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Supporting grouper farming smallholders in Vietnam to improve their SME businesses by engaging with aquafeed companies to produce commercial feeds

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1 Acknowledgments

We would like to acknowledge the contribution of Richard Knuckey from The Company One, Michael Leger from Skretting Vietnam and Marc Campet from ADM/Ocialis to the Expert Working Group. Their contributions all placed invaluable context to the status and future directions of the research presented in this report.

2 Executive summary

Hybrid grouper is the most profitable marine aquaculture sector in Vietnam, while the paternal component of this hybrid, the giant grouper is the basis of a developing aquaculture industry in Australia. Both industries have been limited by a lack of appropriate, manufactured feeds. The hybrid grouper industry has developed with wild-caught small fish as feed, which is of limited supply, variable quality and has been questioned in terms of sustainability. In Australia, formulated feed is used. However, until recently, the available feeds have been largely formulated for barramundi and not appropriate for grouper growth, health, and feed efficiency. There is therefore a need to better understand the nutrient levels required for optimal growth, health and feed efficiency for grouper to provide feeds that allow this industry sector to grow effectively in both geographies.

This research project therefore established some key nutrient requirement levels to remove some of the limitations of the existing feed supply and lay a foundation for further development of grouper feeds. While this was a short-term project, it was developed with a bigger picture in mind and a secondary aim of the project was to establish a pathway to implementation of these feeds. The project has therefore engaged with feed suppliers in Australia and Vietnam and has involved these industry players in longer-term planning for development of these feeds. The project has highlighted that feed supply capacity is not a limitation in Vietnam. Approximately 25 000 tonnes of formulated grouper feed would be required to support the current industry size if all farmers move to formulated feeds. This represents only 1.25% of the total milling capacity in Vietnam. This capacity may not all be suited to marine fish production, as it has largely developed around the pangasius catfish farming industry in the Mekong Delta, however it is clear that the feed industry has the capacity to accommodate increased demand of grouper feeds. The willingness to engage in this relatively small, but growing sector, is also an important factor for commercialization. Our engagement with feed manufacturers in Australia and Vietnam has provided a pathway to commercialization in both countries as well as engagement with commercialization activities in future research activities, such as feed benchmarking.

These research outcomes are critical to optimise grouper health and growth potential, however implementation and farmer appreciation of advancements in feed technology are also needed to facilitate the shift from 'trash fish' to formulated feeds.

We have further built capacity development material including a poster and a sampling video to better prepare for future nutrition experimentation and facilitate education of future researchers in Australia and Vietnam.

3 Background

Grouper farming is the most profitable marine fish aquaculture sector in Vietnam due to a high market price, excellent growth performance and relative tolerance to environmental stressors (Dennis et al 2020; Peterson et al 2015). Hybridisation of giant grouper *Epinephelus lanceolatus* male and the tiger grouper *Epinephelus fuscoguttatus* female has led to improved growth and disease resistance, making it more desirable for farming (Dennis et al 2020; Rimmer and Glamuzina 2019). There are an estimated 2000 smallholders in Vietnam specialising in either the hatchery stage or the growout stage of hybrid grouper farming.

A previous ACIAR project FIS/2012/101 (and a prior SRA FIS/2012/037) developed new technologies for giant grouper broodstock maturation and sperm cryopreservation, and an understanding of prey selection by larvae. Other ACIAR projects on marine finfish and grouper (but not hybrid grouper) aquaculture in SE Asia (FIS/97/073; FIS/2002/068; FIS/2002/077; FIS/2006/141) provided fundamental knowledge on appropriate hatchery technologies and alternative growout diets. Despite these outcomes, the hybrid grouper sector continues to be constrained by an undersupply of locally produced, quality fingerlings and the reliance on a variable supply of 'trash' fish in the absence of an alternative affordable formulated feed. Since these earlier projects were completed the demand for hybrid grouper has increased and addressing these bottlenecks in future ACIAR investment is critical to underpinning the expansion of profitable SMEs in Vietnam's aquaculture sector.

A literature review in relation to state-of-the-art nutritional knowledge for hybrid grouper specifically, and groupers more generically, has identified a major bottleneck in production that manifests enlarged and fatty livers (Nankervis et al 2022). This condition is consistent with results from giant grouper aquaculture in Australia and is generally considered an indication of impaired health and lost growth opportunity through suboptimal energetics. The review yielded several hypotheses on the aetiology of this issue, which are investigated in this research in parallel to defining potentially limiting nutrient requirements.

This SRA is focussed on feed, as it is the major cost (up to 74%) in hybrid grouper aquaculture production (Dennis et al 2020). Sustainability, production cost and profitability will be improved by deriving size-specific nutrient criteria, supporting nutritional requirements at critical fast-growing juvenile stages, while filling key gaps that limit feed efficiency and cost. This SRA will begin to fill some of these gaps and further elucidate the pathway towards formulated feed development for grouper aquaculture.

3.1 Research and/or development strategy and relationship to other ACIAR investments and other donor activities

Research Strategy

The research strategy aims to tackle key nutrition bottlenecks to development of grouper aquaculture. This research will build the foundation of formulation criteria for species-specific formulated feeds for grouper, which is critical for reducing reliance on 'trash' fish which is both more polluting and less abundantly available than formulated feed. The longer-term goal of moving SME's away from 'trash' fish onto a species-specific feed formulation is expected to further impact livelihoods through optimised production and decreased feed cost per unit production, therefore increasing the cost-effectiveness of grouper aquaculture.

