of discrete component raw materials. In contrast to those studies developing calibrations for single raw materials, the present study did not use any cross-experiment reference diet. While it might be argued that lack of such a common reference weakens the capacity to use such datasets across multiple experiments, the present data shows that successful calibrations can still be developed for compound diet digestible nutrient and energy values. It is doubted whether this could be extended to component raw material evaluations, where a greater degree of cross-experiment fidelity is required because of the increased level of error associated with calculating component raw material digestibilities.

Parameters governing the constraints to an acceptable calibration have been the subject of some debate. However, a common agreement is that they should have a regression R^2 value > than 0.8 and an accuracy >1.5 times the value reported for the standard error of the reference method used to determine that parameter, a value referred to as the RPD. Clearly both calibrations in the present study had R^2 values exceeding the suggested regression criteria. Using this assessment the digestible protein calibration had a RPD of 30.4 and the digestible energy a RPD of 0.32. Therefore this would suggest that the digestible protein calibration is acceptable, but that the digestible energy calibration still needs further refinement, despite having a R^2 > 0.80. Importantly, the SECV of the parameters investigated were generally commensurate with the variation in the standard error of each parameter seen across all the diets in this study. Notably the SECV for digestible protein was 0.0382 which was 30 times smaller than the SEM for the same data set. In contrast the SECV for digestible energy was 0.8643, which was three times larger than the SEM for the same data set. As such the RPD values obtained from the present study are at or close to those values considered indicated of robust calibrations for both digestible protein and digestible energy.

Conclusions

The cross validation tests used in this study clearly demonstrate the potential of DA-NIRS to predict the digestible protein or digestible energy values of compound diets when fed to barramundi. Although correlations have been observed between the digestibility values of barramundi and rainbow trout (*Oncorhynchus mykiss*), it would be of value to test the capability of using DA-NIRS to estimate digestible protein and digestible energy for a second species when derived from a calibration such as the present one. An independent study with in vivo and DA-NIRS estimates would enable such a test and should be seen as one priority to follow from the present study.

7.19 - An analysis of the effects of different dietary macro-nutrient energy sources on the growth and energy partitioning by juvenile barramundi, *Lates calcarifer*, reveal a preference for protein derived energy.

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This study used a series of two experiments to examine the effects of the three primary macronutrient sources (protein, lipid and starch) on the bioenergetic value of diets fed to a carnivorous fish. The study initially sought to define the digestible nutrient and energy value of the ingredients to be used so as to enable a more accurate formulation of the experimental diets. Those digestible nutrient and energy specifications where then used to formulate diets where the total digestible energy was kept constant, but the relative proportions of the macronutrient suppling that digestible energy varied. This has enabled an insight into the roles that these macronutrients play in contributing to energy supply in this species.

Effects of digestible energy density on growth and feed utilisation

Classic bioenergetic dogma dictates that fish will eat to an energetic demand to grow to a target weight, subject to being able to consume enough feed to provide that energy and the diets including minimum levels of essential nutrients. A classic test of this hypothesis is reinforced in the present study where two diets of the same ratios of protein:lipid:starch ratios were fed, each with the same DP to DE ratio, but one about 20% lower in DE than the other. In the present study, not only did the fish fed the lower DE diet consume more, but they were also unable to consume enough feed to compensate fully for the lower energy density and therefore also grew less than their counterparts fed the higher DE diet. These results show that aspects of the basic dogma of bioenergetic theory are clearly right. However, this also assumes that the ratio between protein:lipid:starch is kept constant and therefore the roles of each of the macronutrients in energy supply does not vary.

Effects of macronutrient source on growth and feed utilisation

The main focus in the present study was the observation that there were substantial effects of different dietary macronutrients on the growth and feed utilisation by barramundi. Despite being fed diets that were isoenergetic on a digestible basis, it was clear that there was a preference for energy in the order of protein > lipid > starch. This can be seen by the subtle differences in growth and the clearer effects on FCR of the 'Protein', 'Lipid' and 'Starch' diet treatments. It could be argued that this demonstrates that the metabolisable energy value (or more specifically the net energy value) of protein is greater than lipid which is greater than starch. However, the observation that a greater level of lipid deposition but an equivalent level of energy deposition occurs between protein and starch diet fed fish suggest that it is primarily the metabolic 'fate' of these nutrients that differs. Protein, whilst being able to be metabolised for both energy and as a nutrient source, clearly differs from starch which has only energetic value. Furthermore, in a species evolved to derive its energy almost exclusively from protein and lipid, the supply of energy from starch clearly causes metabolic complications.

Many studies on carnivorous fish have demonstrated that the digestible value for starch by these species can be substantial. However, few studies have followed up to examine the metabolisable energy value of this energy source. A range of studies have endeavoured to examine the 'ratios of lipid to starch' in diets for fish though usually this has not been done on a DE basis. The present study demonstrates that, despite the starch content of the diet being highly digestible, that this starch energy it not translated into efficient 'growth' as defined by improved efficiencies of protein deposition. Instead, what occurred was a large increase in the lipid deposition efficiency but only a marginal increase in the energy deposition efficiency. What this indicates is that a large portion of the starch is being converted to lipid, but little of it is directly used to sustain energy needs for protein deposition within the animal. Indeed, the contrast of the 'Starch' diet fed fish to the 'Lipid' diet fed fish show that there are clearly problems with the effective metabolism of starch/glucose in this species. Similar observations have been reported before in other carnivorous fish.

A bias towards supply of energy by lipid did result in an increase in the efficiency of protein deposition, though the relative lipid deposition efficiency declined substantially. This can be easily interpreted by the fact that with the other diets the other macronutrients (which are in greater relative supply) are being actively converted to lipid as energy reserves. In contrast, fish fed the 'Lipid' diet, do not need to synthesise lipids from either starch or protein, as there is adequate supplies provided as dietary lipids. This effect has also been noted in other carnivorous fish.

Results reported with rainbow trout have indicated that the inclusion of starch as an energy source depressed growth and also feed intake. In the present study, in diets balanced for DE intake we also saw a depression in growth from the fish fed the 'Starch' diet, but in contrast an increase in feed intake was observed. Therefore, in contrast to rainbow trout, barramundi in this study attempted to compensate for the differences in the diets, despite the diets having been formulated at equivalent DP and DE levels.

By ensuring that the DP:DE ratio exceeded the established requirements for this species at the size of animal being fed, it can be assured that the responses observed are solely due to energetic constraints and not potential nutrient limitation constraints. In other words, the metabolisable energy value of the different macronutrients is not consistent with their DE basis and that this difference could also explain some of their observations. Indeed, the authors stated that they believe "control of DE intake might be a function of heat production". However, based on our results we observed an improved relationship as we moved the focus from DE Intake against HP ($R^2 = 0.59$) to NEI ($R^2 = 0.63$) of the diets, suggesting that perhaps it is more the NE value of the diet that dictates both performance and feed intake. In addition, observations from the present study also reaffirm the lack of a 'lipostatic' effect, with the relationship between body lipid content and DE intake being very poor ($R^2 = 0.02$).

