Investigación e Innovación en Nutrición Acuícola

Editores: Lucía Elizabeth Cruz Suárez, Mireya Tapia Salazar, Martha Guadalupe Nieto López, David A. Villarreal Cavazos, Julián Gamboa Delgado, y Carlos A. Martínez Palacios

Investigación e Innovación en Nutrición Acuícola
2022, Monterrey, Nuevo León, México

Editores: Lucía Elizabeth Cruz Suárez, Mireya Tapia Salazar, Martha Guadalupe Nieto López, David Alonso Villarreal Cavazos, Julián Gamboa Delgado y Carlos A. Martínez Palacios.

Programa Maricultura
Facultad de Ciencias Biológicas
Universidad Autónoma de Nuevo León 2022

Copias disponibles en:
Universidad Autónoma de Nuevo León
Facultad de Ciencias Biológicas
Programa Maricultura
Cd. Universitaria
San Nicolás de los Garza, Nuevo León
C.P. 66455
Tel.+Fax. 818352 6380
E-mail: lucia.cruzsr@uanl.edu.mx

Para citar alguna parte de ésta obra siga el siguiente estilo:

- Autores del escrito. 2022. Nombre del artículo. Editores: Lucía Elizabeth Cruz Suárez, Mireya Tapia Salazar, Martha Guadalupe Nieto López, David Alonso Villarreal Cavazos, Julián Gamboa Delgado y Carlos A. Martínez Palacios. Investigación e innovación en nutrición acuícola, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp.473 ISBN: 978-607-27-1732-9. El cuidado de la presente edición, así como su realización estuvo a cargo de los editores.

Los editores hacemos extensivo nuestro profundo agradecimiento:

- A las personas que colaboraron en la edición técnica de estas memorias

Directorio

Editores
Lucía Elizabeth Cruz Suárez, Denis Ricque Marie, Mireya Tapia Salazar, Martha Guadalupe Nieto López, David Alonso Villarreal Cavazos, Julián Gamboa Delgado, y Carlos A. Martínez Palacios.

Dirección de edición: Programa Maricultura, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Dra. Lucía Elizabeth Cruz Suárez, Av. Universidad S/N, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León, C.P. 66455
Email: elicruz@hotmail.com, lucia.cruzsr@uanl.edu.mx
Teléfonos: 52 8183526380

ISBN:978-607-27-1732-9. El cuidado y edición estuvo a cargo de los editores. El contenido es responsabilidad de los autores.

Aquaculture Nutrition Research at the Commonwealth Scientific and Industrial Research Organisation: Maintaining Productivity and Competitiveness in Challenging Times

Artur Rombenso*[1], Ha Truong[2], and Cedric Simon[3]

Commonwealth Scientific and Industrial Research Organisation, CSIRO, Livestock & Aquaculture Program, Queensland, Australia

[1]Bribie Island Research Centre, 144 North Street, Woorim, Queensland, Australia 4507 – artur.rombenso@csiro.au

[2]Bribie Island Research Centre, 144 North Street, Woorim, Queensland, Australia 4507 – ha.truong@csiro.au

[3]Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland, Australia 4067 – cedric.simon@csiro.au

Abstract

The Australian aquaculture industry has faced new challenges brought about by the global pandemic and the consequences of which are only beginning to be fully recognised. In regards to the R&D sector, shifts in strategies for undertaking research are becoming apparent. In light of these new challenges, we aim to share our recent research and insights on maintaining productivity and competitiveness within the shrimp and fish nutrition research context.

Keywords: *research opportunities, strategy, CSIRO, aquaculture nutrition*

Introducing the Aquaculture Nutrition Research at CSIRO

The Commonwealth Scientific and Industrial Research Organisation (CSIRO; Australia's Government Research Agency) Nutrition & Production Systems group within the Livestock and Aquaculture Program is responsible for the aquaculture nutrition research, which aims to refine and develop novel aquafeeds and value-added products to improve the diversity, sustainability, and efficiency of the current and prospective aquaculture industry. Our main nutrition research areas include (1) novel functional feeds and formulations, (2) digital technologies for nutrition, mostly near-infrared spectroscopy, (3) circularity in aquaculture nutrition, (4) *in vitro* models of digestion and absorption, (5) hatchery technologies for prawns, and (6) novel phenotypes including feed efficiency, resilience to stress and product quality.

Our footprint has impacts in Australia and in the last decades, overseas. More information about the CSIRO Aquaculture Research can be found at our website - https://research.csiro.au/aquaculture/.

Background and aims

The growth potential of the Australian aquaculture industry is driven by the ongoing investment in seawater leases for sea-cage farming, land mainly for pond production, and RAS facilities for aquaculture commercial enterprises as well as the geographic proximinity to Asia which fuels demand for premium aquacultured products such as abalone and tuna. In the last two years, the Australian aquaculture industry has faced new challenges brought about by the global pandemic and the consequences of which are only beginning to be fully recognised. In regards to the R&D sector, shifts in strategies for undertaking research are becoming apparent. Inn light of these new challenges, we aim to share our recent research and insights on maintaining productivity and competitiveness.

Challenges and new opportunities

For most research organisations, the main challenges faced during the pandemic have beenreduced or no new R&D funding opportunities, difficulties in recruiting internationally staff and students, postponed milestones/activities of current projects, difficulties with face to face communication internally and with clients, lockdowns of laboratories and research facilities , and slow turnaround of reagents, materials and commercial analyses

Indeed, we have faced most of those with the exception of the absence of international students which fundamentally affected all universities. Fortunately, we experienced only a mild delay in activities, such as experiments and analysis, which were critical to delivering ongoing project milestones. As most new project opportunities were put on hold or lost as ventures reduced severely their R&D budget, we have a renewed focus on strategic internally funded research and adapting our capabilities in the face of the new priorities of the aquafeed industry, domestically and overseas. At the corporate level, CSIRO stepped up efforts to support staff working remotely with the launch of a highly anticipated suite of equipment and resources for working from home in 2020. The new work from home package 'provides our people with the equipment, resources and support to work more seamlessly between their home and a CSIRO site. This will enable us to continue this flexibility into the future'. CSIRO staff received a range of kit and equipment to support working from home including a laptop, keyboard, mouse, headset, webcam and USB hub, height adjustable desk, an ergonomic chair and a computer monitor. This has helped tremendously in maintaining productivity in a safe working environment.

One successful approach has focused on domestic small and medium enterprises, mainly ingredient companies interested in entering the aquafeed industry. For example, we have worked with companies producing insects, aquaculture by-products and food waste, more details about those will be presented in the research projects section. Another approach has been incorporating nutrition expertise into projects led by other aquaculture science domains such as breeding, production systems and health to work at the intersection of various disciplines to create greater impact. Finally, we have aligned our strong nutrition analytical capability to secure small fee-for-service projects beyond aquaculture. More details about our approaches and ongoing projects will be discussed.

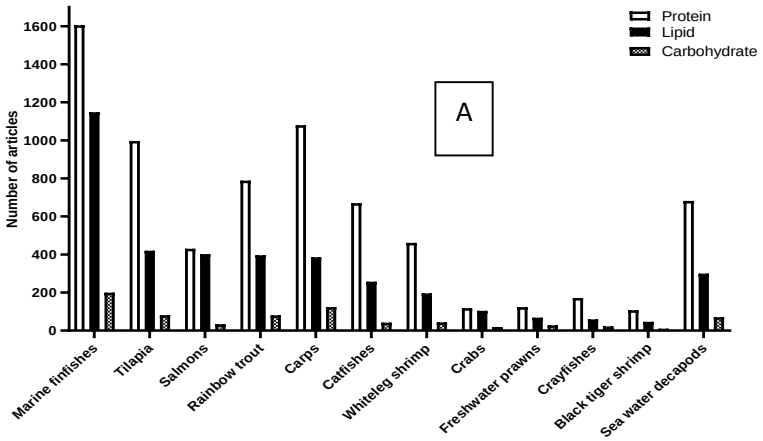
A silver-lining of the travel restrictions and loss of projects could be seen as the freeing of time. This gained time has allowed for more desktop-based activites, and has allowed researchers to work on writing material which had been 'shelved' due to lack of time as well as provide an opportunity to shine new light on the intensive research effort to date. The increased output of reports, website articles, technical and peer-review articles has been evident worldwide, which we have contributed to with an increase in the number and diversity of publications during this period. Strategies within authorship, more specifically to strengthen and nurture collaborations, is key.

Research projects and outcomes

This section summarises our ongoing projects and recent publications, including magazine and peer-review articles in shrimp and fish nutrition.

Aquaculture nutrition:

Early 2021, in collaboration with colleagues from Australia, Brazil, China and Mexico, we published a technical review about macronutrient research (protein, lipids and carbohydrates) in aquaculture nutrition (Rombenso *et al*., 2021). We summarised the scientific literature on macronutrient research between 1990 and 2020, linking with the most produced species worldwide. This was an opportunity to reflect on the status of knowledge in each of these key macronutrients, provide insight into the drivers behind the research effort and identify gaps for future focus (Figure 1). Some of the highlights includedthe number of peer-reviewed published papers from 1990 to 2020 investigating protein was higher than lipid and carbohydrate in most countries; in marine fish and cold-water species, the difference between lipid and protein research is reduced, but protein was still the priority;considering the volume of major aquacultured species produced, lipid- and carbohydrate-based ingredients are worth at least half of the global aquafeed production costs; the authors encourage a shifting focus from protein-rich sources and increasingly consider the role of lipids and carbohydrates in future formulations.