The SRA strategy is to significantly improve the growth performance of hybrid grouper fed formulated pellet feeds to create the commercial drivers for adoption by smallholders and the large-scale aquaculture sector. The project engages representatives from the feed industry, research groups in Vietnam and Australia and a grouper hatchery in an Expert Working Group to develop a better understanding of the grouper industry in Vietnam, potential feed supply pathways and mechanisms of adoption of pellet feeds.

In response to COVID-19 related travel restrictions and ACIAR Foundation Assumptions for modified project operations, this SRA was designed with emphasis on nutrition research with giant grouper based in Australia.

Development Strategy

The project aims to develop capacity in research and implementation of nutrition developments in Australia and Vietnam to lay the platform for further effective research activities in these locations. The project pilots information dissemination platforms in poster and video formats to evaluate the best mechanisms of disseminating fundamental concepts in nutrition experiment design. It is envisaged that posters and video material will become ongoing training tools for training staff new to fish nutrition study and sampling.

4 Objectives

Key nutritional research needs are established through foundational work conducted as part of a scoping tour in 2020 and supported through a review of the current state-of-the-art in nutrition knowledge and impediments to implementation of formulated pellet feeds to replace ‘trash’ fish (Nankervis *et al* 2022). This review highlighted knowledge gaps that must be filled in order to fulfill the need for species-specific feed formulation that is critical for farmer confidence in adopting formulated feeds. This shift will facilitate industry growth while reducing dependence on ‘trash’ fish that is attributed to significant environmental pollution. The project thus has objectives in livelihoods and environmental outcomes.

Interview and literature review processes have further revealed that grouper farmers are not averse to implementing formulated feeds, but they prefer that they are species-specific. By providing a sound scientific platform for grouper-specific feeds, we expect farmer confidence to be increased, which will be further reinforced in the long term by higher growth rates and feed efficiency that precipitate higher returns on farming efforts and improved livelihoods. The shelf-life stability of formulated feeds will ensure that feed is available when the fish are ready to eat, further improving production gains. Shifting away from ‘trash’ fish will further improve the environmental footprint of grouper farming, reducing wastage and pollution of uneaten ‘trash’ fish feed.

In order to contribute to these longer-term objectives, the present study aims to establish research pathways to improve formulated feed quality and pathways to the dissemination of nutrition research for grouper in Australia and Vietnam.

To achieve the fastest outcomes and deliver optimal input to improved feed formulation, the present research focuses on identifying nutrient requirements relating to liver health and alterations in fat metabolism for giant grouper. This was identified in the literature review process as a major bottleneck in appropriate species-specific formulated feed development.

Specifically, we aim to fulfill the following objectives:

- **Objective 1: To establish an Expert Working Group on grouper culture and its supply chain in Vietnam**
- **Objective 2: To define critical nutrient requirement values for grouper**
 - Identifying optimal methionine levels and methionine-aurine interactions for giant grouper. These key nutrients are critical to define appropriate species-specific formulation, contributing to feed cost reduction, growth optimisation and pollution minimisation through more appropriate nutrient profile.
 - Identifying the impact of choline, inositol and lecithin on liver fat levels, lipid digestibility and metabolism. These are critical nutrients for fat digestion, absorption and metabolism. Determination and implementation of optimal values are expected to improve feed efficiency, growth and liver function.
 - Determining the impact of dietary starch on the speed of digestion and liver size through glycogen deposition. By determining the trade-off inherent with starch as a technical aid in feed manufacture and fish growth performance, we intent to guide aquafeed companies toward production methodologies that optimise digestive capability and therefore growth and feed efficiency for farmed grouper.
 - Ensure that nutrition research is implementable and informed by the industry in Vietnam and Australia. Capacity building in aquafeed research techniques.

- ***Objective 3: To establish a research pathway to implementation***
- ***Objective 4: To develop a pilot method of remote capacity building***

5 Methodology

1. Vietnamese grouper industry status, bottlenecks and pathways to impact

An Expert Working Group was established in order to elucidate likely bottlenecks to the continued expansion of grouper aquaculture and pathways toward their removal. This group has compiled information on areas of grouper aquaculture and feed mill locations in order to better visualise the supply chain. This group has brought together the status of grouper farming and the key research opportunities to stimulate further development. Data were collected from questionnaire interviews of local authority and reports of Fisheries Departments, the National Agriculture Extension Center, and Department of Agriculture and local provinces.

2. Nutrition research

Research into nutrition input related to liver and intestinal health and lipid metabolism were conducted at JCU. These studies involved formulation and manufacture of feeds that differed in the nutrient in question with minimal change in other nutrients. These feeds were given to triplicate groups of giant grouper, held in 500 L fibreglass tanks, until the control groups reached approximately three times their initial weight. Fish were weighed and length measured at the start and end of the experiments, while at the end, samples of blood plasma and organs for histological and morphometric analysis were also taken.

3. Implementation of research

We have engaged two feed companies within our Expert Working Group, who have been integral in development of research pathways towards improving formulated feeds and commercialisation of research. These companies have been receptive to advice coming from research outcomes, as have feed companies in Australia, one of whom has developed a commercial grouper feed through the course of this project. This network and working alignment has provided the platform for implementation of future research outcomes, as well as engaging on future commercialisation activities such as feed benchmarking.