Conclusions and future directions

The outcomes of this study demonstrate that each of the three key macronutrient classes; protein, lipid and starch, clearly have different net energy values, which means that simplistic digestible energy based models need some reconsideration based on the actual metabolic fate of that energy. To assess the discrete energy values of each macronutrient, and to determine the partial efficiencies of utilisation of each energy source is the obvious next step in this regard. The observation that the fish fed the 'Starch' diet are depositing substantial amounts of lipid could be further confirmed by assessing the fatty acid composition of the fat deposited in the fish, or even from discrete tissues in the animal like the liver, the dominant site of lipid synthesis. The observation that performance can be substantially improved through the increasing of protein content of the diet (notably the 'lipid' diet also had no starch) raises some considerations for improving commercial diet formulations, though putting this into practice in modern extruded feed designs will be a challenge. Further exploration in the use of cereals with high amylose contents relative to amylopectin provides some scope in this regard.

7.20 - Analysis of partial efficiencies of energy utilisation of different macronutrients by barramundi (*Lates calcarifer)* shows that starch restricts protein utilisation in a carnivorous fish

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The present study sought to define the relative contributions of each of the macronutrients suppling digestible energy in diets fed to juvenile barramundi. This has enabled an insight into the roles that these macronutrients play in contributing to energy supply in this species. Understanding this relationship is critical to fish nutrition due to the strong intrinsic link between fish growth, energy demand and diet energy density.

Effect of macronutrient energy bias on growth, feed utilisation and body composition

Clear effects of each of the diets, with equivalent levels of digestible energy but differences in the proportions of protein, lipid or starch providing that energy, were seen in this experiment. For each of these treatments, the strategy of allowing feed intake to vary each day according to feed demand and restrictively pair-feeding each diet has allowed us to build substantially on earlier findings from these same diets fed over a much longer-term basis. We therefore only discuss here the effects within ration levels to focus on the diet specific effects. At the highest ration level, the responses of growth were only partially consistent with an earlier similar study. In that earlier study the best growth was seen with Diet P, where as in the present study the best growth was seen with Diet L. However in both studies the poorest growth was seen with Diet S. At the lower ration levels (M and L) the growth is not consistent with this pattern seen at the H ration level. At the lower ration levels the best growth is seen from Diet P, followed by Diet L and fish fed Diet S still performed the poorest. These results are directly comparable to those from our earlier study and suggest that at the highest ration level, which was fed to apparent satiety, that feed intake variability may have altered the responses. In another similar study with rainbow trout fed either high or low protein diets with energy biased towards either starch or lipid, the fish down regulated their feed intake when fed the starch biased diets. This observation was a direct contrast to the present study where barramundi increased their satietal intakes of the starch biased diets. Differing again were the observations of the omnivorous species tilapia that growth was not compromised with the use of starch as an energy source relative to that growth seen when lipid was used instead. We suggest that these differences are directly linked to the ability of tilapia to digest and utilise glucose from starch, whereas starch digestion by barramundi is comparatively poorer and its ability to regulate blood glucose questionable. Clearly there appears to be different nutritional capacity among different fish species to respond to a utilise starch as an energy source.

In the present study, specific effects of both diet and ration on feed efficiency (FCR) were also observed. The responses to ration within each diet are consistent with observations of most studies on restricting nutrient/energy supply to fish, and the present findings are consistent in this regard with other findings from this species. An advantage of using this pair-feeding regime is that it allows for a very clear examination of the effect of the diet composition on performance criteria independent from feed intake variability. As such some of the clearest implications from the variation in energy supply by different macronutrients can be seen by the cross-diet comparison of FCR at each of the two lower ration levels. A key observation from this is the superior performance of Diet P, a result which is consistent with earlier findings. Similarly, the poorer performance by Diet S is also consistent with these earlier findings.

Effects of each of the diets on fish body composition were noted primarily in terms of the whole body lipid, dry matter and protein concentrations among each dietary treatment. One of the most notable compositional effects at the highest ration level (H) was the difference in lipid concentrations of those fish fed Diet L relative to the other treatments, and that Diet P had the lowest lipid concentrations. Within this ration there was also an effect of diet on protein concentration, with Diet S being significantly lower than the other diets, but no differences among the remaining treatments. These observations from the present study contrast those from an earlier study using these same diets, in that the lipid concentration in the fish fed Diet S are considerably lower and those of Diet L are higher. At lower ration levels in the present study this effect of the diets with considerable starch content (Diet C and S) on the lipid concentration in the body is more consistent with our earlier work. Reasons for this discrepancy at the satiety (H) ration level is unclear. These present results (from the H ration) are however consistent with those noted from higher levels of lipid in the whole body of fish (Tilapia) fed diets high in lipid, but less so in fish fed diets high in starch.

Effects of macronutrient bias on energy utilisation

The efficiency of energy utilization (i.e. the ratio of gross energy gain as a function of digestible energy intake over a range of intake levels, expressed as k_E) differed among each of the treatments. In this study, the relationship between energy intake and gain was observed to be linear, with a calculated energy utilisation constant value that varied between $k_E = 0.507$ and $k_E = 0.730$, subject to diet. For Diet C (the most analogous to a commercial diet) the $k_E = 0.607$, which is generally consistent with other k_E values that have been determined for this species. In earlier work, a range in the values of k_E of 0.61 to 0.76, with an average of 0.68 was determined and shown to be marginally affected by fish size. In subsequent work the k_E values were also shown to be influenced by temperature, with k_E values ranging from 0.42 to 0.59 and being lower outside optimal thermal regimes. Among these comparison studies the diets used to determine these k_E values were generally analogous to Diet C in the present study.

In the present study however, a range of k_E values was observed and clearly related to the variation in macronutrients used to supply equivalent levels of digestible energy in each of the diets. Those diets higher in starch had the poorer k_E values, with Diet C (135 g/kg starch) $k_E = 0.607$ and Diet S (225 g/kg starch) $k_E = 0.507$, compared to Diet P (17 g/kg starch) $k_E = 0.715$ and Diet L (29 g/kg starch) $k_E = 0.730$. From these observations a clear negative relationship between the k_E values and diet starch concentration is seen. The differences in energy utilization among each of the diets allows for attribution of those differences to the vagaries in diet composition among the treatments. The impact of this will be discussed later. A key difference between these studies was that in the present one we can isolate this effect from differences in digestible energy concentration of the diets, and clearly ascribe the effects solely to macronutrient supply differences. Some significant differences in maintenance energy demands (HEm) were also observed among the different diets. For those diets largely devoid of starch the HEm was estimated to be 36.8 to 40.8 kJ/kg$^{0.8}$/d, where as those diets with starch had HEm values estimated at 14.3 to 17.4 kJ/kg$^{0.8}$/d. However, an important constraint is that these are estimated values derived from extension of the linear regression functions to their intercept of the X-axis, and given that there were no ration levels below the HEm values these estimations are beyond the bounds of the data. It makes little sense that the diets influence this value if the nature of the HEm value represents that energy intake where no gain, nor any loss is observed. As such we suggest that this is an artefact of the extrapolation of data set.

Effects of macronutrient bias on protein and lipid utilisation

The protein utilisation efficiency was determined as the amount of dietary digestible protein (g /kg$^{0.7}$/d) required to deposit a gram of protein in the body of the animal. In the present study values (k_P) determined in the present study ranged from k_P = 0.412 to 0.580. The values also compare well to other carnivorous marine species like the European seabass (*Dicentrarchus labrax*) for which a value of k_P = 0.52 was reported.