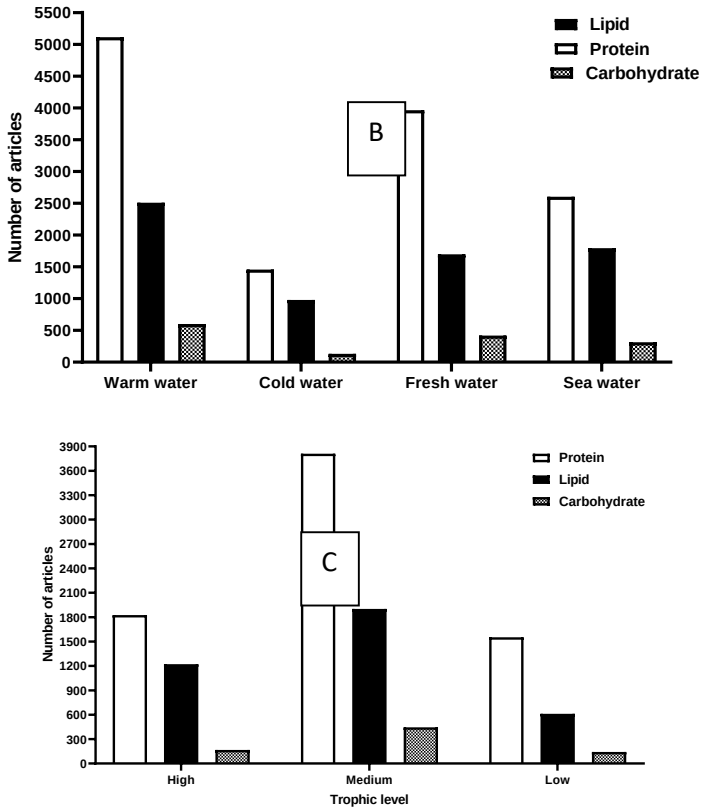


Figure 1. Number of peer-reviewed published articles since 1990 related to protein, lipid and carbohydrate in the top produced species (A), all species collated grouped by their water temperature and salinity preferences (B), and all species collated grouped by their trophic level (C), position of an organism in a food web (High = top carnivores 4-5, Medium = mid-low carnivores 3-4, Low = omnivores and herbivores 2-3). Extracted from Rombenso *et al.*, 2021 – https://www.globalseafood.org/advocate/macronutrient-research-in-aquaculture-nutrition/. FishBase (http://www.fishbase.org/search.php)

Shrimp nutrition:

In Australia, we strictly work with the native black tiger shrimp *Penaeus monodon* and in China, Thailand and Vietnam with Whiteleg shrimp *Litopenaeus vannamei*. *L. vannamei* is considered a pest species in Australia, along with tilapia. Our capability in shrimp nutrition is broad. Enabled by the flexibility of our facility, we research all life stages from broodstock to harvest size animals in various systems, including various sized tanks, net pens, raceways and ponds. Although most of our research is done in clear water, we have run trials in culture conditions dominated by

autotrophic and heterotrophic organisms. We see our research focus as being aligned with two areas: i) shrimp digestive physiology and nutritional requirements; and ii) the role of nutrition for breeding, health and production. This focus allows us to engage in activities that are of priority to the Australian aquaculture industry while ensuring we are able to satisfy the delivery of impactful science as an R&D organisation.

Feed and feed additives

Novacq

CSIRO has been working in the feed additive space for decades, specifically on the development and characterisation of Novacq™, a dry microbial biomass for shrimp nutrition. CSIRO published research has highlighted the benefits of this feed additive illustrated by enhanced growth (20-60%) and health at 5-10% dietary inclusion rates, and has contributed to the sustainability of aquafeeds enabling successful replacement of marine-origin ingredients and reducing dietary protein content. Recent studies have focused on different life stages (postlarvae and juveniles), species (black tiger prawn and white leg shrimp), culture conditions (clear water, dominated by heterotrophic or photoautotrophic organisms) and growth performance, survival, feed efficiency, health and immunostimulation (Simon *et al*., 2020; Rombenso *et al*., 2020; 2021; Noble *et al*., 2022).

We demonstrated the suitability of custard diets in *P. monodon* postlarvae (PL3 to PL30) and that Novacq™ supplementation (12.7%) in custard diets appears to be a beneficial ingredient in PL nutrition, improving survival and enhancing the growth of better performing PLs (Rombenso *et al*., 2019). Similarly, survival and growth of PL12 and early juvenile (0.3-0.4g) white leg shrimp was enhanced by the supply of Novacq™ at 10% inclusion level in practical and commercial feeds (figure 2; Rombenso *et al*., 2021). Additionally, whole-body composition was not altered by Novacq™ supply.

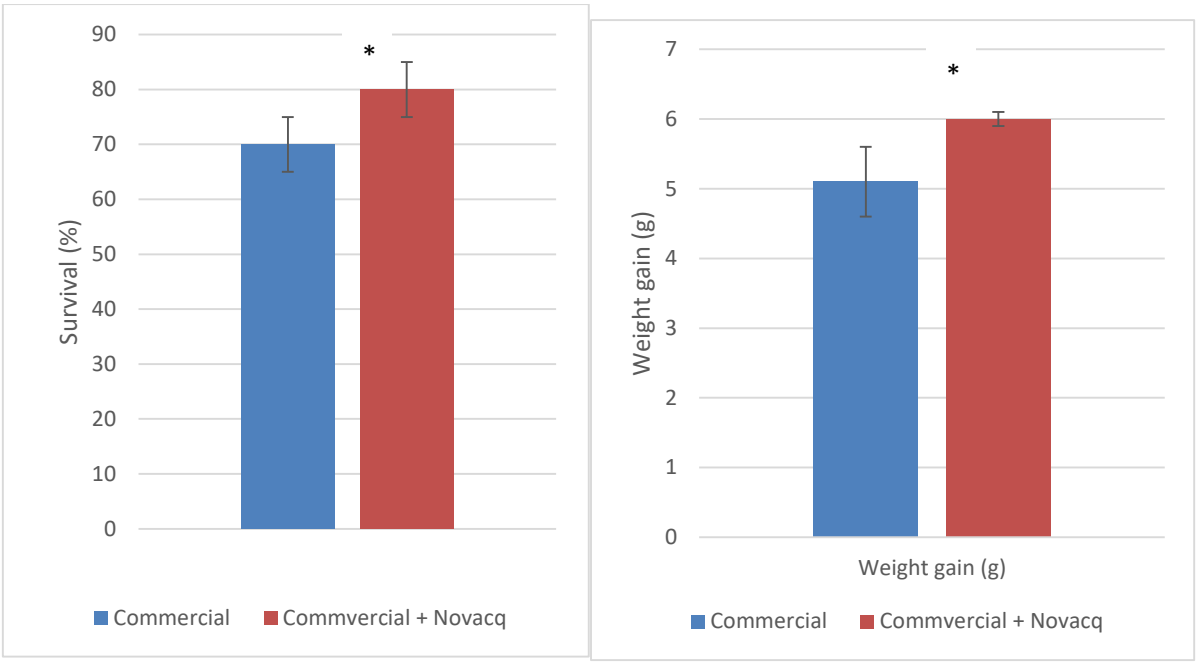


Figure 2. Survival (%) and weight gain (g) of early juvenile white lega shrimp fed commercial feeds with and without Novacq™ supplementation. * represents statistical differences.

To better understand the mode of action of Novacq™, we investigated the performance, digestibility, digestive enzyme activity, nutritional condition and gut microbiota of black tiger prawn fed commercially relevant marine-origin ingredients/additives including krill meal, krill hydrolysate, whole squid and Novacq™ at 10% inclusion, to apparent satiation or restriction (~60% of control diet satiation; Simon *et al*., 2020). Shrimp fed Novacq™ feeds confirmed previous findings, displaying greater production performance, feed intake, feed efficiency and nutrient retention. Novacq™ appears to enhance the efficient use of nutrients by increasing the retention of protein and the catabolism of non-protein energy. Insights into shrimp physiology were obtained where protease activity in the hepatopancras were correlated with FCR and retention of non-protein energy (Figure 3).

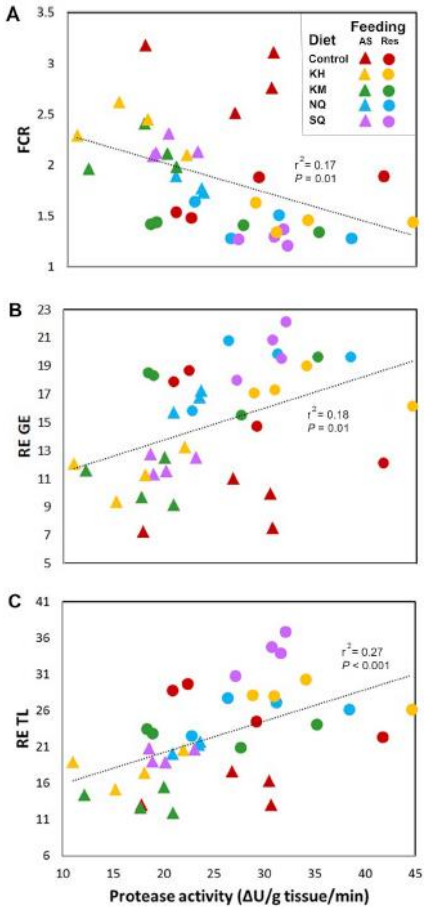


Figure 3. Significant linear relationship between protease activity in the hepatopancreas and FCR (r2=0.17, *P*=0.01), RETL (r2=0.27, *P* < 0.001) and REGE (r2=0.18, *P*=0.01). Triangles represent satiety (AS) and circles represent restricted (Res) ration, while colours represent the Control (red), krill hydrolysate (KH, yellow), krill meal (KM, green), Novacq™ (NQ, blue) or whole squid (SQ, purple) diets, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.) Extracted from Simon *et al*., 2020 – https://doi.org/10.1016/j.aquaculture.2019.734679.

We also demonstrate the advantage of supplementing Novacq™ to promote shrimp performance in different culture conditions dominated by heterotrophic or photoautotrophic organisms, and particularly if it continues to be delivered in a diet throughout the feeding period (figure 4; Rombenso *et al*., 2020).

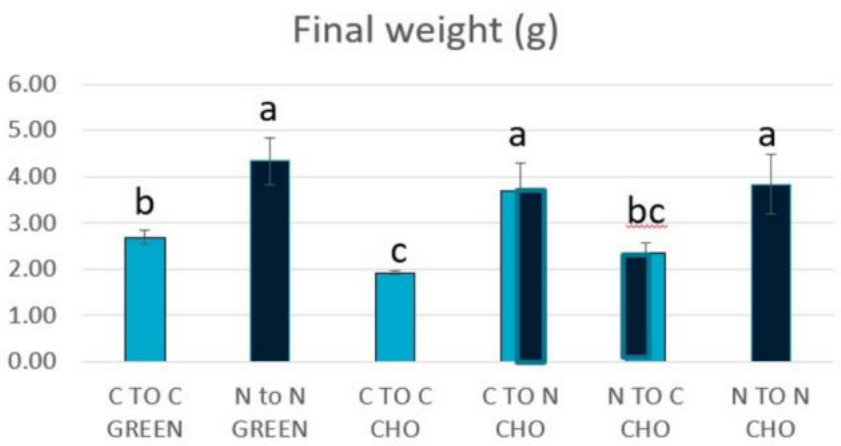


Figure 4. Individual final weight (g) of shrimp fed control (C) and/or Novacq™ (N) feeds in nursery phase under two culture conditions (Green = culture condition dominated by autotrophic organisms and CHO = culture condition dominated by heterotrophic organisms) and clearwater feeding trial for a total of 135 days (from postlarvae 19 to 2-4g) . The first letter (C or N) corresponds to the feed during nursery phase and the second to the feed in the clear water feeding trial. Extracted from Rombenso *et al*., 2020 – https://www.globalseafood.org/advocate/commercial-microbial-biomass-additive-evaluated-in-black-tiger-shrimp-diets/

The findings collectively justify Novacq™ as a key strategic feed additive in shrimp (*P. monodon* and *L. vannamei*) formulations. Further information about these findings and their implications for future research on prawn nutrition will be discussed.