6 Achievements against activities and outputs/milestones

Objective 1: To establish an Expert Working Group on grouper culture and its supply chain in Vietnam

no.	activity	outputs/ milestones	completion date	comments
1.1	Map existing supply chains and impediments in the aquafeed industry in Vietnam PC	1a. Mandate for working group documented 1b. Report, mapping and visualisation of aquafeed supply chain and bottlenecks	August 2021 January 2022	Complete Complete
1.2	Engage aquafeed companies, smallholders and integrators that have business ambitions with grouper aquaculture and the appetite to engage in research to develop the industry PC/A	Stakeholder commitment documented (letters of support and commitment) for broader hybrid grouper project	June 2023	Letters of support received from TCO, JCU and RIAs. Business engagement with Skretting, Biomar, ADM/Ocialis, Cargil, Tuan Nguyen (academic and grouper farmer)

PC = partner country, A = Australia

Objective 2: To define critical nutrient requirement values for grouper

no.	activity	outputs/ milestones	completion date	comments
2.1	Define the impact of dietary sulphur amino acids on growth, feed conversion, liver and intestinal health. (Australia, with dissemination in Vietnam)	Journal publications Recommendations on key nutrient values disseminated to Expert Working Group and industry stakeholders.	June 2023	One publication in final review and another in preparation. Recommendations have been disseminated to commercial feed companies within Australia and Vietnam
2.2	Define the impact of dietary starch on physical aspects of feed and digestive capacity. (Australia, with dissemination in Vietnam)	Recommendations on key nutrient values disseminated to Expert Working Group and industry stakeholders.	June 2022	Recommendations have been disseminated to commercial feed companies within Australia and Vietnam. Publication in preparation.

2.3	Developing understanding of dietary phospholipids and components thereof and liver health. (Australia, with dissemination in Vietnam)	Impact of physical aspects of aquafeeds on digestive functions defined and disseminated.	June 2022	Recommendations have been disseminated to commercial feed companies within Australia and Vietnam. Publication in preparation.

PC = partner country, A = Australia

Objective 3: To establish a research pathway to implementation

no.	activity	outputs/ milestones	completion date	comments
3.1	Final Workshop directed to stakeholders across the private and public sectors of Vietnam and Australian feed supply	Delivery of research outputs and outcomes in formats suitable for uptake by key stakeholders	Various	All results have been disseminated to feed companies in Australia and Vietnam (Skretting, Cargil, ADM/Ocialis, Biomar) as well as The Company One and all three RIAs. This has occurred in various fora, both in person (Australia) and via several online meetings rather than a single workshop as originally envisaged. Results were further presented at the World Aquaculture 2022 conference.
3.2	The mandate of the Expert Working Group reviewed	Implementation strategy developed with Expert Working Group	March 2023	The Expert Working Group has been integral in developing and refining the ongoing project, which incorporates engagement with farmers to enable them to understand the outcomes of this research.

Objective 4: To develop a pilot method of remote capacity building

no.	activity	outputs/ milestones	completion date	comments
4.1	Develop a video training module for methods in aquatic nutrition research.	Video produced, disseminated to RIA staff and feedback received to optimise further delivery.	June 2023	Poster and video material developed and disseminated to RIAs for comment in June 2023. Some constructive feedback was received and will be useful for further production.

7 Key results and discussion

Expert Working Group establishment and grouper feed supply chain

An Expert Working Group (EWG) has been established and is made up of key stakeholders across the grouper farming industry, government and feed companies. The EWG's role is to map the feed supply chain of the grouper aquaculture industry, identify bottlenecks to the growth of the industry and to support the implementation of research findings.

The EWG has consolidated and analysed existing data from official in-country sources (Fisheries Departments, the National Agriculture Extension Centre, and the Department of Agriculture of respective provinces). The data shows the industry now produces in excess of 10 000 tonnes of grouper, split evenly between the three broad regions; North, Mid and South (Figure 1). Approximately 1 tonne of fish is produced per seacage or pond, and the industry is dominated by smallholders, owning on average 5 ponds or cages per family. This indicates some 2000 smallholders are engaged with grouper farming. This industry is in a period of rapid growth. For example in the central region of Vietnam grouper farming represents 40% of the marine fish farming sector (Khanh Hoa Department of Fisheries), which underwent 325% growth between 2015 and 2020.

The feeds industry is largely located in the south of Vietnam, with a heavy focus on the pangasius farming activity around the Mekong Delta (Figure 1). It is estimated that there is more than 2 million tonnes of feed capacity in Vietnam, however the focus on pangasius feed means that the majority of factories are unlikely to be suitable for grouper feed manufacture. The project has engaged with ADM/Ocialis and Skretting Vietnam, both of which have current grouper feed activities in Vietnam and an interest in developing this further.