In the present study, however, a focus was made on the energy retention as protein energy retention. This was estimated based on its energy equivalent, in this case 23.6 kJ/g protein, and expressed relative to the metabolic body weight (W$^{0.8}$) of the animal rather than its protein body weight (W$^{0.7}$). The calculated energy cost as DE (kJ) for deposition of protein from each diet varied and was shown to be significantly higher as a result from the inclusion of starch in the diet. The energy cost values ($1/k_{PE}$) determined in the present study for protein deposition ranged from = 1.72 to 2.43 kJ per kJ of protein energy deposited, with the higher cost values of 1.87 to 2.43 being from those diets high in starch. This further supports that protein synthesis in the presence of higher dietary starch levels is more energetically expensive. In comparison to other marine fish species (e.g. *Sparus aurata, Dicentrarchus labrax* and *Epinephelus aeneus*) which had $1/k_{PE}$ values ranging 1.79 to 1.90 and in carp (*Cyprius carpio*) a $1/k_{PE}$ was estimated at 1.78 (8, 33).

The lipid utilisation efficiency (data not shown) was determined as the amount of digestible dietary lipid (g /kg$^{0.9}$/d) required to deposit a gram of lipid in the body of the animal. In the present study the lipid utilisation efficiency values (k_L) determined ranged from k_L = 1.07 to 1.55. These values were closer to one for those diets with higher lipid levels (Diets C and L) and greater than 1.3 for Diets P and S. No other comparative values of k_L for barramundi were found in the literature. The utilisation of dietary lipid energy for lipid energy deposition to determine the partial efficiencies of lipid energy utilisation (k_{LE}) was also examined. What appeared unusual about these values is that they were all greater than one. This implied that there was greater lipid energy deposition than lipid energy intake resulting in a net energy gain from this macronutrient and clearly indicating synthetic activity. While for protein this would be impossible, for lipid it simply demonstrates that there is lipid being synthesised from other macronutrient substrates (e.g. starch or protein). In particular, from those diets low in lipid it can be seen that the relative contribution to lipid synthesis from these other macronutrients is enhanced.

The energy cost ($1/k_{LE}$) for lipid gain in the present study ranged from 0.65 to 0.93 kJ per kJ of lipid deposited. This was similar to the range of values (0.83 to 0.86) with rainbow trout (*Oncorhynchus mykiss*), but was substantially lower than that the 1.10, 1.11 and 1.31 reported for three marine species (*Sparus aurata, Dicentrarchus labrax* and *Epinephelus aeneus*). In carp the efficiency was estimated at 1.39, demonstrating that lipid accumulation from lipid energy intake was a highly efficient process in barramundi, like that seen in other carnivorous species. That the energy cost of lipid gain is below one also demonstrates that this is an energetically efficient process in terms of energy storage. In contrast with the values of the energy cost of protein deposition, which showed that the energetic cost of protein deposition was almost twice that of the energetic value of what was being synthesised support the reason why lipid is so much more useful in terms of its storage mechanisms, because it uses less energy for storage than its own energetic value. One particular observation of note was the slight differences in the $1/k_{LE}$ values, with Diet S having the lowest value of $1/k_{LE}$ = 0.65 showing that lipid storage from starch to be very efficient.

Effects of macronutrient bias on component energy utilisation

Because energy retention in fish consists almost exclusively of protein or lipid deposition, the efficiency of energy gain in terms of protein and lipid gain can be considered separately using multiple regression analysis. The comparison of the four diets in this study showed that the inclusion of starch in the diet had a significant effect on the gain of either protein or lipid relative to digestible energy intake, and a clear reduction of protein synthesis with the inclusion of this macronutrient in the diets.

When examining the components of energy utilisation, we have worked on the premise that it is the sum of the digestible value of protein, lipid and starch, their relative energetic proportions (% of total) in the diet and the discrete component utilisation (θk_{PE}, θk_{LE} or θk_{SE}) of each macronutrient that combines to provide the overall k_E value for any particular diet. Using this premise we observed that the component protein energy utilisation value (θk_{PE}) was significantly impaired with high inclusion levels of dietary starch (Diet S $\theta k_{PE} = 0.412$ cf. Diet L $\theta k_{PE} = 0.582$). With lower levels of digestible starch (e.g. Diet C $\theta k_{PE} = 0.534$; 111 g/kg), although a numerically lower θk_{PE} was observed, it was not significantly reduced relative to those diets with nominal levels of starch (e.g. Diet P $\theta k_{PE} = 0.557$).

The component lipid energy utilisation value (θk_{LE}) was highly variable compared to component energy utilisation values (θk_{PE} or θk_{SE}) the other macronutrients with θk_{LE} values ranging from 0.821 to 1.345. These determined values appear to reflect both the inclusion of dietary starch (e.g. Diet S $\theta k_{LE} = 0.821$ cf. Diet P $\theta k_{LE} = 1.345$), and influences of dietary lipid level on the component lipid energy utilisation (e.g. Diet P $\theta k_{LE} = 1.345$ cf. Diet L $\theta k_{LE} = 1.036$). We suspect that the variability in this component utilisation value reflects the responsive nature of the metabolism of lipids by this animal in response to variable nutrient supply. In effect, what we are observing is an enhanced capacity of the animal to produce lipid from protein energy sources. Although it is less efficient than that from lipid or protein, there is still substantial lipid synthesis from starch energy occurring.

The component starch energy utilisation values (θk_{SE}) determined from using the multiple regression approach were determined to be the same across all diets ($\theta k_{SE} = 0.438$). Energy deposition from this component of energy in the diet was clearly the least efficient of all the macronutrients (although a poorer θk_{PE} was noted for Diet S). We suggest that barramundi has limited metabolic capacity to utilise starch derived energy. While it can produce lipids from glucose precursors, it clearly does so at a less efficient rate than that seen from either protein or lipid directly.

Effects of macronutrient bias on oxygen consumption

Traditional dogma has suggested that the metabolism of dietary protein by fish was energetically "expensive" in so much as an increased level of oxygen consumption was required to metabolise this macronutrient and in contrast dietary lipid placed less demands on oxygen requirements during post-prandial metabolism. In the present study we did not observe this effect. Indeed our results support that provided the digestible energy is kept similar among the diets, then such a macronutrient bias does not generally appear to affect oxygen consumption, with some exception to starch, which may marginally lower the oxygen demand when consumed on an isoenergetic basis. However, we did observe strong relationships between oxygen consumption and net energy intake (NEI; $R = 0.994$, $p = 0.006$), retained energy (RE; $R = 0.989$, $p = 0.011$), heat production (HP; $R = -0.916$, $p = 0.084$) and the heat increment (HiE; $R = 0.911$, $p = 0.089$). Generally heat production and oxygen consumption have been positively linked, however our observations were counter to this in barramundi, with a clear negative relationship observed between the calculated heat production (HP = MEI – RE) and the measured cumulative oxygen consumption over a 24 h post-prandial for each treatment. This does cast some doubt over the suitability of oxygen consumption as a guide to HP for barramundi. It was proposed that heat production in fish might be linked to digestible energy intake (DEI) and as such play a direct role in regulating feed intake. However, there was limited variability in HP by the different treatments and as such it was difficult to clearly link such variables. In the present study, however, HP within the satiety fed replicates ranged from 852 to 1475 kJ. This broader range of HP values from our observations has allowed us to show that there was a significant relationship (R= 0.783, p = 0.021) between HP and DEI in barramundi.