Organic acids

We investigated the use of organic acids individually or in combination at 1% in feeds for juvenile black tiger prawns (Rombenso *et al*., 2020). Combination of butyrate, succinate and fumarate improved survival, feed intake, growth, and nutrient retention efficiency. Individually, butyrate appears to be the most potent organic acid followed by succinate, whereas fumarate seems to be dispensable in black tiger prawn diets.

Digestive physiology

Our focus on shrimp digestive physiology allows us to investigate the utilisation of nutritional inputs and identify factors which are important for efficient conversion of feed in shrimp. Thus,

feed and ingredients are major influential factors in this regard. Advances in feed formulations have been achieved in shrimp nutrition. However, the reliance on marine-origin ingredients (i.e. fishmeal, squid by-product meal, mussel meal, krill meal) in *P. monodon* appears to be greater than in *L. vannamei*, in which complete replacement has limited success. Accordingly, we aimed to understand the effects of key marine ingredients (the microbial biomass Novacq^{TM}, krill meal and squid meal) on feed intake and nutrient utilisation (Truong *et al*., 2021). Feed intake, rate of digesta travelling through the gut and post-prandial concentration of free amino acids in haemolymph varied across the tested marine ingredients.

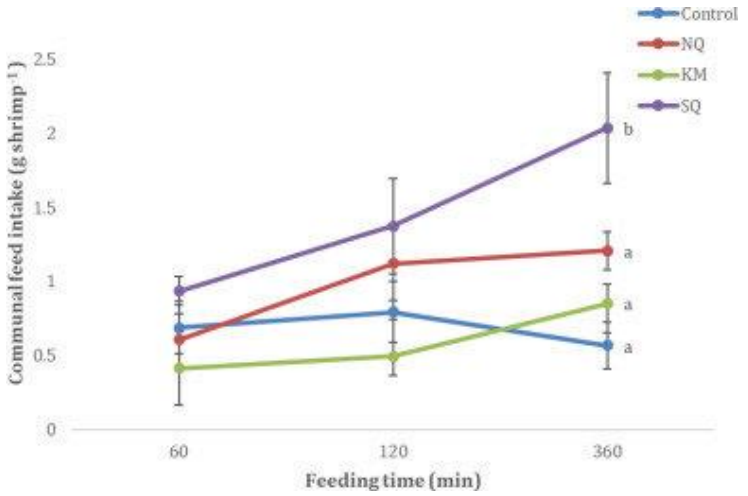


Figure 5. Communal feed intake (g/shrimp) per feeding time of shrimp fed diets containing marine-origin ingredients. Extracted from Truong *et al*., 2021 – https://doi.org/10.1016/j.aquaculture.2020.736171

This paper identied key digestive mechanisms which are likely to contribute to the enhanced growth observed when marine meals are added to shrimp feed (Simon *et al*., 2020). Ingredients which increased feeding rate and gut transite rate, such as the microbial biomass (Novacq^{TM}, NQ), were also shown to prolong feeding time as shown in Figure 5. This provides insights into the drivers behind feed intake and gut mobility in shrimp as well as supports the growing literature on the effectiveness of marine meals in shrimp feed.

Additionally, we examined the feeding behaviour, crystalline amino acid (CAA) leaching loss and amino acid uptake in adult *P. monodon* fed a fishmeal control and alternative terrestrial meal-based formulation enriched with CAA (Simon *et al*., 2021). No differences in attractiveness, palatability

and apparent digestibility were noticed. However, water stability of diets differed from those supplemented with CAA resulting in sub-optimal levels of essential amino acids within 2 hours in water (figure 6). The growth implications associated with leaching losses need to be investigated, and feed management becomes more important in CAA supplementation.

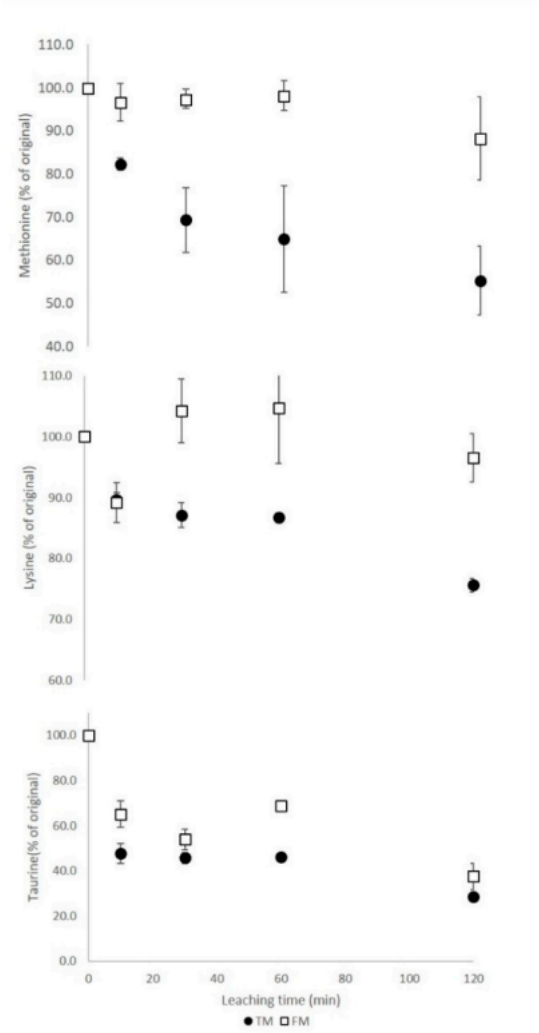


Figure 6. Leaching of amino acids (methionine, lysine and taurine) through time. Extracted from Simon *et al*., 2021 – https://doi.org/10.3390/ani11030847.

Gut microbiota

The effects of diets containing high and low fishmeal levels on microbiota of different sections of the gastrointestinal tract (stomach, intestine and digesta) was investigated in black tiger prawn (Noble *et al*., 2021). Bacterial communities were influenced by dietary treatments and different sample types. Diets largely modified the stomach and digesta communities, while intestine was less affected (Figure 7). Selection of gastrointestinal segments should be carefully considered in nutrition studies focusing on gut bacterial communities.

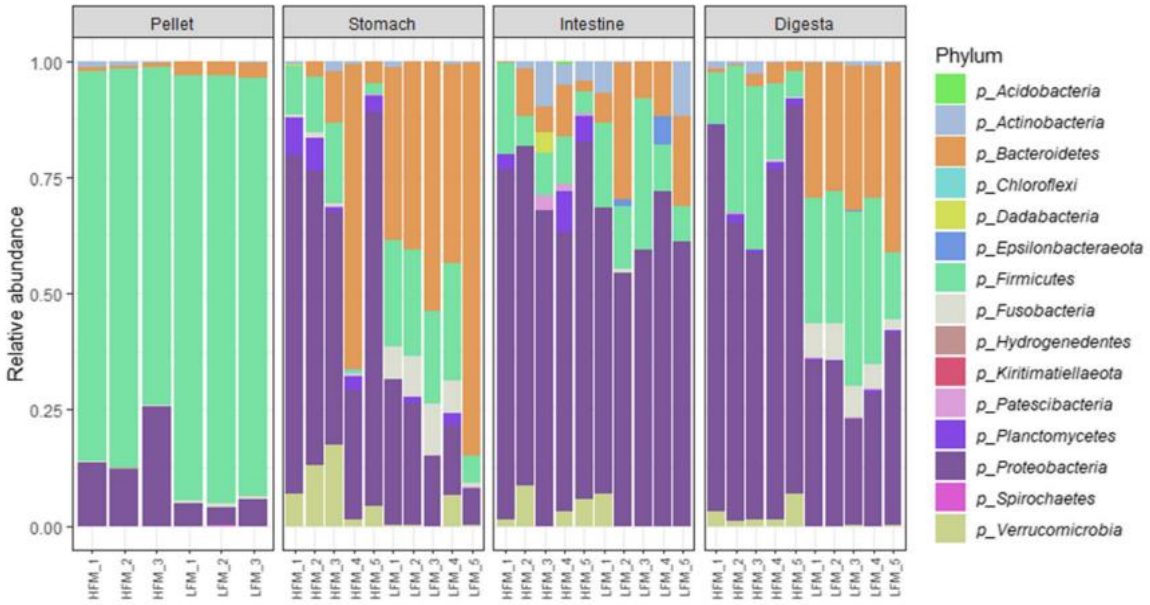


Figure 7. Taxonomy of bacterial communities of dietary treatments and gastrointestinal tract sections (stomach, intestine and digesta) of shrimp fed dies containing high or low fishmeal. Extracted from Noble *et al*., 2021 – https://doi.org/10.1007/s00253-020-11052-6.

Apparent digestibility and digestive physiology research

Unlike fish, where abdominal stripping is considered to be a reliable method for collecting faecesfor the measurement of apparent digestibility, in shrimp, current methods rely on collection of excreted faeces by settlement. The later is likely to result in over-estimation of apparent digestibility due to leaching of soluble nutrients from the faeces. Accordingly, we tested the efficacy of different faecal collection methods based on dissection, screening and decanting in a bucket on the apparent digestibility of commercially relevant ingredients rich in protein, lipid and starch (Truong *et al*., 2022). Protein and amino acid apparent digestibility values were influenced by collection methods, whereas dry matter, starch and lipid were not. Traditional collection

methods (screen and bucket) over-estimated protein and total amino acids apparent digestibility due to leaching (Figure 8), suggesting standard practices for evaluating digestible protein of protein-rich ingredients in shrimp needs to be reconsidered. Dissection method provides a similar method to fish stripping that can be applied to shrimp.

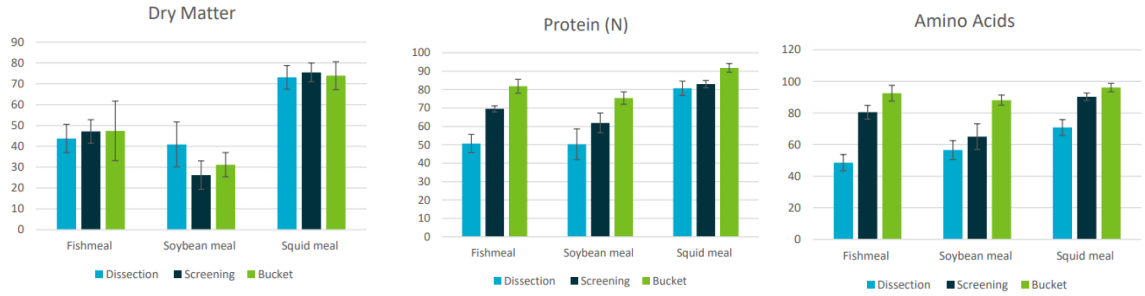


Figure 8. Dry matter, protein and amino acids apparent digestibility of protein-rich ingredients (fishmeal, soybean meal and squid meal) determined by three faecal collection methods (dissection, screening and bucket).