Through discussions, the following bottlenecks to industry growth have been elucidated:

- Pellet feed price is considered high (AUD 2-2.40/kg) compared to 'trash fish' (AUD 0.84/kg). Although cost of production is ultimately more important for farmers, the high moisture content of trash fish makes them attractive on a cost per kg basis.
- The Feed Conversion Ratios (FCR) of pellet feed is approximately 2.5 and trash fish is 7.25 (i.e. it takes 2.5 kg of pellet feed to convert to 1kg of grouper growth, compared to 7.5 kg of trash fish required to convert to 1 kg of growth).
- Thus the FCR of current pellet feeds limits their cost-effectiveness to farmers; at \$2.40/kg and FCR of 2.5, cost per unit growth is equal to trash fish at \$0.84 and FCR 7.25.
- Growth therefore appears to be the main limitation currently in the adoption of pellet feeds, limiting the return on investment for a 9-18 month growout time when trash fish are perceived to support superior growth. Optimising growth of grouper fed formulated feeds requires a supply of pellet-weaned fingerlings, clear feeding methods and recommendations, as well as species-specific nutrient-replete formulations (reviewed by Nankervis et al 2022)
- Low volumes of feed limit financial returns for feed companies, making independent R&D investment unattractive. Business is incentivised towards further development of existing income streams, making R&D investment into emerging industries challenging. Funding from ACIAR and other public funding sources is therefore critical to determine species-specific feed characteristics required to facilitate industry growth and transition to formulated feeds.

- Uncertainty of trash fish quality and its consistent availability: Trash fish may be stored for an extended time (2-3 days common) which has, as yet, unknown impact on fish growth, feed efficiency and welfare. Better understanding trash fish quality is required to help inform farmer choices over feed types and their impact over production and economics.
- Health and survival of grouper in growout is still an issue and the link to feed quality is unknown. Potential vitamin degradation and bacterial spoilage of trash fish may interact with health status. Fingerling cost and availability is an ongoing issue. There is a balance between domestic supply and importation. Fingerlings are largely imported January to April when waters are at their coolest, which substantially increases fingerling cost during this period.
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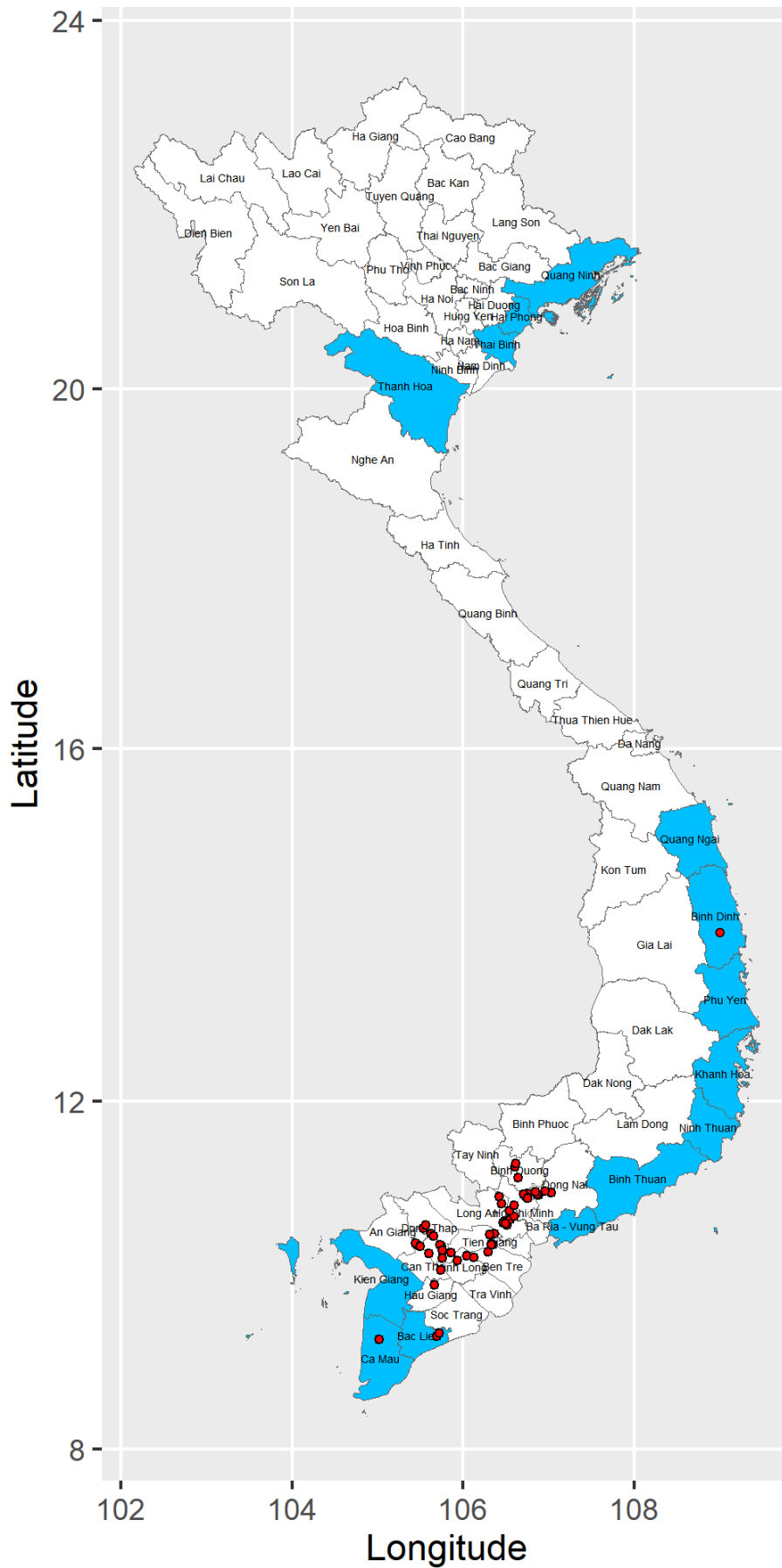


Figure 1. Locations of Vietnamese provinces that have known grouper farms (blue) and aquafeed factory locations (red dots).