Conclusions

The results from this study show that barramundi have clear metabolic inefficiencies associated with the inclusion of starch in their diet. With the increasing inclusion of this macronutrient in the diet of this species there was a reduction in the partial efficiency of protein (protein energy) utilisation and this contributed to an overall decline in the partial efficiency of energy utilisation. In the absence of starch, protein utilisation was constant and it was unaffected by its concentration in the diet. However, the partial efficiency of lipid utilisation was also affected and appeared to respond to its concentration in the diet. It was also shown that the component energy utilisation efficiencies could be determined from a multiple regression approach involving each of the diets. From this it could be seen that the component utilisation values of protein energy were significantly affected by the presence of high levels of starch, but less so at lower starch inclusion levels. The component utilisation efficiency of lipid energy was variable and appeared to be influenced by the lipid inclusion level in the diet and the presence of starch. Collectively, the findings of this study support the notion that the concentration and type of macronutrient mix in a diet for barramundi has a significant effect on the ability of the fish to use those nutrients for energy. This finding suggests the existence of a metabolic mechanism that influences the ability of fish to utilise discrete nutrients for energy, independent of total energy intake.

7.21 - Hepatic molecular reprogramming that underlies macro-nutrient energy utilisation in juvenile barramundi, *Lates calcarifer*

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In many studies where the effect of manipulating dietary macronutrients on fish has been investigated, the true digestible energy content of the diet is either unknown or not equal across each diet, or the amount of one nutrient (such as protein) becomes limited. This creates significant variability in the response to different dietary formulations, and often precludes the ability to assess the nutritional fate of various macronutrient classes. In past studies where, digestible energy has remained constant, the types of macronutrients that make up that digestible energy were not utilised equally by various fish species, resulting in significant changes in growth performance, feed utilisation and body composition. This clearly indicates that each of the three macronutrient classes: protein; lipid and starch, have different net energy values. In this study, we sought to further define how this might be occurring by measuring the levels of circulating plasma metabolites, the proportion of lipids in the liver, the expression of key hepatic metabolism genes and the activation of growth signalling cascades. This was performed using fish fed one of four iso-energetic diets for 84 days, then sampled 2 hours after a final meal fed to satiation.

Responses to elevated dietary protein

Barramundi fed an elevated proportion of energy supplied in the form of protein showed significantly improved growth performance, FCR and protein energy retention. No differences in plasma glucose or TG were recorded compared with control fish, but there was a significant elevation in plasma FAA as has been observed in several other fish species in response to high protein diets. In barramundi, there was no significant modification in the expression levels of hepatic aminotransferases. In addition, there were no changes in the proportion of lipids in the liver, as might be expected given the dietary lipid levels were similar between the control and protein diets. Higher energy supplied in the form of protein triggered a significant up-regulation of *LcFBPase*, *Lc FAS*, *Lc G6Pase*, *Lc G6PDH* and *Lc PK* expression, and a significant down-regulation of *Lc mPEPCK* expression.

In other fish, the response to elevated protein has been variable, and comparison is difficult due to the confounding effects of feed intake, dietary energy density or nutrient limiting effects in some studies. An increase in hepatic ALAT and FBPase activities has been suggested to be indicative of increased dietary protein catabolism and gluconeogenesis. However, no elevation of plasma free amino acids was recorded, nor did these changes result in improvements in growth or protein retention. In trout, hepatic ASAT or ALAT activities were unchanged by increasing dietary protein, the activities and mRNA levels of gluconeogenic enzymes (G6Pase, FBPase) increased, while GK and PK enzyme activities were reduced. It has been hypothesised that *FBPase* is potentially regulated by amino acids, having shown decreased expression in response to low protein diets. However, *FBPase* expression was neither modified by dietary addition of alanine, asparagine or glutamine, nor was it activated by a pool of amino acids in primary cell culture. Expression of *Lc FBPase* was unaffected after a single meal in trout, as well as in barramundi. We cannot discount the fact that in fish, FBPase may be regulated at both the transcriptional and post-translational levels, as it is in mammals. Despite showing a significant response to elevated dietary protein in this and other studies, at present the role of FBPase in fish nutrient utilisation requires further clarification.

The hormonal (insulin and/or IGF1) regulation of Akt, mTOR, FoxO1 and FoxO4 activation is known to be a major mediator of growth related gene expression in skeletal muscle in mammals, and has also been suggested to exist in rainbow trout. In addition, free amino acid levels have been linked with the nutrient-sensing capacity of the Akt / mTOR signalling cascade in mammals. This has also been demonstrated in trout, but very few studies have been performed in other fish. Surprisingly, barramundi showed no significant differences in any of the signalling pathways that respond to nutritional status or regulate growth. This is despite having shown a strong activation in this signalling pathway after a single feeding event, which clearly indicated these pathways are present and active in this species. In our previous work, mTOR activity and plasma free amino acids peaked 1 hour and 4 hours post-feeding, respectively. Despite elevation of plasma FAAs in the plasma of high protein fed fish, there was no complementary elevation of growth signalling cascades. The trend across diets for mTOR and FoxO1-3 phosphorylation was like the overall trend in growth response of these fish. However, these differences were not significant and there was no correlation between growth data and phosphorylation status of individual fish (data not shown). The fact that fish in this study showed significant growth differences may indicate that small changes to signalling pathways may cause large effects on growth over time. In addition, this study assessed only one site of phosphorylation for each molecule signalling molecules, whereas Akt, mTOR and GSK are known to be phosphorylated in multiple locations, each of which can affect activity of specific downstream pathways. Alternatively, peak activation of various pathways may be occurring at different times in response to different diets, or the effects may be masked by the fact that feed intake was not equal across diets. Feed intake did not entirely account for the differences in growth observed in the fish in this study. These caveats exist for the results of this study irrespective of diet, and are clear areas that need to be addressed in future work.

Overall, results from elevating dietary amino acids suggest that barramundi utilise these amino acids for improved growth, and not metabolised for energy production. This concept is supported by the improved protein retention, yet distinct from results in other fish such as dentex and trout. Intermediates of glucose metabolism may be minimised through increased expression of glycolytic genes (*Lc PK*), gluconeogenic genes (*Lc FBPase*, *Lc G6Pase*) and pentose phosphate pathway genes (*Lc G6PDH*). These data suggest there may be a potential link between the expression of these genes and improved growth performance, FCR and protein energy retention. There are a large number of genes involved in the regulation of amino acid metabolism, most of which have not been covered in this study. From the study, we can clearly establish that expression levels of alanine and aspartate aminotransferase were not significantly modified by dietary energy manipulation. This species shows a clear preference for nutrients supplied as dietary protein, and this protein energy is strongly directed towards growth and not utilised for energy production.

Responses to elevated dietary lipid

When a significant proportion of dietary energy was supplied in the form of lipids, barramundi showed similar growth and FCR to those fed control diets, but a vastly reduced lipid retention efficiency of 40%. In the plasma, TG levels were significantly reduced, in the order of 30% compared with controls. In the liver, there was a significant elevation in the proportion of lcPUFAs (particularly EPA, DPA and DHA) with a concomitant decrease in the proportion of saturated and monounsaturated FAs. This was largely reflective of the lipid proportions in the diet that contained elevated levels of fish oil containing higher amounts of lcPUFAs. At the molecular level, barramundi displayed a pronounced ability to simultaneously down-regulate the expression of lipid metabolism genes (*Lc acyl*; *Lc SCD*; *Lc CPT1a*) and glycolytic genes (*Lc GK*). The expression patterns of different lipogenic genes (*Lc acyl*; *Lc FAS*; *Lc SCD*; *Lc SREBP*) were strongly correlated with each other. This result is supported by our previous studies in the species, where the expression of each of these genes was correlated over time after a single meal. Interestingly, a diet high in lipid caused a significant decrease in the expression of *Lc CPT1a*, a major regulator of ß-oxidation. However, like protein, no changes were recorded in the activation of signalling cascades.