We also tested the assumption that nutrient absorption predominately occurs in the hepatopancreas and not in the posterior gut sections. Using the dissection method, we determined the apparent digestibility of three gastrointestinal tract regions (foregut, proximal and distal hindgut). Higher values of dry matter and protein apparent digestibility were measured in hindgut (proximal and distal) than foregut (Figure 9). Digestibility did not increase further down the hindgut. Combined, the results provide substantial evidence that the hepatopanceas is the main site of nutrient digestion and absorption in shrimp.

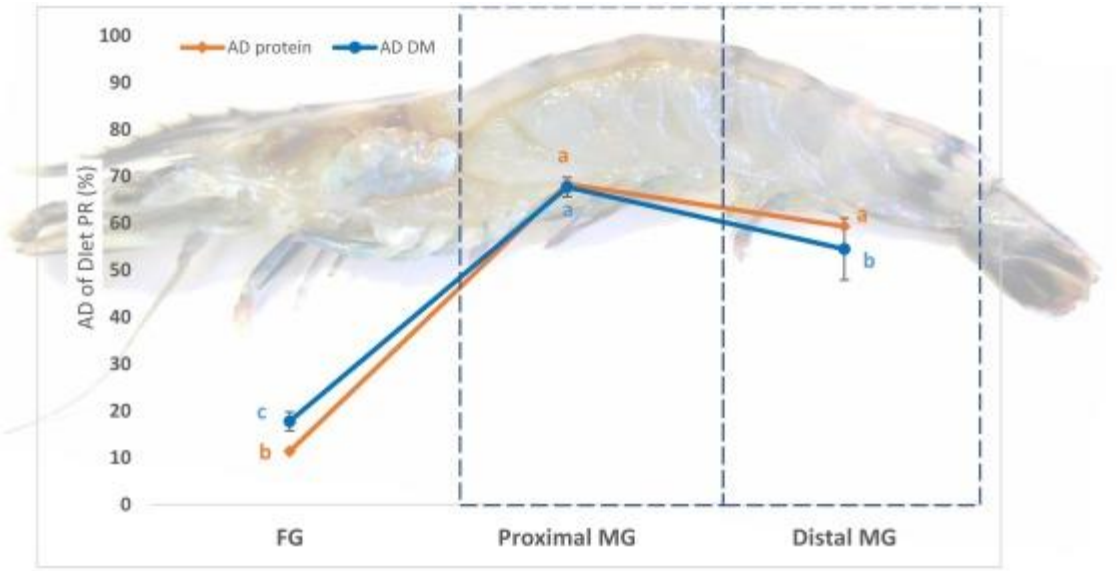


Figure 9. Dry matter and protein apparent digestibility of three regions of the gastrointestinal tract. Extracted from Truong *et al*., 2022 – https://doi.org/10.1016/j.aquaculture.2022.737957

Nutrient essentiality

Knowledge of mineral essentiality is limited in shrimp nutrition, especially for *P. monodon* (NRC 2011). We aimed to assess the importance of a large number of minerals which had or had not been assessed in shimp previously. We adopted a novel Plackett Burman screening design to more efficiently understand the influence of twelve dietary minerals for optimal growth in *P. monodon* (Truong *et al*., 2020). Addition of calcium:phosphorus at 1:1 ratio, magnesium, boron, manganese, selenium and zinc yielded greater weight gain, feed conversion efficiency, biomass gain and nutrient/energy retention. More specifically, boron and manganese demonstrated higher retention of crude protein, total lipids and gross energy. We also brought some insights on mineral chemical form and its effect on diet stability and bioavailability, and highlighted the need to refine mineral requirements in shrimp.

Nutrition, production system, breeding and health review

Shrimp farming is intensifying, aiming for more control and biosecurity to mitigate risks (Figure 10). Advances in nutrition and breeding linked to production systems have positively contributed to the development and adoption of intensive systems. Our recent review discusses the role of nutrition in shrimp intensification, including feed cost (relationship between prices of raw materials and shrimp, and raw materials and their respective protein content), feed quality, feeding

management, nutrient requirements (Table 1), tailored feeds, feed additives, and marine and microbial-based growth promoters (Emerenciano *et al*., 2022). We additionally explored the links among nutrition, breeding, and health towards intensification of shrimp farming.

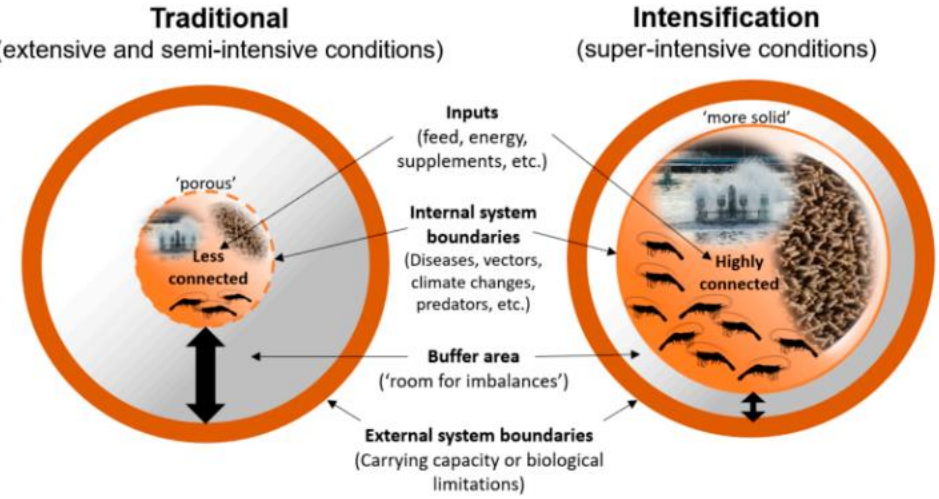


Figure 10. Comparison between traditional and intensive systems. Extracted from Emerenciano *et al*., 2022 - https://doi.org/10.3390/ani12030236.

Table 1. Recommended nutrient requirements for *L. vannamei* under different production systems and intensity. Data adapted from Internation Aquaculture Feed Formulation Database (IAFFD - https://www.iaffd.com/). Extracted from Emerenciano *et al*., 2022 - https://doi.org/10.3390/ani12030236

Nutrient Requirements (%)	*L. vannamei*		
	RAS	**Semi-Intensive**	**Intensive**
Crude protein	38–44	33–42	40–46
Crude lipid	9–11	7	8
Dig. energy (kJ/kg)	15,820–16,292	14,033–15,380	15,079–15,874
Amino acids (%)			
Arg	2.56–2.94	2.58–2.92	2.69–2.99
His	0.73–0.83	0.73–0.82	0.77–0.84
Ile	1.51–1.71	1.52–1.70	1.59–1.73
Leu	2.52–2.99	2.53–2.98	2.64–3.06
Lys	2.76–3.18	2.72–3.14	2.83–3.22
Met	0.97–1.11	0.98–1.11	1.01–1.13
Phe	1.74–1.97	1.76–1.96	1.83–2.00
Thr	1.31–1.56	1.31–1.54	1.37–1.58
Trp	0.34–0.39	0.34–0.39	0.36–0.39
Val	1.7–2.01	1.72–2.00	1.79–2.04
Fatty acids (%)			
Sum n-3	0.89	0.83	0.87
Sum n-6	0.6	0.6	0.6
EPA + DHA	0.71–1.01	0.67–0.94	0.69–0.98
Cholesterol	667–834	521–727	540–752
Phospholipids	1.1–1.5	1–1.4	1.1–1.4

Ongoing Shrimp research projects

Our future research will encompass more and more considerations that extend beyond nutrition in order to consider interactions with health, breeding and production systems. All these areas are interacting and need to be considered together for the research to be truly adaptable to the commercial environment–the expectation for relatively high return on investment from R&D is becoming more apparent in a post-pandemic era.

In the shrimp broodstock space, we have been investigating the key nutritional inputs of traditionally used fresh feeds, polychaetes fortification strategies, and replacement of fresh feeds with formulated moist feeds.

We are investigating the impact of dietary nutrients, specifically minerals, when rearing shrimp in systems with low water replenishment or mineral-deficient water.

We are continuing to support start-ups and small/medium enterprises by assessing the usefulness of complementary/alternative ingredients such as food waste, Greenshell mussel powder, algae-based ingredients, microbial biomass feed additives, fermented meals and Australian sourced canola mealproducts for shrimp feeding. These relatively under-explored raw materials are not only relevant as locally available nutrient source but also play a role in the development of functional feeds with immunomodulatory properties and some brings an important sustainability angle via therecycling of waste nutrients from other industries.

Fish nutrition:

The core of our fish nutrition research has focused on barramundi *Lates calcarifer* and Atlantic salmon *Salmo salar*. The latter represents the bulk of the aquacultured fish production in Australia (circa 70,000 tonnes) and therefore is of major research focus in Australia. Similar to shrimp nutrition, our research scope and footprint in fish nutrition is broad and focus on lipid nutrition, novel ingredients, digital technologies (near-infrared spectroscopy – NIRS), metabolism, in vitro ingredient characterisation, and linking nutrition with other science areas such as production systems and health.

Formulation optimisation

One of the biggest challenges of lipid research in fish nutrition consists of decreasing the reliance of fish oil in aquafeeds while maintaining fish survival, performance and levels of omega-3 fatty

acids (DHA and EPA) in the edible flesh. Despite decades of searching and developing alternative and complementary lipid sources, fish oil remains a cost-effective source of long-chain polyunstaturated fatty acids, mostly DHA, EPA and ARA. We wrote a piece on the current main challenges around fish oil and lipid sources in fish nutrition, describing and discussing strategies the aquafeed industry can take (Rombenso *et al*., 2021). We introduced and discussed in depth the pros and cons, and future perspectives of an approach that has received little attention the "omega-3 sparing effect" – phenomenon based on saturated fatty acids-rich feeds that won't proportionally accumulate in tissue resulting in similar or higher omega-3 fatty acids deposition, metabolically sparing the omega-3 fatty acids (Rombenso *et al*., 2021).