Definition of critical nutrient requirement values for grouper

- a. Identifying optimal methionine levels and methionine-aurine interactions for giant grouper. These key nutrients are critical to define appropriate species-specific formulation, contributing to feed cost reduction, growth optimisation and pollution minimisation through more appropriate nutrient profile.
- b. Identifying the impact of choline and lecithin on liver fat levels, lipid digestibility and metabolism. These are critical nutrients for fat digestion, absorption and metabolism. Determination and implementation of optimal values are expected to improve feed efficiency, growth and liver function.
- c. Determining the impact of dietary starch on the speed of digestion and liver size through glycogen deposition. By determining the trade-off inherent with starch as a technical aid in feed manufacture and fish growth performance, we intent to guide aquafeed companies toward production methodologies that optimise digestive capability and therefore growth and feed efficiency for farmed grouper.
- d. Ensure that nutrition research is implementable and informed by the industry in Vietnam and Australia. Capacity building in aquafeed research techniques.

Experiment 1: Methionine and taurine

Methionine is an essential amino acid that is commonly in low supply in feed formulations in relation to requirement values. In addition to its role as a building block of proteins, methionine is metabolically active, with activities related to protein accretion, activation of DNA and lipid metabolism. Methionine is available to be added to feeds as a concentrated powder and knowledge of its requirement values for optimal production is essential for effective formulated feed development. Methionine is also a metabolic precursor for taurine, and there remains significant uncertainty whether taurine is essential for marine fish where dietary methionine supply is sufficient.

To elaborate a species-specific feed formulation for giant grouper, dietary methionine was modulated in a dose-response experiment to achieve five graded levels from 9.5 to 21.5 g/kg, including an additional diet with methionine at 18.6 g/kg supplemented with 8 g/kg taurine. Each diet was randomly allocated to triplicate tanks, each containing 14 fish (83.9 ± 8.4 g).

The best-fit regression for growth showed that the optimal dietary methionine content was 15.8 g/kg. Inadequate dietary methionine content triggered physiological responses, including hepatic hyperplasia and hypoplasia at 9.5 and 21.5 g/kg, respectively, and high aspartate transaminase levels at 18.9 g/kg. Moreover, inadequate dietary methionine contents resulted in higher densities of mixed goblet cell mucin and less absorptive surface area of posterior intestinal villi. Our results suggest that adequate levels of methionine, but not taurine, improved posterior intestinal condition while decreasing relative liver weight by 17%. Commercial feeds available for grouper farming in Australia and Southeast Asia are typically in the range of 9-11 g/kg methionine, which our results suggest as suboptimal, and

at the lower end these formulations may limit growth to 60% of optimum. When the nutrient balance in the feed is suboptimal, fish often have behavioural and physiological mechanisms of adapting. Enlarged livers are a symptom of the fish attempting to cope with methionine deficiency. Given that commercial feeds are routinely at the lower end of methionine levels tested in this study, it is reasonable to suspect that this is at least part of the enlarged liver issue commonly reported for farmed grouper. This study additionally demonstrates for the first time that optimising methionine levels has implications for intestinal health, as well as showing that dietary taurine is dispensable for giant grouper when methionine is optimised.

This research has been published as Candebat, C.L., Eddie, T., Marc, A.F., Fernando, F. and Nankervis, L. 2023. Exploring the physiological plasticity of giant grouper (*Epinephelus lanceolatus*) to dietary sulfur amino acids and taurine to measure dietary requirements and essentiality. *Fish Physiology and Biochemistry*, 1-23. <https://link.springer.com/article/10.1007/s10695-023-01222-4>

Experiment 2: Choline and methionine requirement interactions.

Methionine and choline are two nutrients that share several metabolic functions, both being methyl donors and contributing to lipid metabolism. To elucidate this relationship and provide clear formulation guidance for giant grouper, this study investigated two series of diets, including one that is replete with methionine and varies in choline, and another supplemented to a constant 2.71 mg/g choline and increasing levels of methionine. The purpose of these diets was to establish a requirement for choline and the other to determine whether choline has an influence on the previously established requirement for methionine for *E. lanceolatus*. This experiment utilised two series of diets, one series formulated at the methionine requirement (12.7 mg/g) with increasing levels of choline and the second series with an estimated choline requirement (2.71 mg/g) with increasing levels of methionine. Both methionine and choline supplementation increased feed efficiency, with concurrent shifts in circulating low-density lipoproteins (LDL), and triglycerides (TAG) demonstrating that both of these nutrients contribute to the liver's ability to process lipids and make them available as energy substrates for growing tissues. From this investigation, it is recommended that juvenile *E. lanceolatus* diets are supplemented with both dietary methionine and choline no less than 12.7 mg/g and 4.3 mg/g, respectively. The inclusion of choline may decrease the need for methionine slightly, due to their combined roles as methyl donors. Given that these results demonstrate that choline and methionine levels alter lipid mobilisation, they raise the potential to capture the growth and efficiency effects of higher lipid levels than previously found optimal for this species.

This research is in preparation for publication.