In mammals, the activity SREBP is a known activator of proliferation, downstream of mTOR and S6K1 signalling. This link has also been established in trout, whereby the administration of rapamycin inhibits mTOR phosphorylation and its downstream effectors (S6K1 and S6), followed by decreased SREBP expression that leads to decreased expression and activity of *FAS* and *GK*. Similarly, lines of trout genetically selected for higher muscle fat content showed elevated TOR activity and lipogenic gene expression. In barramundi, our results suggest that the expression of lipid metabolism genes (*Lc acyl*, *Lc FAS*, *Lc SCD*), and potentially the first step of glucolysis (*Lc GK*), may be directly regulated by the expression of *Lc SREBP*, as it is in other fish. However, there was no change to Akt or mTOR phosphorylation in response to elevated dietary lipid energy. Combined, this implies that *Lc SREBP* expression may be regulated by an additional pathway independent of mTOR activity, potentially linked to lipids. The results of this study were unable to establish a clear link between mTOR activity and *SREBP*, although a link between *SREBP* and downstream lipid metabolism genes was evident. As noted earlier, the ability to equalise feed intake as well as assess changes at several time points post feeding may allow a deeper understanding of the gene regulatory networks to be identified. In addition, the effects of mTOR inhibition on *SREBP* and lipid gene expression have not been investigated in this species.

Carnitine palmitoyltransferase 1a (CPT1a) is considered the rate-limiting enzyme of fatty acid catabolism. Expression of *CPT1a* is elevated during starvation and reduced upon refeeding, in mammals and fish. Surprisingly, the expression of *Lc CPT1a* in barramundi followed that of FA synthesis genes, and showed reduced expression in response to high dietary lipids in this study and increased expression 4-12 hours after a meal. There was no correlation with Akt activity , as has been observed in trout. Inhibition of Akt/mTOR signalling may further define the regulatory relationships between insulin signalling and lipid metabolism. In a separate experiment in trout, increasing dietary fat intake for 14 days caused prolonged hyperglycaemia and reduced insulin sensitivity, along with reduced activity of hepatic hexokinase, FAS and G6PDH. The reduction in activity of these enzymes was similar to the decreased *Lc GK*, *Lc FAS* and *Lc SCD* gene expression observed for barramundi in this study. However, no hypoglycaemia was observed in barramundi as it was in trout. This could potentially explained by the fact that the increased G6Pase activity in trout was not matched by an increase in *Lc G6Pase* expression in barramundi, and that endogenous glucose production is not regulated in the same way in these two species.

Overall, results from elevating dietary lipids suggest that barramundi strongly downregulate endogenous lipid production. This result underlies the reduced plasma TG levels and may partially explain the vastly decreased lipid retention. Barramundi showed co-ordinated regulation of lipid synthesis genes, likely controlled by the nuclear receptor SREBP. However, in this study there was no evidence of Akt regulation of SREBP expression, as seen in trout. In addition, barramundi display evidence of co-regulation of genes controlling lipid synthesis and breakdown.

Responses to elevated dietary starch

When a significant proportion of dietary energy was supplied in the form of starch, barramundi showed poorest growth and FCR, and a significantly increased lipid retention efficiency of 182%. No significant changes were evident in the plasma, with no elevation of plasma glucose levels in response to elevated starch intake. However, the liver accumulated a significant proportion of SFAs and MUFAs (particularly 16:0, 18:0 and 18:1n-9) with a concomitant decrease in the proportion of lcPUFAs. The lack of lcPUFAs was largely reflective of the lack of these lipids in the diet, but SFAs and MUFAs were present in significantly greater excess than controls. At 2 hours after feeding their respective diet, barramundi displayed few modifications to gene expression or signalling pathways in response to high starch diets. The expression of *Lc G6Pase*, *Lc mPEPCK*, and *Lc G6PDH* was elevated in the livers of animals fed high starch diets. The only significant response in phosphorylation of signalling pathways was a decreased activation of S6 two hours after feeding a diet high in digestible starch energy. This study represents the first assessment of the underlying molecular mechanisms that respond to feeding elevated dietary levels of carbohydrate in barramundi.

Most carnivorous fish display an elevation of blood glucose levels and prolonged periods of hypoglycaemia in response to feeding starch, that is often associated with increased glycolytic and lipogenic enzyme activity and gene expression, and also a fatty liver [45-47]. In trout, elevated dietary CHO levels of more than 20% have been shown to significantly elevate both plasma glucose and GK gene expression and activity. The response to dietary carbohydrates of the species most closely related to barramundi, European seabass (*Dicentrarchus labrax*), common dentex (*Dentex dentex*) and gilthead sea bream (*Sparus aurata*), was like other fish, although considerable variation exists among species. Sea bream fed 20% pregelatinised or waxy maize starch that showed no hypoglycaemia or elevated glycolysis. Sea bass have shown elevated plasma glucose GK activity and expression were increased when fed 28% gelatinised maize starch. However, gelatinised starch had no effect on GK or G6PDH activities in sea bass, but enhanced PK and FBPase activities. Common dentex fed 28% maltodextrin showed significant elevation of hepatic GK, FBPase and ALAT activities, but no response in plasma glucose or the activities of hepatic G6PDH, PK or ASAT activities. This research suffers from a lack of consistency, whereby different sets of experiments use diets of different energy density, composition, starch source or starch treatment method, fed using various feed regimes and analysis methods that measure different sets of enzymes using different techniques. Absolute activities for individual enzymes, in particular PK and FBPase, vary markedly between studies. Initial molecular studies were hampered by the inability to identify gene homologs and isoforms in some species, as well as techniques with poor sensitivity to detect changes in gene expression. In instances where the physiological responses are shared, an increasing number of studies are identifying that the mechanisms by which these responses are controlled are unique among fish species.

The inability to down regulate gluconeogenesis has been suggested as a cause of the deficiencies in postprandial glucose regulation in fish. However, considerable variability exists in G6Pase regulation between species. The expression of *G6Pase* was down-regulated in sea bream fed 20% CHO. Similarly, the expression of *G6Pase* expression was down-regulated after feeding in both barramundi and sea bass, suggesting the ability to repress glucose production when not required. However, sea bass fed 28% gelatinised maize starch showed no effect on G6Pase activity, and levels of G6Pase mRNA were unaffected in sea bream fed 13-28% gelatinized corn starch. Indeed, *Lc G6Pase* expression was upregulated in barramundi fed high levels of dietary starch, similar to the upregulation of G6Pase activity in sea bass fed glucose. These data suggest that gluconeogenesis is not regulated by dietary CHO level in this fish, and that other mechanisms of regulation may be at play.

Interestingly, simple sugars such as glucose often showed a different result to more complex carbohydrates such as starch. Sea bream fed 20% glucose showed elevated plasma glucose levels as well as increased glycolytic enzyme activity, compared with fish fed 20% pregelatinised or waxy maize starch that showed no hypoglycaemia or elevated glycolysis. A similar disparity in hypoglycaemic response has been observed in barramundi. Elevated plasma levels were observed when injected with 1g/kg glucose or fed 30% glucose. But no complementary increase in plasma glucose occurred when barramundi was fed 30% raw starch or pre-gelled starch, or in this study when fed 20% pregelatinized starch. This is despite the fact that certain forms of carbohydrate are highly digested by barramundi, including greater than 85% digestibility of the pregelatinized starch in this study. The precise mechanisms by which barramundi regulate blood glucose levels and metabolise glucose are currently poorly understood, and the effects of glucose injection on hepatic regulation of glucose metabolism has not been investigated to date.