Digestibility of astaxanthin, the most commonly used carotenoid pigment in salmonids is not consistent and sub-optimal. We compared the digestibility of synthetic or algal carotenoids in diets containing different lipid sources such as poultry oil, beef tallow or canola oil, as well as feed intake and diet palatability (Courtot *et al*., 2022). Algal carotenoids were more digestible than the synthetic product. Fatty acid composition of diets influenced astaxanthin apparent digestibility with those rich in monounsaturated fatty acids, mainly 18:1n-9, resulting higher astaxanthin apparent digestibility than those rich in saturated fatty acids (Figure 11). No differences in intake or palatability were observed across the dietary treatments. These findings suggest the relevance of tailoring dietary fatty acid composition to water temperature and the respective influence on astaxanthin digestibility and the suitability of algal carotenoids as natural pigments in salmon feeds.

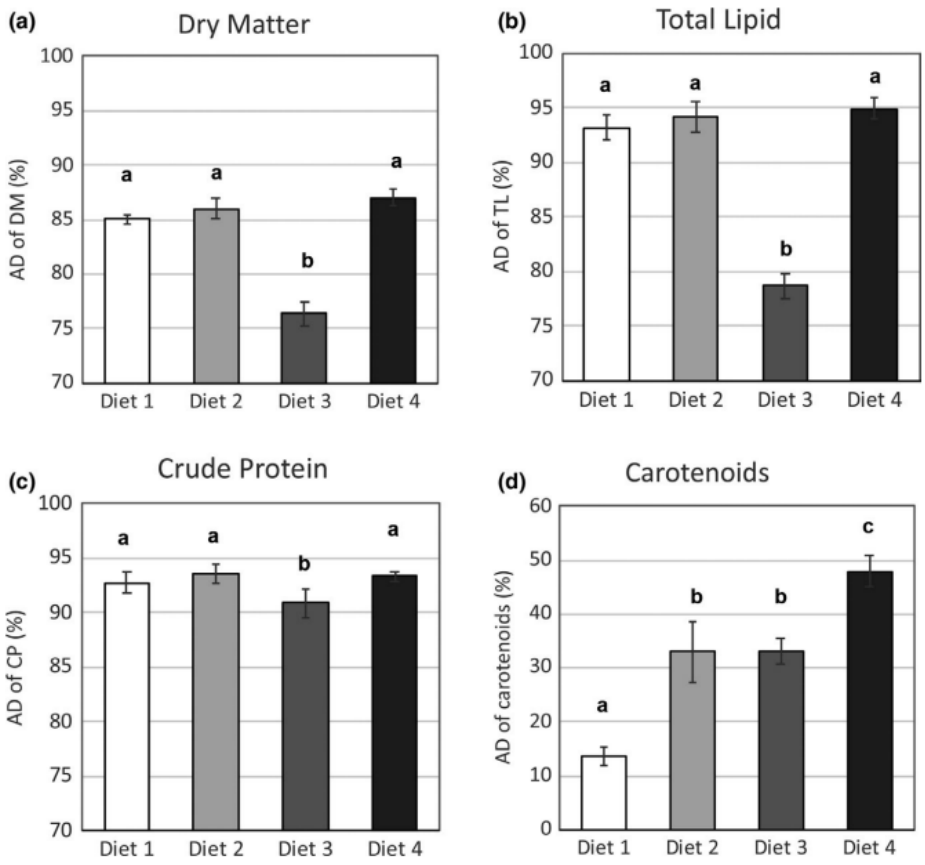


Figure 11. Apparent digestibility of dry matter (a), total lipid (b), crude protein (c) and carotenoids (d) of juvenile Atlantic salmon fed diets containing algal or synthetic astaxanthin with distinct lipid sources. Diet 1 = poultry oil with synthetic astaxanthin, Diet 2 = poultry oil with algal astaxanthin, Diet 3 = beef tallow with algal astaxanthin, and Diet 4 = canola oil with algal astaxanthin. Extracted from Courtot *et al.*, 2022 - https://doi.org/10.1111/are.15753.

Heterotrophic cultivation of thraustochytrids is a promising alternative to sustainably produce the health promoters omega-3 long-chain polyunsaturated fatty acids. CSIRO thraustochytrids strains can contain high DHA of as much as 14 g/L (40% total fatty acids, cell dry weight at 69 h). We investigated the potential of thraustochytrid whole cell biomass to replace fish oil in Atlantic salmon feeds (Chang *et al.*, 2020). No negative effects in production performance and whole-body total lipid and fatty acid composition were observed (Figure 12). The Australian thraustochytrid strains grown heterotrophically appears to be suitable for aquafeeds, although further on-farm research is needed to validate these findings.

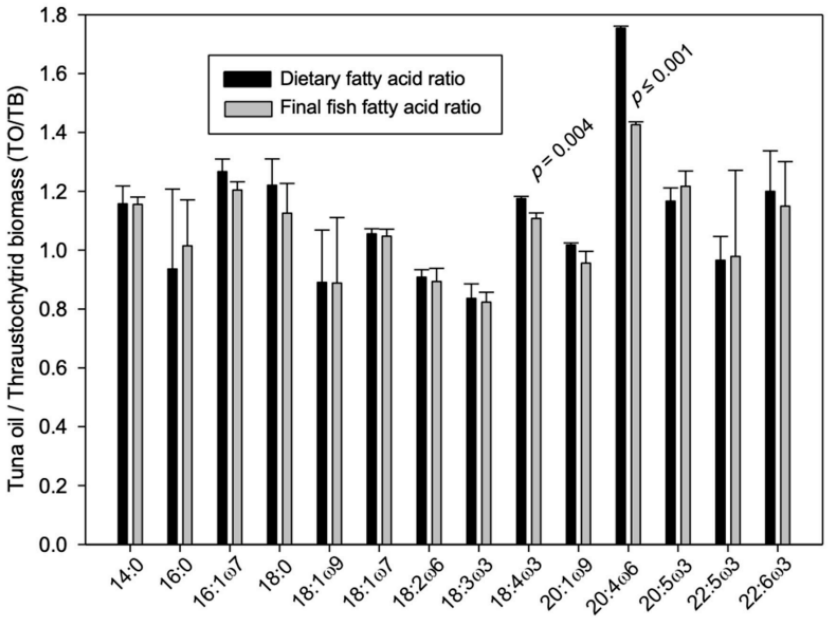


Figure 12. Dietary and final fish fatty acid ratio (fish oil/thraustochytrids whole cell biomass). Extracted from Chang *et al*., 2020 - https://doi.org/10.3390/jmse8030207

Long-chain n-3 rich oils from crops GM with algal genes are also a promising new sources for the industry. We engaged with NOFIMA and Nuseed in a collaboration assessing the use of a newly developed n-3 canola oil (DHA-CA) in diets of Atlantic salmon fingerlings in freshwater (Bente *et al*., 2019). The DHA-CA oil has high proportions of the n-3 fatty acids (FA) 18: 3n-3 and DHA and lower proportions of n-6 FA than conventional plant oils.

Two feeding trials were conducted to evaluate effects of two dietary levels of DHA-CA compared with two dietary levels of FO at two water temperatures, in Australia (16°C) and Norway (12°C) with respective fish genetics and local ingredients used in comparable formulations. Fish increased their weight approximately 20-fold at 16°C and 12-fold at 12°C during the experimental periods, with equal growth in salmon fed the FO diets compared with DHA-CA diets. Salmon fed DHA-CA diets had approximately the same EPA+DHA content in whole body as salmon fed FO diets. Gene expression, lipid composition and oxidative stress-related enzyme activities showed only minor differences between the dietary groups, and the effects were mostly a result of dietary oil level, rather than the oil source. The results demonstrated that DHA-CA is a safe and effective replacement for FO in diets of Atlantic salmon during the sensitive fingerling life-stage (Bente *et al*., 2019).

Table 2. Growth and survival data. Extracted from Bente *et al*., 2019 - https://doi.org/10.1017/S0007114519002356

	16°C water temperature trial									12°C water temperature trial								
	Low FO		High FO		Low DHA-CA		High DHA-CA			Low FO		High FO		Low DHA-CA		High DHA-CA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	ANOVA, *P*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	ANOVA, *P*
Body weight (g)																		
Day 0	0·83	0·01	0·84	0·02	0·86	0·03	0·83	0·02	0·74	2·04	0·09	2·16	0·04	2·08	0·04	2·05	0·01	0·46
Period 1	4·63	0·12	4·49	0·06	4·49	0·14	4·44	0·13	0·68	4·47	0·19	4·79	0·07	4·52	0·10	4·38	0·03	0·14
Period 2	10·35	0·08	10·14	0·28	10·17	0·09	9·94	0·20	0·48	6·82	0·29	7·33	0·10	6·81	0·16	6·67	0·03	0·11
Period 3	15·49	0·47	15·29	0·31	16·22	0·77	15·25	0·22	0·47	23·82	0·94	25·41	0·80	23·28	0·62	23·74	1·02	0·38
SGR (%/d)																		
Period 1	5·04	0·09	4·94	0·02	4·90	0·04	4·94	0·03	0·18	2·95	0·03	3·01	0·12	2·93	0·07	2·87	0·03	0·64
Period 2	3·66	0·08	3·72	0·05	3·73	0·10	3·67	0·06	0·90	3·05	0·03	3·08	0·03	2·97	0·07	3·03	0·08	0·57
Period 3	2·87	0·26	2·91	0·12	3·18	0·20	3·06	0·04	0·65	3·02	0·03	3·00	0·05	2·97	0·01	3·07	0·11	0·73
SGR total	4·17	0·04	4·20	0·02	4·20	0·02	4·16	0·02	0·72	3·01	0·01	3·02	0·07	2·96	0·02	3·00	0·04	0·76
TGC total	1·36	0·02	1·35	0·01	1·38	0·027	1·35	0·00	0·73	1·59	0·02	1·63	0·04	1·57	0·02	1·59	0·04	0·50
Survival (%)	99·7	0·40	99·0	0·40	98·0	0·49	99·3	0·40	0·14	97·2	1·2	97·0	1·5	96·7	1·4	93·7	3·0	0·55

FO, fish oil; DHA-CA, *n*-3-rich modified canola oil; SGR, specific growth rate; TGC, thermal growth coefficient.
* The period from 0 to 34 d is denoted Period 1, from 34 to 56 d is denoted Period 2 and from d 56 to 70 d is denoted Period 3 in the 16°C trial. The period from 0 to 27 d is denoted Period 1, from 27 to 41 d is denoted Period 2 and from d 41 to 83 d is denoted Period 3 in the 12°C trial.