Experiment 3: Lipid and lecithin interactions for giant grouper

Of the macronutrients included in fish feed formulation, lipid inclusion is particularly variable between species. For species that effectively metabolise lipids, they promote growth and improve feed efficiency due to their high energy density. In contrast, research with *E. lanceolatus* to date indicates that neither growth or feed efficiency are improved with lipid levels greater than 10% of the feed. A possible reason for this lack of response to higher lipid inclusion levels is digestion and absorption over the intestine or transport from the liver to the circulatory system, both of which are dependent on phospholipids. This study therefore aimed to investigate the inclusion of lecithin in diets with low and high levels of lipids in diets for juvenile *E. lanceolatus*. Six isonitrogenous and isoenergetic diets were formulated, three diets contained low levels of lipids formulated at 10% lipid inclusion and increasing levels of lecithin at 0%, 0.5%, and 1%, the remaining three diets contained 15% lipid inclusion and the same three levels of lecithin. Increasing lipid from 10% to 15% of the feed resulted in 13.8% increase in weight gain when 1% lecithin was included in the feed, however no positive effect of similar lipid increments was recorded when lower levels of lecithin were included. The higher growth for the high lipid, high lecithin treatment coincided with an elevation of circulating triglycerides, while both lecithin and lipid inclusion also altered circulating cholesterol and glucose levels. These results together suggest that lecithin improves lipid transport as circulating triglycerides, thereby mediating the growth-promoting effects of dietary lipid supply. Increased dietary lipid significantly increased ($p < 0.05$) feed intake and decreased relative liver size. This investigation shows that with increased supplementation of lecithin, there is potential to improve the growth of juvenile *E. lanceolatus*. In light of these new findings, it is pertinent to re-evaluate appropriate lipid inclusion levels for this species.

This research is in preparation for publication.

Experiment 4. Starch inclusion and grinding size influences growth and intestinal morphometrics

Starch is included in aquafeed to provide energy, physical shape, and durability. Starch further facilitates expansion and, therefore, floatation of feeds. High levels of starch are therefore used in floating feeds that are favoured by farmers who use the floating characteristics of feed to aid feed management. Visual assessment of feed on the water surface is often used by farmers in order to understand appetite and limit over-feeding. However the implications of the consequently elevated feed starch levels are not widely studied. The extent to which feed is ground is a further mechanism of manipulating feed

properties, however the implications of the degree of grinding and fish digestive and growth performance are largely unstudied for any species, let alone for grouper. Therefore, a six-week growth trial was conducted to elucidate the effects of dietary starch and feed raw material grinding sizes on growth, morphometrics, and histo-morphometrics of key organs to elucidate growth and physiological aspects of this feed manipulation. Seven diets were formulated to contain low (10%), medium (13%), and high (15.5%) starch levels for both 1 mm and 0.5 mm raw material grinder screens, respectively, while an additional very high starch level (16.5%) was also included for the 0.5 mm screen size. Each dietary treatment was fed to triplicate tanks (500 L), each containing 15 fish. Fish fed the diets made from RM ground to 0.5 mm had a significant reduction in specific growth rate (SGR). Dietary starch elevation above the minimum resulted in intestinal histology changes characteristic of inflammation. Increased dietary starch levels also increased the liver size due to increased glycogen deposition in the hepatocytes. This study suggests that dietary starch levels should be minimised, particularly when grinding raw materials finely. This conflicts with the preferred method of manufacturing floating feeds, and further research should be directed towards mitigating this inflammatory effect of starch inclusion without compromising the technical integrity of pellets or their floating properties.

This research is in preparation for publication.

Establishing a research pathway to implementation

Meetings with the Expert Working Group have established the need for cost effective feeds that provide growth comparable or better than trash fish. The pathway from research to community benefit requires several steps to be in place:

- Integration of research results into formulation practice
- Cost-effective supply of feeds to farmers
- Farmer acceptance and willingness to implement feeds
- Feeding practice that optimises the benefits of feeds for farmers

Our mapping of grouper farming activity and feed mills has established that there is no deficit of feed milling capacity in Vietnam. The project has identified two feed mills in Vietnam that are committed to in-kind support and implementation of project results. We have also engaged with two feed mills in Australia and have disseminated results. The research results are largely able to be implemented directly in existing feed mills, with the potential exception of lecithin, which may not be a standard raw material in all feed mills. Further development in this space is envisaged with a longer-term project that aims to engage further with farmers through developing benchmarking between formulated feed

and trash fish, better-understanding farmer attitudes toward formulated feeds, and development of models to enable cost-benefit analysis. This will enable grouper farmers to better understand their farming system and make informed decisions on their feed inputs. The full project will further develop feed formulation criteria to ensure that the developed feeds provide high growth performance at optimised cost.

Development of a pilot method of remote capacity building

This project developed a poster on external and internal morphometrics and organ identification for grouper, as well as a sampling methods training video. This has been evaluated by RIA staff and has received positive feedback in training potential of these approaches.

8 Impacts

8.1 Scientific impacts – now and in 5 years

This project has aided the understanding of nutrient requirements and interactions of nutrients to support production of grouper and their intestinal and hepatic health. It has laid the platform for optimised feeds, and has disseminated the results to a scientific and industry audience. These results are in the process of being published in international peer-reviewed journals.

Specifically, we now know two mechanisms by which grouper livers can be enlarged through nutritional manipulation. We have further highlighted that optimising methionine and (likely) choline can help alleviate this issue, and that these nutrients work together to optimise feed efficiency. Our results have shed light on the functional responses of intestinal and liver structure to feed manipulations and nutrient required for optimised lipid metabolism and transport.