In this study, the effects of feed intake could not be differentiated from the effects of dietary composition. Variability in feed intake prior to their final meal, and across the entire experiment, may significantly impact the gene expression response. Dietary treatments that cause prolonged or delayed expression of a particular gene may also not be detected between treatments. For example, high protein diets may rapidly trigger expression of some genes, while high starch diets may delay or prolong others. Although timed to coincide with the peak time of gene expression based on previous studies, measurement of gene expression at 2 hours post feeding may not identify all changes that are occurring in response to dietary macronutrient composition. Further studies are under way using a paired fed design and post-feeding time series for all dietary treatments.

The purpose of the present study was not to test the ability of barramundi to tolerate different levels of dietary carbohydrates, but to identify regulatory responses of key nutritional pathways to different energy sources. Results showed that, in response to elevated dietary starch levels, barramundi do not up-regulate genes controlling glycolysis or fatty acid synthesis, but may increase the production of metabolic intermediates through the pentose phosphate pathway. As highlighted by the poor growth performance of fish fed high dietary starch level, the consequences of the metabolic processing of glucose in this way would appear to be quite wasteful. Despite accumulating significant levels of endogenous lipids in the liver, barramundi neither up- or down-regulated lipid production when excess energy was supplied in the form of CHO, even though lipid production was markedly reduced when excess energy was supplied in the form of lipid. These changes also explain the significant accumulation of visceral fat deposits, and the massive increase in lipid retention in these fish. These data demonstrate that barramundi metabolise dietary CHOs predominantly for use in lipid production, which may then be utilised for energy, but not as a direct energy source for growth.

7.22 - Postprandial molecular responses in the liver of the barramundi, *Lates calcarifer*

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Carnivorous fish are recognized for their low efficiency to use digestible carbohydrates and are often characterized as glucose intolerant. However, after being fed a single meal containing appreciable levels of a highly digestible starch source, the barramundi in this study showed the ability to effectively regulate blood glucose levels, since plasma glucose reached it maximum value 2h after refeeding but returned to basal level at the most 2h after that. This result mimicked the peak in plasma glucose levels at 2 hours post-feeding recorded previously in this species when using a diet containing 30% pre-gelatinised starch. However, in the same study, extremely high levels of dietary glucose (up to 30%) or intraperitoneal injection of over 1g kg^{-1} of glucose caused pronounced elevation of plasma glucose levels for at least 8 hours. The findings from the present study demonstrated that there was no persistent post-feeding hyperglycaemia using a diet that is composed of approximately 10% highly digestible starch, indicating that these fish can tolerate dietary carbohydrates at this level. However, the strict classification of barramundi as a carbohydrate tolerant or intolerant species still requires clarification.

In addition, the mechanism by which plasma glucose is regulated and utilised in barramundi appears to be very different to other fish species, despite its carnivorous status. Glucokinase (GK) and pyruvate kinase (PK) are the two first enzymes involved in hepatic glucose utilization, along with the entry of glycolytic pyruvate to the TCA cycle via the pyruvate dehydrogenase complex (PDC). The ability of barramundi to rapidly and simultaneously up-regulate *Lc GK, Lc PK* and down-regulate *Lc G6Pase* and *Lc PDK* may underlie the lack of post-prandial hyperglycaemia in this species. The rapid induction of *Lc GK* and *Lc PK* gene expression during the first 2 hours following the meal is consistent with the ability of barramundi to utilise digestible carbohydrates. Induction of glucokinase after a meal has been reported in many fish species, as well as in response to increased levels of dietary carbohydrates. However, the regulation of *Lc PK* gene expression in barramundi after a meal seems to be closer to that observed in mammalian species, rather than in other fish. In mammals, regulation of PK occurs at both transcriptional and post-transcriptional levels (Assimacopoulos-Jeannet and Jeanrenaud 1990), whereas *PK* gene expression has been shown to be poorly regulated in trout. Pyruvate dehydrogenase kinase (PDK) phosphorylates and inactivates the pyruvate dehydrogenase complex (PDC), a group of enzymes that converts pyruvate to acetyl-CoA as an entry point into the TCA cycle. The expression of *Lc PDK* was lowest 4 hours after a meal, suggesting that there was also an increase in both the activity of the PDC and the entry of glycolytic pyruvate into the TCA cycle. However, the PDC is also utilised during metabolism of gluconeogenic amino acids such as alanine, serine and glycine. With the observed increase in *Lc cALAT* expression, also indicating increased metabolism of gluconeogenic amino acids, it is not possible to distinguish the proportion of PDC activity that is due to glycolysis or amino acid metabolism. In some fish species, such as trout, the lack of post-prandial *G6Pase* repression has also been proposed to be a contributing factor towards the prolonged elevation of plasma glucose levels, although some fish have shown the ability to reduce *G6Pase* expression in response to dietary stimuli. More recently, trout *G6Pase1* expression was shown to be strongly down regulated post-feeding, but not until 4-8 hours after the feeding event. Barramundi in this study showed the ability to down-regulate *Lc G6Pase* expression after a meal, a result consistent with the low requirement for endogenous glucose production while it is available in the diet. However, down regulation of *G6Pase* gene expression does not automatically mean inhibition of gluconeogenesis. A recent study performed in trout indicated a post-prandial inhibition of G6Pase gene expression without any modification of the enzyme activity.

The mammalian target of rapamycin (mTOR) is a central controller of the growth in response to nutrients, growth factors and cellular energy status. Recent studies performed in mammals and fish indicate that TOR signalling pathway controls *de novo* lipogenesis by regulating the expression and processing of sterol regulatory element-binding protein 1c (SREBP1c), a master regulator of sterol- and lipogenic gene transcription. Genes that code the enzymes glucokinase (GK), ATP-citrate lyase (acyl), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) have been thus identified as targets of the transcription factor SREBP-1c. In this study, we observed strong correlations between the activation of the mTOR signaling pathway, *Lc SREBP-1c* expression and glycolysis (*Lc GK* and *Lc PK*), the pentose phosphate pathway (*Lc G6PDH*) and lipogenic gene expression (*Lc acyl*, *Lc FAS* and *Lc SCD*). As such, our data confirm the link between the mTOR pathway and *SREBP-1c* as the master regulator of fatty acid and triglyceride synthesis in barramundi, resulting in increased expression of glycolytic and lipogenic genes. Whereas Akt phosphorylation is mainly controlled by insulin in trout, *in vivo* activation of the mTOR pathway seems to require both insulin and amino acids. We observed a small increase in circulating free amino acids in the plasma that coincided with the activation of the mTOR pathway, but this was not significantly correlated. Although likely to be the same as other species, further studies are required to confirm the role of amino acids in the regulation of the TOR signalling pathway in barramundi.