Table 3. Whole body fatty acid composition (% of total). Extracted from Bente *et al*., 2019 - -

https://doi.org/10.1017/S0007114519002356

	16°C water temperature trial									12°C water temperature trial								
	Low FO		High FO		Low DHA-CA		High DHA-CA			Low FO		High FO		Low DHA-CA		High DHA-CA		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	ANOVA, *P*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	ANOVA, *P*
14 : 0	3·7^{a}	0·02	4·05^{b}	0·03	3·4^{c}	0·03	3·5^{c}	0·02	<0·001	3·4^{b}	0·03	3·9^{a}	0·09	3·0^{c}	0·03	3·0^{c}	0·03	<0·0001
16 : 0	17·0^{a}	0·13	18·5^{b}	0·17	15·8^{c}	0·10	16·0^{c}	0·12	<0·0001	13·0^{b}	0·03	13·7^{a}	0·03	12·2^{c}	0·23	12·1^{c}	0·03	<0·0001
18 : 0	4·3^{a}	0·05	4·7^{b}	0·01	4·2^{a}	0·01	4·3^{a}	0·05	<0·001	2·9	0·03	2·9	0·03	2·9	0·03	3·0	0·03	0·44
20 : 0	0·3^{a}	0·00	0·3^{b}	0·00	0·3^{a}	0·00	0·3^{a}	0·00	<0·001	0·3	0·00	0·3	0·03	0·3	0·03	0·3	0·00	0·33
Σ SFA*	26·3^{a}	0·18	28·8^{b}	0·22	24·8^{c}	0·20	25·1^{c}	0·13	<0·0001	20·0^{b}	0·06	21·3^{a}	0·13	18·9^{c}	0·30	19·0^{c}	0·07	<0·0001
16 : 1*n*-7	4·0^{a}	0·05	4·5^{b}	0·03	3·7^{c}	0·03	3·7^{c}	0·01	<0·0001	3·2^{b}	0·15	3·6^{a}	0·10	2·3^{c}	0·09	2·2^{c}	0·00	<0·0001
18 : 1*n*-7	3·4^{a}	0·01	3·4^{a}	0·01	3·3^{b}	0·02	3·3^{b}	0·01	<0·01	3·7^{c}	0·07	3·6^{c}	0·00	4·0^{b}	0·03	4·3^{a}	0·03	<0·0001
18 : 1*n*-9	34·9^{a}	0·16	30·3^{b}	0·20	34·0^{c}	0·07	28·2^{d}	0·09	<0·0001	31·6^{a}	0·20	26·7^{b}	0·20	31·3^{a}	0·12	26·2^{b}	0·07	<0·0001
20 : 1*n*-9	2·4^{a}	0·01	2·2^{b}	0·03	2·3^{c}	0·03	2·1^{d}	0·00	<0·0001	6·3^{b}	0·06	6·8^{a}	0·03	5·6^{c}	0·03	5·4^{d}	0·00	<0·0001
22 : 1*n*-11	nd		nd		nd		nd			6·1^{a}	0·06	6·5^{a}	0·24	4·8^{b}	0·24	5·3^{b}	0·06	0·0005
Σ MUFA†	45·7^{a}	0·17	41·4	0·252	44·2^{c}	0·11	38·1^{d}	0·09	<0·0001	54·0^{a}	0·23	51·3^{b}	0·26	51·9^{b}	0·18	47·8^{c}	0·09	<0·0001
18 : 2*n*-6	6·4^{a}	0·05	5·7^{b}	0·03	6·4^{a}	0·03	5·9^{c}	0·07	<0·0001	5·4^{a}	0·06	4·5^{c}	0·00	5·5^{a}	0·10	4·9^{b}	0·00	<0·0001
20 : 4*n*-6	0·5^{a}	0·01	0·6^{b}	0·01	0·3^{c}	0·01	0·3^{c}	0·01	<0·0001	0·3	0·00	0·3	0·00	0·3	0·00	0·3	0·00	
Σ *n*-6‡	7·8^{a}	0·06	7·3^{b}	0·11	7·6^{a}	0·03	7·1^{b}	0·11	<0·0001	6·8^{a}	0·03	5·9^{c}	0·09	6·9^{a}	0·09	6·4^{b}	0·03	<0·0001
18 : 3*n*-3	3·1^{a}	0·05	2·6^{b}	0·06	5·9^{c}	0·02	8·5^{d}	0·12	<0·0001	3·2^{c}	0·06	2·6^{d}	0·00	5·7^{b}	0·10	7·8^{a}	0·03	<0·0001
18 : 4*n*-3	0·9^{a}	0·01	0·9^{a}	0·01	1·3^{b}	0·01	1·8^{c}	0·03	<0·0001	nd		nd		nd		nd		
20 : 3*n*-3	0·2^{a}	0·00	0·2^{b}	0·00	0·5^{c}	0·00	0·7^{d}	0·00	<0·0001	0·2^{b}	0·00	0·2^{b}	0·00	0·5^{a}	0·03	0·6^{a}	0·12	0·003
20 : 4*n*-3	0·5^{a}	0·00	0·5^{a}	0·01	0·8^{b}	0·00	1·2^{c}	0·00	<0·001	0·6^{b}	0·09	0·8^{a}	0·01	0·6^{b}	0·01	0·6^{b}	0·00	0·02
20 : 5*n*-3	3·0^{a}	0·05	3·2^{b}	0·03	2·9^{a}	0·02	3·2^{c}	0·04	<0·0001	2·8^{b}	0·00	3·5^{a}	0·03	2·4^{c}	0·00	2·5^{c}	0·03	<0·0001
22 : 5*n*-3	1·4	0·00	1·5	0·02	1·2	0·21	1·4	0·23	0·55	0·9^{b}	0·03	1·2^{a}	0·00	0·9^{b}	0·03	0·9^{b}	0·03	0·0002
22 : 6*n*-3	11·0^{a}	0·16	13·1^{b}	0·15	10·7^{a}	0·05	12·8^{b}	0·10	<0·0001	9·7^{d}	0·19	10·8^{b}	0·07	10·4^{c}	0·12	12·2^{a}	0·03	<0·0001
Σ *n*-3	20·5^{a}	0·26	22·6^{b}	0·22	23·7^{b}	0·23	29·9^{c}	0·09	<0·0001	17·6^{d}	0·15	19·3^{c}	0·20	20·6^{b}	0·23	24·7^{a}	0·17	<0·0001
n-6:*n*-3	0·38^{a}	0·00	0·32^{b}	0·00	0·32^{b}	0·00	0·24^{c}	0·00	<0·001	0·38^{a}	0·00	0·31^{c}	0·00	0·33^{b}	0·01	0·26^{d}	0·00	<0·0001
EPA+DHA	17·1^{a}	0·25	20·1^{b}	0·20	16·6^{a}	0·08	19·6^{b}	0·15	<0·0001	12·5^{b}	0·19	14·3^{a}	0·10	12·8^{b}	0·12	14·7^{a}	0·06	<0·0001

FO, fish oil; DHA-CA, *n*-3-rich modified canola oil; nd, not determined.
^{a,b,c,d} Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* Includes 15 : 0, 17 : 0, 19 : 0 and 21 : 0.
† Includes 17 : 1, 20 : 1*n*-7, 20 : 1*n*-11, 22 : 1*n*-7, 22 : 1*n*-9 and 24 : 1*n*-9.
‡ Includes 18 : 3*n*-6, 20 : 2*n*-6, 20 : 3*n*-6 and 22 : 4*n*-6.

Novel ingredients

We investigated the intestinal health of juvenile Atlantic salmon fed CSIRO engineered soybean genotypes with reduced antinutritional factors known to cause gut irritation, including lipoxygenases and oligosaccharides (Rombenso *et al*., 2021). This soybean meal also contained a more desirable glycinin profile. High levels of dietary SBM resulted in mild intestinal inflammation indicating enteritis progression. Speciality soybean types lacking lipoxygenases, oligosaccharides and altered glycinin profile did not improve intestinal health suggesting these antinutrients are not drivers of the intestinal inflammatory process in this species (Figure 13; Table 4). No additional benefits in terms of production performance or blood biochemistry were noticed in the speciality soybean types compared to the traditional soybean. The present findings demonstrates a targeted genomic approach to modifying soybean meals for aquaculture feeding. Clearly, more research in this space is required to identifying the most impactful antinutritional factors impacting salmon gut enteritis.

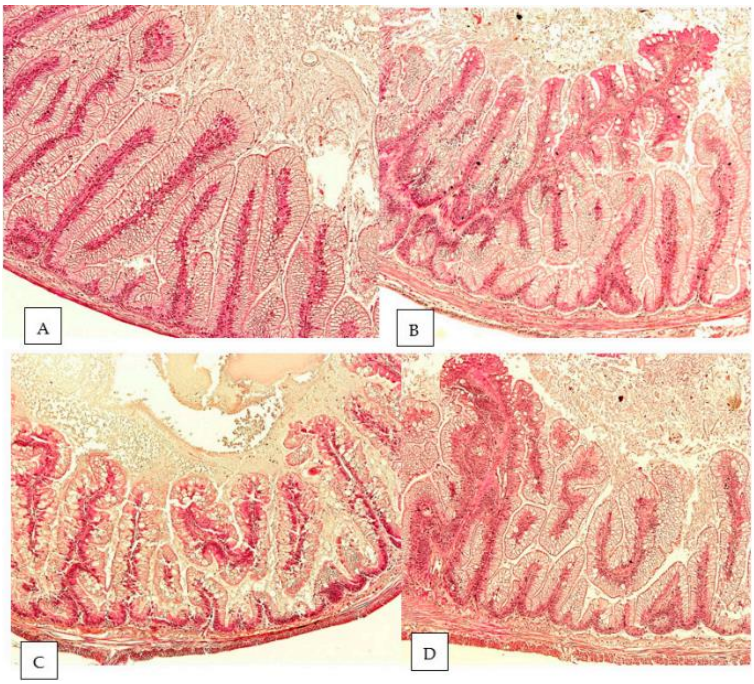


Figure 13. Histology of distal intestine of juvenile Atlantic salmon illustrating mild intestinal inflammation. A = control fishmeal diet, B = standard soybean meal, C = soybean meal absents of seed lipoxygenases and homozygous for the rs2 allele conditioning and near absent of seed raffinose and stachyose, and D = soybean meal conditioning null 11sA4 and 11sA5 globulins of glycinin.

Table 4. Summary of the main findings and parameters investigated of soybean meal-induced enteritis studies with Atlantic salmon. Extracted from Rombenso *et al*., 2021 - https://doi.org/10.3390/app11199327.