Scientific impact is felt through immediate impacts of research, but also through highlighting unknowns that will be addressed in the future. Our research has highlighted that lipid levels can be increased for giant grouper feeds through lecithin addition; however we do not yet know the level of dietary lipid that can be added for optimised grouper feed, using updated nutrient requirement criteria. We have also highlighted that dietary starch induces intestinal inflammation in grouper, posing a potential welfare issue which is difficult to avoid while maintaining control over pellet integrity and density. Ideal lipid levels and mitigation of starch-induced inflammation will be important topics for future research that were instigated by this project.

In five years, it is envisaged that this, and subsequent ACIAR investments, will have developed grouper-specific feeds that are demonstrated economic alternatives to trash fish use, presenting a more scalable and sustainable alternative feed input.

8.2 Capacity impacts – now and in 5 years

Student projects: this project supported two Masters minor projects and one Honours research project. The research skills and networks inherent with being involved with a project like this have been key to kickstarting their careers in research, systems design and fisheries management in Australia and internationally.

Educational materials: A pilot video and poster have been developed to facilitate knowledge transfer for nutritional study.

The current SRA was part of the development of a full project with ACIAR, which will contribute to capacity in research and industry over the next 5 years in the following areas:

1. New HDR students; three international PhD students will be associated with the developed project in Australia. Each of these comes from ACIAR partner countries; Vietnam, Indonesia and Bangladesh.
2. Increased research capacity in Vietnam; working with the three RIA groups, we will develop new research outcomes in Vietnam for hatchery methods and nutrition. This will develop capacity in research at all three RIAs.
3. Increased farmer capacity for feed management through development of growth models, workshops and training opportunities.

8.3 Community impacts – now and in 5 years

Community impacts are expected to flow out of this project through facilitating expansion of the grouper industry. Feed development is a current limitation and quality of formulated feed

limits its uptake. The current results indicate large growth improvements compared to current commercial formulation practice. These growth improvements range from 18% compared to a commercial formulated feed and 58% increase compared to the common formulation practice of inadequate methionine content. The most important test for these improvements though is the implementation of the developed feed formulation criteria into feeds that provide economically advantageous outcomes for farmers. This will be achieved through a 3.5 year ACIAR project, which will further develop formulation criteria and demonstrate to farmers the efficacy of these improved feeds on grouper growth and business profitability.

8.3.1 Economic impacts

Economic results stem through improved growth outcomes of farmed grouper, improved feed conversion and improved capacity for grouper farming through decreased reliance on trash fish. The formulation criteria developed here form the basis of formulated feeds that are likely to perform well against trash fish and will be evaluated in the full ACIAR project. The impact of this project will therefore be recorded in more detail as the full project engages with the farming community. The project has engaged with Australian feed producers, one of which has implemented a grouper-specific feed. Anecdotal evidence suggests that this feed is performing well for giant grouper in Australia.

8.3.2 Social impacts

This project will enable expansion of the grouper farming activity in Australia and Vietnam. The continued implementation and improvement of specific feeds for grouper is a critical step in the development of this industry, removing the barrier of limited trash fish supply and facilitating the development of smallholders toward SME scale of production.

8.3.3 Environmental impacts

Continued development of formulated feeds will facilitate transition of farmers from trash fish, which is more polluting and has more chance of disease transfer than formulated feed. Improving the efficiency of production allows more to be produced with less input, allowing increased production with the same environmental and supply-chain footprint.

8.4 Communication and dissemination activities

This project integrated key users of the data, including The Company One hatchery in Australia, Skretting Vietnam and ADM/Ocialis Vietnam. Thus key users were integrated into project activities and received data first-hand through project meetings.

In addition, results were disseminated more broadly through specific presentations made to Skretting Australia, Biomar Australia, ADM and The Company One.

Project results were presented in the World Aquaculture conference in Singapore in 2022.

9 Conclusions and recommendations

9.1 Conclusions

Grouper farming is increasing in Vietnam, placing increased pressure on 'trash fish' supply as a feed source. We have laid down fundamental feed formulation criteria in this research that has been disseminated to feed companies. Feeds for grouper should contain at least 12.7 mg/g methionine and 4.3 mg/g choline for optimal production. Starch should be limited, but more research is required into optimal feeding methods for sinking feeds for grouper. Alternatively, physiological mechanisms for minimising the detrimental effects of starch on intestinal health and growth may be a productive pathway to maintain technical capacity for feed manipulation while not compromising fish production or health.

These research outcomes are critical to optimise grouper health and growth potential, however implementation and farmer appreciation of advancements in feed technology are also needed in order to facilitate the shift from 'trash fish' to formulated feeds.

9.2 Recommendations

- Further research should cover updated lipid requirements for grouper in light of the current research results.
- Further research on mitigating intestinal inflammation in grouper that have been given feeds containing different dietary starch levels is needed.
- Farmer attitudes toward formulated feeds will be important to understand and target with communications material in future projects.
- Implementation of formulated feeds likely requires tools that allow farmers to understand their business cost-benefit from decisions they make regarding feed choice.