Expression of genes regulating β-oxidation in barramundi were in stark contrast to the response seen in fish, mammalian and avian species. Fasting has been shown to be a potent activator of β-oxidation in the liver. As such, refeeding has been shown to strongly down regulate CPT1A and the master regulator of hepatic lipid catabolism, PPARα, expression in fish. However, in barramundi both *Lc CPT1A* and *Lc PPAR*α elevated over a prolonged periods after refeeding. These results imply that the lipolytic pathway, along with the glycolytic and lipogenic pathways, are for some reason active at the same time, or that one of these pathways is perhaps constitutively active or poorly regulated. One potential explanation is that certain classes of polyunsaturated fatty acids (PUFAs), particularly n-3 and n-6, are known to act as direct ligands to activate PPARα and hence other fatty acid oxidation genes. In a similar way, supply of dietary n-3 and n-6 PUFAs may also be triggering fatty acid catabolism through PPARα in barramundi, although this requires further confirmation through other genes targets such as acyl Co-A oxidase. What remains clear is that the pathways that modulate fatty acid oxidation in barramundi are nutritionally regulated, and that PPARα and downstream targets of PPARα are involved in fatty acid catabolism in barramundi.

The results of the glycogen gene expression data were also counter intuitive, in that glycogen breakdown appeared to be occurring at times when glucose and energy were abundant, since the expression of the glycogen synthase gene underwent a continuous decrease during the 12 hours following the meal. In general, an increase in liver glycogen has been observed once fish are refed after periods of starvation, or in response to elevated dietary carbohydrates. Barramundi injected with D-(^{14}C)-glucose incorporated ^{14}C into muscle glycogen in response to injection of insulin-like growth factor I, but the proportion incorporated into fats or glycogen was not investigated. In the European seabass, glycogen replenishment after prolonged starvation was immediate and was produced predominantly through increased gluconeogenesis rather than glycolysis. These observations in other species do not explain the apparent down regulation of glycogenesis in barramundi after a meal, even though glycogen is not thought to be a major contributor to the storage of excess energy in fish. At the very least, the data from the present study indicate that in barramundi the expression of genes controlling glycogen turnover is regulated by nutritional status.

Although these types of functional genetic correlations performed in this study do not indicate that these genes are directly co-regulated, they do provide an indication of the trends that may be occurring in the regulation of various nutritional pathways. A higher degree of confidence in biological function could be attributed to some of the gene expression responses if a corresponding change in enzyme activity was also confirmed. These include the cytosolic form of alanine aminotransferase (*Lc cALAT*), which exhibited an extremely strong and rapid post-prandial response in gene expression level with very little response in the expression of the other aminotransferases. Although frequently measured in the literature, a change in ALAT enzymatic activity or gene expression has rarely been reported in any fish in response to either a single meal or dietary manipulation. In one recent example, ALAT activity was depressed in the liver of the common Dentex after prolonged starvation. Primary used as a blood marker to indicate liver damage, ALAT provides a TCA cycle entry point for some of the gluconeogenic amino acids, and is also involved in the glucose alanine cycle, which is an alternative pathway for removing unwanted lactate produced by anaerobic glycolysis. Cytosolic ALAT may play a critical role in nutrient utilisation in barramundi, a relationship that remains poorly characterised.

Most often, the focus of current studies of fish intermediary metabolism is directed towards the modification of gene expression in response to different dietary carbohydrate sources. It is paramount that this type of post-feeding regulation be interpreted in conjunction with knowledge of how different species to modify gene expression of intermediary metabolism after a single meal. Differences in the timing of activation of, or the ability to regulate, specific metabolic pathways may play a central role in the metabolic consequences of dietary carbohydrate inclusion for each of these different fish species.

8. Impacts

Scientific impacts – now and in 5 years

A suite of scientific impacts was delivered from this project. Many of these are delivering impact now, others will realise further industrial impact over the next five to ten years. Notable advances were made in the science underpinning key areas of aquaculture nutrition, including;

Digestibility – methods were validated, and some of the underlying assumptions tested to ensure the highest quality data was obtained (Blyth et al., 2015). Digestibiity models were developed that explained the impact of different classes of non-starch polysaccharides on protein and energy digestion by fish, this will help explain the constraints of using some plant protein resouces in fish feeds (Irvin et al., 2016). This also extended into the development of NIRS calibrations to predict the digestible protein and digestible energy value of whole compound feeds (Glencross et al., 2017c).

Energetics – The project produced data on the estimates of basal protein and energy demands, estimates of maintenance protein and energy demands, estimates of protein and energy utilisation capacities for most of the five key marine species studied (Glencross et al., 2017a). Such energetics parameters are used as key components in nutritional models used to estimate feed demands and optimise feed composition estimates (Tien et al., 2016).

Modelling – The project provided the opportunity to further the science of nutritional modelling by exploring various assumptions underpinning those models (Glencross et al., 2014; Tien et al., 2016). These assumptions where then tested further by extending their assessment to redefine some key energetics parameters used in the models (Glencross et al., 2017a).

Nutrigenomics - The project also furthered the science of nutrigenomics by allowing the development of a suite of tools to assess nutritional function of barramundi using modern genomic technologies (Wade et al., 2014). These techniques were then used to underpin further research in this and other projects nationally (e.g. Phd theses; Dr Michael Salini and Dr David Poppi and MSc theses; Ms Ngo Thi Diu).

Applied data on five species – A comprehensive data set on the nutritional physiology of five marine species (*Lates calcarifer, Epinehpehlus cooidides, Rachycentron canadum, Scylla serata* and *Panulirus ornatus*), including variation in composition (protein, lipid, ash and moisture contents) with size, estimates of key bioenergetic parameters, as well as digestibility data on a comprehensive suite of ingredients for most species (Glencross et al., 2012; Glencross et al., 2017b)

There was also close industry engagement in this project, both in Australia and Vietnam. Industry representatives partipated in some of the research, and made notable contributions to the direction of much of it (Glencross et al., 2012).

Capacity impacts – now and in 5 years

The partners in this project were integrally involved in the development of all research protocols. Through this they gained experience in the design constraints placed on a range of studies from socio-economic survey development and implementation to the design and preparation required for ingredient digestibility studies. The partners were also engaged in the analysis of the socio-economic data and manuscripts for publication and several have already been published with the Vietnamese scientists as coauthors.

The six Regional Aquafeed Forums have allowed the partners in the project to present aspects of their scientific background and refine their scientific presentation standards and capabilities. These forums have continued beyond the ACIAR project are now into their 10^{th} year. As the now pre-eminent forum for this domain in Vietnam they constitute a major capacity and technical knowledge exchange for the region.

An Aquafeed Extrusion Masterclass was held in June 2010 and a second held for June 2011. Through this not only has there been critical capacity development in the project scientific partners, but also the feed production sector in Vietnam as well. This training improved the understanding of both sectors (research and industry) of critical limitations in the feed production process and helped the two sectors better engage with each other. Additionally, an Aquaculture Nutrition Masterclass was held in March 2014.

The project partners were also involved in the development of the animal experimental protocols. Through this they gained experience in the design constraints placed on nutritional trial development and implementation. The partners were also engaged in the analysis of the nutritional trial data and preparation of manuscripts for publication and several have already been published with the Vietnamese scientists as co-author and in some cases as lead author.

A John Allwright Fellowship application was submitted in 2010 for Ngo Thi Diu from RIA-1. The application was successful, and the applicant has now completed her fellowship and graduated with an MSc, including publishing two first-author papers from her work.

Community impacts – now and in 5 years

Community impacts from this project were linked to the Regional Aquafeed Forums and the Aquafeed Extrusion and Aquaculture Nutrition Masterclasses. The socio-economic analysis work has underpinned the indentification key performance indicators and issues that will further underpin the impacts of the remainder of the project work (Petersen et al 2013a; 2013b; 2013c; 2015; 2016).