Growth Impairment	Feed Intake	Level of DI Inflammation	Enzymes	Time Sampling (Days)	Tissues Analyzed	Parameters	Ref.
SBM yes SPC no		SBM +++			PI and DI	MSA/LSC, ESA/LSC, LPSA/LSC, ESA/LPSA, GC/E, LM	[16]
Yes		+++		2, 7, 14 and 21	DI	MF	[17]
		++	5′ N, Mg-ATPase, ALP, ACP, NSE, LAP, AAP	21	MI and DI		[3]
Low SBM no High SBM yes	No changes	+, ++ and +++	ALP, LAP, maltase, isomaltase, lactase and sucrase		MI and DI	MF, SNV, LP, leycocyte	[4]
		+, ++ and +++			DI	MF, SNV, LP, CT	[28]
		++ and +++			DI	MF, GC, LP SNV, EG, SM	[29]
		++ and +++			DI	MF, GC, LP SNV, EG, SM	[10]
		+, ++, and +++	Pancreatic (trypsin, chymotrypsin, elastase, and lipase), chyme (LAP), brush border membrane (LAP and maltase)	0, 1, 2, 3, 5, 7, 10, 14, 17, and 21	PI, MI and DI		[30]
		+, ++, and +++		1, 2, 3, 5 and 7	DI		[31]
Low saponins no Mid-high saponins yes	Low saponins no Mid-high saponins yes	+, ++, and +++	Trypsin activity, bile acids, brush border membrane enzyme activity (LAP)		PI and DI	MF, LP, enterocyte vacuolization, GC, nucleos position within the enterocytes	[20]
		+++			DI	MF, SNV, LP, CT	[32]
		+, ++, and +++		7, 14 and 21	DI	Inflammation score, SM and microbiome	[18]
No	No changes				DI	MF, GC, LP, SNV, EG, SM	[33]
No	No changes	+ and ++	Brush border LAP, trypsin activity		DI	MF, SNV, SM, LP, microbiota, gene expression	[9]
		+ and ++			PI, MI and DI	MF, SNV, LP, CT,	[19]
SBM 17 no SBM 34 yes						Body composition and blood biochemistry	[27]
Yes	No changes	++ and +++			DI	MF, SNV, LP, CT,	[34]

Ref. = references; Level of enteritis: + mild, ++ moderate, and +++ high; 5′ N = 5′-nucleotidase; Mg-ATPase = Mg^{2+} dependent adenosine triphosphatase; ALP = alkaline phosphatase; ACP = acid phosphatase; NSE = non-specific esterase; LAP = leucine aminopeptidase, AAP = alanine aminopeptidase; PI = proximal intestine; MI = mid-intestine; DI = distal intestine; MF = mucosal fold; MSA = mucosal surface area; LSC = length mucosal stratum compactum; ESA = epithelial surface area; LPSA = lamina propria surface area; GC = goblet cells; E = 100 um epithelium; LM = length microvilli; SNV = supranuclear vacuoles; LP = lamina propria; EG = eosinophilic granulocytes; CT = connective tissue; SM = sub-epithelial mucosa.

In Australia, cowpea is a significant rotation crop which demonstrated promising value as an emerging plant-based protein for aquaculture markets (Bell *et al*., 2021). However it is severely underutilised. Therefore we reviewed cowpea-related aquaculture nutrition research discussing its ingredient characteristics, nutritional composition and value, and potential strategies to be used in aquafeeds (Tables 5 and 6).

Table 5. Protein, lysine and methionine content and price of various protein ingredients. Extracted from Bell *et al*., 2021 - https://doi.org/10.3390/agronomy11081654.

	Protein (%)	Lysine (%)	Methionine (%)	Price of Commodity (USD/MT-2020–2021) [e,f]
Fishmeal (herring) [a]	72	7.30	2.20	1500
Soybean meal [a]	44	2.83	0.61	~440
Soy protein concentrate [a]	64	3.93	0.81	~565
Soy protein isolate [a]	81	3.02	1.15	~1000
Canola meal solvent extracted [a]	38	2.02	0.77	~400
Canola protein concentrate [a]	69	3.10	1.26	-
Cottonseed meal solvent extracted [a]	42	1.60	0.58	178
Sunflower meal solvent extracted [a]	32	1.20	0.82	~300–500
Lupin meal [a]	30	1.54	0.27	~80–210
Wheat flour [a]	12	0.58	0.19	~240
Barley whole grain [a]	11	0.53	0.18	~80–120
Dehulled cowpea meal [b]	21.3–25.6	7.0–7.5	1.4–2.2	~360
Cowpea protein isolate [c,d]	75 [c]	6.8 [d]	1.4 [d]	-

Table 6. Summary of cowpea-related aquaculture research. Extracted from Bell *et al*., 2021 - https://doi.org/10.3390/agronomy11081654.

Aquaculture species		Cowpea	Experimental design			
Common name	**Scientific name**	**Scientific name - Type**	**Formulaion inclusion level (%)**	**Design**	**Major findings**	**Reference**
Black tiger prawn	*Penaeus monodon*	*V. unguiculata* - Whole	29.5	D and GT	Dehulling increased the nutritional value of cowpea	[50]
		V. unguiculata - Dehulled	24.68			
Black tiger prawn	*Penaeus monodon*	*V. unguiculata* - Whole	20	GT	Cowpea suitable at 20% contributing to low cost formulation	[51]
Whiteleg shrimp	*Litopenaeus vannamei*	*V. unguiculata* - Whole	15	GT	Cooking and extruding increased the nutritional value of cowpea	[52]
		V. unguiculata - Dehulled	15			
		V. unguiculata - Cooked	15			
		V. unguiculata - Germinated	15			
		V. unguiculata - Extruded	15			
Giant freshwater prawn	*Macrobrachium rosenbergii*	*V. unguiculata* - Whole	15, 30, 45, and 60	FM replacement	Cowpea can replace 30-45% of FM without impairing performance	[62]
Grouper	*Epinephelus coioides*	*V. unguiculata* - Whole	30 (D) | 20.5 (GT)	ID and GT	Cowpea can be included up to 20.5% without adverse growth effects	[54]
Indian carps - mrigal and rohu	*Cirrhinus mrigala* and *Labeo rohita*	*V. unguiculata* - Whole	100	D and GT	Cowpea was the least performing ingredient compared to soybean and mung bean	[48]
		V. unguiculata - Hydrothermically processed	100			
Nile tilapia	*Oreochromis niloticus*	*V. catiang* - Whole	10, 16, 18, 30, 32, 40, and 50	FM replacement with different dietary protein	Cowpea can replace up to 20-33% FM without impairing performance	[53]
Nile tilapia	*Oreochromis niloticus*	*V. unguiculata* - Whole	9, 18, 28, 37, 83 | 35	FM replacement	Cowpea can be added up to 20% without adverse effects. Processing treatments improve the nutritional value of cowpea, however increase cost	[49]
		Heat treated (48C, dry heat)	33			
		Heat treated (70C, dry heat)	33			
		Heat treated (119C, wet heat)	31			
		Dehulled treated (48C, dry heat)	33			
		Dehulled treated (70C, dry heat)	33			
		Dehulled treated (119C, wet heat)	32			
Nile tilapia	*Oreochromis niloticus*	*V. unguiculata* - Cowpea protein concentrate	5, 10, 15, 20, and 25	FM replacement	Highest growth with 20-30% FM replacement and best protein efficiency at 40%	[61]

D: digestibility, ID: in-vitro digestibility, GT: growth trial, FM: fishmeal.

Another novelraw material is insect meal but unlike cowpea, its use in aquafeeds has been more deeply investigated and reviewed. On-farm trials with Atlantic salmon and other fish species have been done and some feed mills seems to be keen in adopting it as an ingredient once its production reaches the volumes required. As part of the growing literature in this space we published a chapter (introductory reading to academic and private sector) discussing the benefits of insects in animal nutrition, describing and discussing the nutritional aspects of different insect meals, some practical developments on aquatic nutrition and constraints on insect use and its future perspective (Freccia *et al*., 2020).

Digital technologies for nutrition: Near-infrared spectroscopy (NIRS)

Near-infrared spectroscopy (NIRS) consists of a non-invasive technique that provides rapid information on a range of chemical and physical properties of scanned samples. In the Aquafeed industry, it is used widely to predict the proximate composition of ingredients and resulting diets. We investigated the NIRS ability to predict the dietary proximate composition and starch cook of various species-specific aquafeeds, two of the most relevant characteristics and properties (Bourne *et al*., 2021). Aquafeed samples from various studies were analysed (Table 7). NIRS models were successfully developed with high R^2 values, low standard error of cross-validation and robust residual predictive deviation (Table 8). Our findings suggest the suitability of NIRS to rapidly and accurately predict proximate composition and starch cook of various aquafeeds. Similar approaches for chemical composition, including proximate composition, of raw materials are routine in feed mills, however calibrations like the one proposed are not as commonly adopted and would promote cost and operational efficiencies by monitoring quality control as well as the deployment of those technologies at farms to cross-check on feed attributes after storage

Table 7. Aquafeed data set used for NIRS model development. Adapted from Bourne *et al*., 2021 – https://doi.org/10.1177/0967033521999116

# samples	Species	Dry matter (%)	Ash (%)	Total Lipid (%)	Crude protein (%)	Gross Energy (MJ/kg)
5	Abalone	90-95	5-9	3-4	30-38	17-18
28	Barramundi	88-98	5-16	6-21	41-61	17-23
8	Salmon	94-97	12-15	16-18	57-60	21-22
17	Kingfish	93-98	10-12	13-18	45-52	21-23
43	Prawn	92-97	4-11	5-12	36-59	19-22
2	Red claw	92-95	6-8	2-5	30-35	18-19
15	Lobster	96-98	11-12	12-14	63-64	21-22
118	All	88-98	4-16	2-21	30-64	17-23

Table 8. NIRS models outputs. Adapted from Bourne *et al*., 2021 – https://doi.org/10.1177/0967033521999116

Component	PCs	R^2CV	SECV	RPD	RER
Dry matter	8	0.78	0.89	10.9	66.7
Ash	13	0.9	1.09	9	55.4
Total lipid	5	0.95	0.85	9.2	56.3
Crude protein	9	0.96	1.9	4.6	28.3
Gross energy	5	0.9	0.5	15.6	95.9
Starch cook	10	0.87	7.77	1.2	7.2
Starch gelatinised via steaming	5	0.93	5.89	1.8	11.2
Starch damaged via extrusion	9	0.92	4.77	1.9	11.5

*PCs = number of principal components used for model generation; R^2CV = coefficient of determination in cross validation; SECV = standard error of cross validation; RPD = ratio of performance deviation; RER = range error ratio.

In another NIRS study, we investigated the suitability of NIRS in predicting diet apparent digestibility, the composition of diets and faeces, and digestibility marker (Simon *et al*., 2022). NIRS models were developed and validated as part of a case-study investigating the accuracy of apparent digestibility results based on a range of prediction scenarios. Successful predictions for most nutrients were achieved for diets and faeces (Tables 9 and 10), except for yttrium oxide (digestibility marker) and a few micronutrients. We demonstrated the suitability of NIRS to assist in calculating apparent digestibility of nutrients in diets by predicting dietary and faecal composition of most nutrients, except for the digestibility marker, which at this stage still needs to be chemically analysed for accuracy. This approach reduces the number of samples to be chemically analysed, has welfare implications and bring opportunities in fish nutrition. Follow up research is planned to predict endogenous digestibility markers that may have a greater capacity for NIRS determination.