10 References

10.1 References cited in report

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Nankervis, L., Cobcroft, J. M., Nguyen, N. V., & Rimmer, M. A. (2022). Advances in practical feed formulation and adoption for hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂) aquaculture. *Reviews in Aquaculture*, 14(1), 288–307. <https://doi.org/10.1111/RAQ.12598>

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10.2 List of publications produced by project

Butler, G. 2022. Dietary methionine, choline, and lecithin supplementation improving lipid metabolism in giant grouper (*Epinephelus lanceolatus*) BSc Honours Thesis, James Cook University, Townsville, Australia

Butler, G., Candebat, C.L., Nankervis, L. Improving the utilisation of lipids through lecithin inclusion in diets for giant grouper (*Epinephelus lanceolatus*) In prep

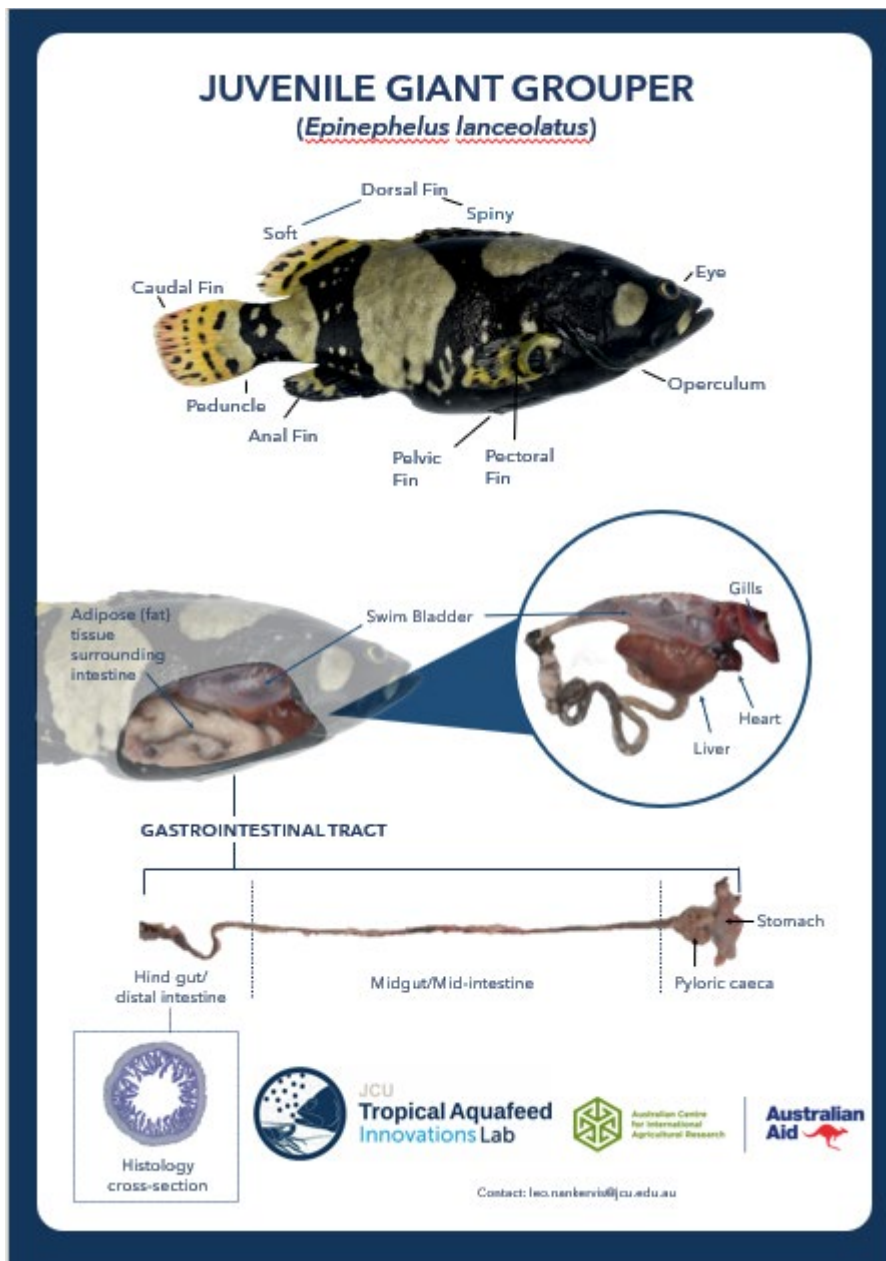
Butler, G., Candebat, C.L., Nankervis, L. Dietary methionine and choline mediate mobilisation of plasma lipids to improve feed efficiency for the diets of giant grouper (*Epinephelus lanceolatus*). In prep

Candebat, C.L., Eddie, T., Marc, A.F., Fernando, F. and Nankervis, L. 2023. Exploring the physiological plasticity of giant grouper (*Epinephelus lanceolatus*) to dietary sulfur amino acids and taurine to measure dietary requirements and essentiality. *Fish Physiology and Biochemistry*, 1-23. <https://link.springer.com/article/10.1007/s10695-023-01222-4> Nankervis, L., Maveto, A., Marc, A.F. and Candebat, C.L. Dietary starch interacts with grinding to affect giant grouper (*Epinephelus lanceolatus*) growth, feed efficiency, intestinal and hepatic histology. *In prep*

11 Appendixes

11.1 Appendix 1: Grouper morphometry poster

This poster is designed to be printed and decorate walls of research labs as an ongoing reference and training aid for new researchers in fish biology and nutrition. It is envisaged as a reference to teach researchers about sampling and organ identification. A .pdf version of this poster accompanies this report.



11.2 Appendix 2: Fish morphology, dissection and sampling.mp4

This video accompanies the report and is intended as a more detailed training aid for those new to sampling for fish nutrition studies.