Economic impacts

Improved technical and scientific aspects of feed production by those companies attending the Regional Aquafeed Forums and the Aquafeed Extrusion and Aquafeed Nutrition Masterclasses has and will continue to lead to economic benefit in Vietnam and Australia. By providing a solid scientific basis from which to develop high-performance feeds the industry has an improved capcity to use a broader range of ingredients and better-defined diet specifications for key species.

Social impacts

A key social impact was the realisation of the key role played by women in many of the aquaculture business activities and decision making. Further work in this area to highlight this observation and publicise it would be worthwhile for many reasons, but will continue to deliver impact now and beyond the next five years.

Environmental impacts

It is anticipated that there will be reduced wastage through improvements in feed manufacturing and nutritional technology adoption by the feed companies attending the Regional Aquafeed Forums and the Aquafeed Extrusion Masterclasses.

Communication and dissemination activities

- Six Regional Aquafeed Forums were held (NhaTrang - August 2009, Hanoi - July 2010, HoChiMinh City - July 2011, NhaTrang - April 2012, VungTau – September 2013; NhaTrang – September 2014). The forums consisted of two days of open symposium involving researchers and industry people within the project / ACIAR / SE Asian region who came together to present results, discuss findings and network. A number of industry personnel attended the second, third, fourth, fifth and sixth forums and there has been robust discussion of the presentations and also a general discussion session that highlighted some of the key issues in this sector in Vietnam. These RAF formum events have continued subsequent to the project and are now in their 10th year.
- A range of conferences were attended and papers presented by staff working on the project, these include:
  - Glencross, B.D., Rutherford, N.E., Jones, J.B. (2009) Assessment of fishmeal replacement options for juvenile barramundi, *Lates calcarifer*. Proceedings of the World Aquaculture Society Conference. Kuala Lumpur, Malaysia, 3rd – 6th November 2009.
  - Smith, D.M., Glencross, B.D. (2010) Harvesting the benefits of grain application in aquafeeds. Aquafeed Horizons, 3rd March 2010, Bangkok, Thailand.
  - Glencross, B.D., Bourne, N., Hawkins, W.,E., Burridge, P., Evans, D.R., Rutherford, N.E., McCafferty, P., Dods, K., Hauler, R., Sipsas, S. (2010) Using Near-InfraRed Spectroscopy (NIRS) to predict the digestible protein and energy value of grain – The lupin case study. In: International Society for Fish Nutrition and Feeding Meeting. 28th – 31st May 2006, Qingdao, China.
  - Glencross, B.D., (2011) Feed management in coastal aquaculture in Southeast Asia – An update on the latest developments. Cage Aquaculture Asia. Kuala Lumpur 16th – 18th November 2011. INVITED SPEAKER.
  - Wade, N., Skiba-Casey, S., Plagnes-Jua, E., Irvin, S., Blyth, D., Glencross, B.D., (2012) Effects of feeding and dietary manipulation on hepatic metabolic regulators in the barramundi, *Lates calcarifer*. In: International Society for Fish Nutrition and Feeding Meeting. 4th – 7st June 2012, Molde, Norway.
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9. Conclusions and recommendations

9.1 Conclusions

The project delivered a suite of outcomes addressing the original seven objectives;

1. *Determine the soci-economic barriers to adoption of manufactured feed*

A series of soci-economic studies were undertaken and published. These studies identified key social and economic constraints to adoption of formulated feeds by the Vietnamese marine aquaculture sector. These observations were used to direct subsequent research foci.

2. *Model the optimal dietary nutrient and energy specifications*

Models defining the optimal protein and energy needs for Asian seabass and Cobia were developed and published. Data underpinning the development of models for Grouper, Lobster and Mud crabs was obtained or attempted. These models remain incomplete (it was never intended to complete them during this project) and need further data to finalise.

3. *Measure the digestibility of diets and specific ingredients*

Methods for measuring the digestibility of diets and ingredients was developed and validated in each of the five test species. In addition to this a series of experiments were undertaken to characteriste the digestibilities of a range of ingredients in both Vietnam and Australia. This included the assembly of a comprehenisive database of ingredient digestibilities being developed and published for Asian seabass.

4. *Benchmark optimal feed specifications and feed management strategies*

Based on the optimised nutrient and energy specifications identified from the models for each species, a series of benchmark diets and feed management strategies were developed and tested in either or both laboratory and/or farm settings. These studies demonstrated the practical value of the refined diet specifications. It also provided a practical situation for applying the outcomes of the digestibility and modelling work.

5. *Improve the capacity for feed manufacturing technology*

Two separate Aquafeed Extrusion Masterclasses were held in Vietnam in Hanoi (north) and CaiBe (south). In thee Masterclasses the Vietnamese project participants helped deliver the courses that were aimed at the industrial sector. Not only did this deliver an advanced level of training to the Vietnamese feed sector, but it also generated improved capacity within Vietnam for knowledge transfer and industrial linkages.

6. *Improve the capacity in Vietnam to undertake industry applicable research*

A key part of this project was to develop the capacity in Vietnam to undertake high quality industrialy relevant research. Through a series of collaborative studies on defining the requirements for protein and energy by five species in Vietnam and additional research to assess ingredient quality the Vietnamese partners developed enhanced skills in nutritional research. Additionally, an annual forum (the Regional Aquafeed Forum) was held to present findings from this work to a scientific and industrial audience in Vietnam.

7. *Explore some of the the mechanistic elements of fish models for barramundi*

The project was also used to explore some frontier elements of nutritional research for barramundi. This included studies on understanding the roles that the three macronutrient classes of protein, lipid and carbohydrates play in energy provision in fish diets. Additionally, the opportunity was also used to develop the capability to undertake nutrigenomics research through the development of a suite of molecular tools to assess nutritional responses and the transcriptomic level.

9.2 Recommendations

Recommendations for post-project activities include:

- Consider maintaining the research collaboration and networking among aquaculture nutrition researchers in Vietnam by:
    - Supporting the Vietnamese scientsts to continue the Regional Aquafeed Forums (RAF). This may include support for international expert participation.
    - Encouraging RAF to facilitate Vietnamese Government/Industry round table interactions to help overcome administrative bottlenecks to the use of aquafeeds, e.g. approval process for new aquafeeds, to assist aquaculture expansion with focus of aquafeed development and policy.
    - Re-visit the Vietnamese research priorities for aquafeed development.
    - Regular repeats of the Extrusion and Nutrition Masterclasses.
- Consider funding a new project to identify those research barriers limiting increased uptake by farmers of new formulated feeds on key marine species identified by Vietnamese government (e.g. barramundi, large size cobia and pompano):
    - Identify and address knowledge gaps in nutritional science.
    - Further improve the nutritional research capacity in Vietnam to construct bioenergetics models to guide aquafeed development.
    - Assist Vietnamese feed companies to improve feed production technology by facilitating improved dialogue between researchers and industry.
    - Exploring better (species and culture method) feed management options, possibly through development of demonstration/involvement in farms and greater focus on extension.
    - Further analysis of the economic feasibility of on-farm use of formulated feeds (e.g. through bio-economic analyses) to capture the opportunity costs, quality-of-life indicies in addition to direct farm performance metrics.

- Consider the support of Vietnamese scientists and University staff to act as extension officers, in key Provinces, to disseminate information on use of formulated feeds to farmers would be well recieved. While some aquaculture sectors (e.g. shrimp and Pangasius) are familiar with using formulated feeds, others like the grouper and Asian seabass sectors are less advanced and could benefit from extension support.

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