Table 9. NIRS models outputs. Adapted from Simon *et al*., 2022 – https://doi.org/10.1016/j.aquaculture.2021.737624

Component	PCs	R^2CV	SECV	RPD	RER
Diet composition	5-14	0.6-0.9	1-119	1-3	3-19
Faeces composition	5-15	0.6-0.9	1.-342	2-4	8-16

*PCs = number of principal components used for model generation; R^2CV = coefficient of determination in cross validation; SECV = standard error of cross validation; RPD = ratio of performance deviation; RER = range error ratio.

Table 10. Successful NIRS models outputs. Adapted from Simon *et al*., 2022 – https://doi.org/10.1016/j.aquaculture.2021.737624

Component	Nutrients
Diet composition	Dry matter, ash, crude protein, total lipid, gross energy and total amino acids
Faeces composition	Crude protein, total lipid, gross energy, total amino acids, methionine and lysine

Nutrition and production system

In a recent book "Biology and Aquaculture of Tilapia", we published a chapter "Biofloc technology (BFT) in tilapia culture" where we introduce, summarise the literature and briefly discuss the relationships between nutrition and BFT for tilapia, including topics such as composition of flocs, nutrient requirements, role of alternative ingredients, feed formulations, and feed management (Emerenciano *et al*., 2021).

Metabolism

Fish is generally very efficient in acquiring energy from protein, which is not different for barramundi. In this context, we investigated the metabolic effects of two diets varying the proportions of digestible protein and starch energy using metabolic tracers. Our findings highlight a unique series of specific hepatic regulatory mechanisms of barramundi to deal with dietary carbohydrates and provide valuable insights to nutritionists.

Ongoing research projects

Our ongoing research has focused on circularity using food waste as aquafeed ingredients and in vitro characterisation of pro- and anti-inflammatory properties of insect meals and other ingredients/additives. We are also working on the development of novel ingredients via pre-processing techniques.

Conclusion

The bulk of R&D related to the illustrated publications was done before the pandemic. However, we managed to finalise them (e.g. analytical work, data analysis and writing) using part of the time "gained" by the uncontrolled changes (e.g. projects and opportunities put on hold, limited access to facilities, etc). The ongoing research projects briefly discussed have been done throughout these challenging times.

We have learned a few lessons so far that could be summarised in (1) utilising technology and more efficient designs to gain more value from samples or data; (2) emphasis on focusing on research areas that can more effectively generate impact for the broad benefit of the Aquaculture industry; (3) even in times where travel is restricted, collaboration and exchange of ideas through online platforms was ongoing and preventeds repeating particular published or planned studies; (4) balancing several research horizons and areas with results being able to be applied straight away commercially (H1) but also longer term improvement in

methodologies (H2-H3) which can assist the aquacultureindustry is responding to present and future challenges.

References

Bell, L.W., James, A.T., Augustin, M.A., **Rombenso, A.,** Blyth, D., **Simon, C.,** Higgins, T.J.V., & Barrero, J.M. (2021). A niche for cowpea in sub-tropical Australia? *Agronomy*, 11, 1654.

Ruyter, B., Sissener, N.H., Ostbye, T-K., **Simon, C.J.,** Krasnov, A., Bou, M., Sanden, M., Nichols, P.D., Lutfi, E., & Berge, G.M. (2019). n-3 canola oil effectively replaces fish oil as a new safe dietary source of DHA in feed for juvenile Atlantic salmon. *British Journal of Nutrition*, 122(12)- 1329-1245.

Bourne, N., Blyth, D., & **Simon, C.** (2021). Rapid prediction of chemical composition and degree of starch cook of multi-species aquafeeds by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy*, 29(4), 216-225.

Chang, K.J.L., Parrish, C.C., **Simon, C.J.,** Revill, A.T., & Nichols, P.D. (2020). Feeding whole thraustochytrid biomass to cultured Atlantic salmon (*Salmo salar*) fingerlings: culture performance and fatty acid incorporation. *Journal of Marine Science and Engineering*, 8, 207.

Courtot, E., Musson, D., Stratford, C., Blyth, D., Bourne, N.A., **Rombenso, A.N., Simon, C.J.,** Wu, X., & Wade, N.M. (2022). Dietary fatty acid composition affects the apparent digestibility of algal carotenoids in diets for Atlantic salmon *Salmo salar*. *Aquaculture Research*. Doi: 10.1111/are.15753.

Emerenciano, M.G.C., **Rombenso, A.N.**, Vieira, F.d.N., Martins, M.A., Coman, G.J., **Truong, H.H**., Noble, T.H., & **Simon, C.J.** (2022). Intensification of Penaeid shrimp culture: an Applied review of advances in production systems, nutrition and breeding. *Animals*, 12(3), 236.

Noble, R.H., Rao, M., Briggs, M., Shinn, A.P., **Simon, C.**, & Wynne, J.W. (2021). Novacq™ improves survival of *Penaeus vannamei* when challenged with pathogenic *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease. *Aquaculture*, 545, 737235.

Noble, T.H., **Truong, H.H., Rombenso, A., Simon, C.J.**, Wade, N.W., & Wynne, J.W. (2021). Effect of sample type and the use of high or low fishmeal diets on bacterial communities in the gastrointestinal tract of *Penaeus monodon*. *Applied Microbiology and Biotechnology*, 105, 1301-1313.

Rombenso, A., Irvin, S., **Truong, H.,** Musson, D., Perrin, T., Blyth, D., Emerenciano, M., Briggs, M., & **Simon, C.** (2020). Commercial microbial biomass additive evaluated in black tiger shrimp diets. *Global advocate aquaculture magazine*, 13 July 2020.

Emerenciano, M.G.C., Fitzsimmons, K., **Rombenso, A.N.**, Miranda-Baeza, A., Martins, G.B., Lazzari, R., Fimbres-Acedo, Y.E., & Pinho, S.M. (2021). Biofloc Technology (BFT) in tilapia culture. *In book: Biology and Aquaculture of Tilapia*. CRC Press/Taylor & Francis Group 258-293.

Freccia, A., Tubin, J.S.B., **Rombenso, A.N.**, & Emerenciano, M.G.C. (2020). Insects in Aquaculture Nutrition: an emerging eco-friendly approach or commercial reality? *InTech Open* Doi: 10.5772/intechopen.90489.

Rombenso, A.N., Truong, H., & Simon, C. (2019). Insights on hatchery feed for *Penaeus monodon* postlarvae: custard diets supplemented with microbial biomass Novacq™ improve survival and enhance growth of larger animals. *Hatchery Feeds*, 7(3), 27-30.

Rombenso, A.N., Truong, H., & Simon, C. (2020). Dietary butyrate alone or in combination with succinate and fumarate improved survival, feed intake, growth and nutrient retention efficiency of juvenile *Penaeus monodon*. *Aquaculture*, 528, 735492.

Rombenso, A.N., Esmaeili, M., Araujo, B., Emerenciano, M., **Truong, H.,** Viana, M.T., Li, E., & **Simon, C**. (2021). Macronutrient Research in Aquaculture Nutrition. *Global Aquaculture Advocate* 8 February 2021.

Rombenso, A.N., Blyth, D., James, A.T., Nikalaou, T., & **Simon, C.J**. (2021). Lipoxygenase enzymes, oligosaccharides (raffinose and stachyose) and 11sA4 and A5 globulins og glyvinin present in soybean meal are not drivers of enteritis in juvenile Atlantic salmon (*Salmo salar*). *Applied Sciences*, 11(19), 9327.

Rombenso, A.N., Turchini, G.M., & Trushenski, J.T. (2021). The omega-3 sparing effect of saturated fatty acids: a reason to reconsider common knowledge of fish oil replacement. *Reviews in aquaculture*, 14(1), 213-217.

Rombenso, A.N., Duong, M.H., Hines, B.M., Ma, T., & **Simon, C.J.** (2021). The marine microbial biomass, Novacq™, a useful feed additive for postlarvae and juvenile *Litopenaeus vannamei*. *Aquaculture*, 530, 735959.

Simon, C., Rombenso, A., Truong, H., Noble, T., Wynne, J., Porras, O., Wade, N., & Briggs, M. (2019). Benefits of feeding microbial biomass on growth performance, feed efficiency, immunocompetence and gut microbiome of prawns. *AquaExpo 2019 Guayaquil*. Book of abstracts.

Simon, C.J., Truong, H.H., Noble, T.H., Osborne, S.A., Wynne, J.W., & Wade, N.M. (2020). Microbial biomass, marine invertebrate meals and feed restriction influence the biological and gut microbiota response of shrimp *Penaeus monodon*. *Aquaculture*, 520, 734679.

Simon, C.J., Truong, H., Habilay, N., & Hines, B. (2021). Feeding behaviour and bioavailability of essential amino acids in shrimp *Penaeus monodon* fed fresh and leached fishmeal and fishmeal-free diets. *Animals*, 11(3), 847.

Simon, C.J., Bourne, N., Hines, B.M., Pirozzi, I., & Booth, M. (2022). Estimation of apparent dietary nutrient digestibility in yellowtail kingfish *Seriola lalandi* by near-infrared spectroscopy (NIRS). *Aquaculture*, 548, part 1, 737624.

Truong, H.H., Moss, A.F., Bourne, N.A., & **Simon, C.J.** (2020). Determining the importance of macro and trace dietary minerals on growth and nutrient retention in juvenile *Penaeus monodon*. *Animals*, 10(11), 2086.

Truong, H., Hines, B.H., **Rombenso, A.N., & Simon, C.J.** (2021). Feed intake, gastro-intestinal transit and haemolymph free amino acids in the shrimp *Penaeus monodon* are influenced by marine meal supplementation. *Aquaculture*, 533, 736171.

Truong, H., Blyth, D., Habilay, N., Bourne, N., Wade, N., Hines, B., **Rombenso, A., & Simon, C**. (2022). Faecal collection methods result in different estimates of nutrient apparent digestibility in *Penaeus monodon*. *Aquaculture*, 551, 737957.

Wade, N.M., Trenkner, L.H., Viegas, I., Tavares, L.C., Palma, M., Skiba-Cassy, S., Dias, K., Vachot, C., Araujo, B.C., Bourne, N., Blyth, D., Irvin, S., & Glencross, B.D. (2020). Dietary starch promotes hepatic lipogenesis in barramundi (*Lates calcarifer*). *British Journal of Nutrition*, 124, 363-373.